



# The prevalence of *Phytophthora* in British plant nurseries; high-risk hosts and substrates and opportunities to implement best practice

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## Abstract

Invasive *Phytophthora* species infect a very broad range of herbaceous and woody hosts globally. The UK alone has experienced a particularly damaging series of outbreaks and epidemics of new, invasive *Phytophthora* species affecting the nation's trees over the last 30 years. The link between *Phytophthora* outbreaks and the importation and spread of infected nursery stock is well established across many countries worldwide. To understand better the pathways of spread of *Phytophthora* in the nursery trade in Britain, we applied a standardized nursery sampling method combined with a refined metabarcoding detection method to capture the diversity of *Phytophthora* species at 134 British plant nurseries representing a range of biosecurity and trading practices over multiple sampling years between 2016 and 2022. This included root and water samples collected from 17 nurseries sampled seasonally and root samples collected from 117 nurseries sampled once as part of plant health inspections. Based on analyses of 1894 pooled samples, DNA barcodes of 85 *Phytophthora* species or complexes were detected, with variation in species' relative frequencies across nurseries. We present the top 20 host-*Phytophthora* associations ranked by relative frequency and report five novel *Phytophthora* records for the UK. We identified surprisingly high-risk hosts (such as Douglas fir) with the greatest number of *Phytophthora* associations and revealed *Phytophthora* nursery niche preferences for water or roots. We discuss the implications of our findings in terms of pathogen diversity and abundance, high-risk

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hosts, our information dissemination approach and resulting advice on nursery practices aimed at reducing risk.

**KEYWORDS**

best practice, diversity, niche preferences, novel host associations, *Phytophthora*, plant nursery

## 1 | INTRODUCTION

*Phytophthora* is a diverse genus of filamentous oomycete plant pathogens that infect a broad range of herbaceous and woody plants with severe economic and ecological impacts in the agricultural, horticultural and forestry sectors worldwide (Barwell et al., 2021). One of the most notable examples is *P. ramorum*, the causal agent of 'sudden oak death' in the United States (Grünwald et al., 2012), 'ramorum blight' in Europe and 'sudden larch death' in the UK (Webber et al., 2010). Other invasive species include *P. cinnamomi*, the causal agent of forest dieback in multiple hosts and regions globally (Burgess et al., 2017), and *P. ×alni*-driven alder (*Alnus glutinosa*) decline across Europe (Aguayo et al., 2014). The UK has experienced a particularly damaging series of outbreaks and epidemics of invasive *Phytophthora* species affecting the nation's trees over the last 30 years (Green et al., 2021) including not only *P. ramorum* and *P. ×alni* but also *P. kernoviae* infecting mainly beech (*Fagus sylvatica*; Brasier et al., 2005), *P. lateralis* killing Lawson cypress (*Chamaecyparis lawsoniana*) in a range of amenity settings (Green et al., 2013), *P. austrocedri* killing native juniper (*Juniperus communis*) in natural woodlands across northern Britain (Green et al., 2015), and most recently *P. pluvialis* infecting western hemlock (*Tsuga heterophylla*; Pérez-Sierra et al., 2022) and Douglas fir (*Pseudotsuga menziesii*) in commercial forestry plantations. Clearly, there is an urgent need to understand the drivers of *Phytophthora* emergence in Britain.

*Phytophthora* has been found to be prevalent in plant nurseries in continental Europe (Jung et al., 2016; Moralejo et al., 2009; Prigigallo et al., 2015) and the United States (Parke et al., 2014). *P. ramorum*, *P. kernoviae*, *P. austrocedri* and *P. lateralis* are regularly intercepted on imported plants by UK statutory Plant Health authorities and other forms of surveillance (Table S1). Routine surveillance within Britain also reveals that these notifiable species are frequently detected in plant nurseries and in the wider environment, the latter defined here as any natural or managed environment outside of a nursery setting (Table S1). The link between *Phytophthora* outbreaks in naïve environments and the importation and spread of infected nursery stock is thus well established, not just in Britain but across many countries (Antonelli et al., 2022; Goss et al., 2011; Green et al., 2021; Migliorini et al., 2015; Schoebel et al., 2014). Spread from nursery settings to the wider environment is a significant risk because nursery stock is planted in public and private gardens, amenity, forestry, landscaping and conservation plantings. For example, in Britain, supplementary planting activity of juniper has been linked to adjacent (<2 km) outbreaks of *P. austrocedri* (Donald et al., 2021) and the novel pathogen *P. tentaculata* was first detected in US plant nurseries growing

and supplying native stock to a failing restoration project (Rooney-Latham et al., 2015). As global trade in plants-for-planting increases, the potential for *Phytophthora* to spread to new hosts and geographic regions is growing (Roy et al., 2014; Seebens et al., 2015), with significant implications for plant health. Given the impacts that *Phytophthora* is already having across the UK, and the current high potential for further introductions, it is imperative to gain an understanding of *Phytophthora* species diversity and abundance across the nation's plant nursery environments so that more effective mitigation measures can be implemented.

Although they resemble fungi in many aspects of their life cycle, *Phytophthora* species are in fact more closely related to brown algae and are taxonomically positioned within the kingdom Chromista, producing motile, free-swimming zoospores that are formed in spore sacs known as sporangia. Many *Phytophthora* species also produce resting structures including asexually produced chlamydospores and hyphal stromata, and sexually produced oospores, all of which are very resilient to degradation, enabling survival in plant residues and soils over years (Crone, McComb, & O'Brien, 2013; Ristaino & Gumpertz, 2000). It is the water- and soil-inhabiting aspects of the *Phytophthora* life cycle that allow these pathogens to thrive and persist in plant nursery environments. In particular, the ability of *Phytophthora* to reside unseen in soil or growing media, cause cryptic infection and even sporulation on host foliage in the absence of obvious symptoms (Denman et al., 2009) is one of the greater challenges to controlling *Phytophthora* in the plant trade, as these species may evade national and international Plant Health surveillance protocols (Green et al., 2021). Additionally, statutory surveillance is focused on regulated pathogens, but many unregulated *Phytophthora* species circulating in traded plants are equally damaging. The *Phytophthora* genus comprises over 240 described species worldwide, currently divided into 11 major and five minor phylogenetic clades (Abad et al., 2023; Brasier et al., 2022; Jung et al., 2017, 2024). To understand better *Phytophthora* spread in the nursery trade, a diagnostic method must be applied that can potentially capture the full diversity of species present across all clades in a range of sample types without reliance on the presence of symptoms.

Previous studies involving *Phytophthora* detection in nurseries (e.g., Moralejo et al., 2009; Parke et al., 2014; Sims et al., 2019) have used direct isolation methods from symptomatic tissues and baiting from water or growing media samples. The latter method relies on the ability of zoospores to swim from an inoculum source to infect a susceptible bait plant and tends to favour fast-growing species that readily produce zoospores under the baiting conditions (Sarker et al., 2021; Scibetta et al., 2012).

High-throughput metabarcoding sequencing is a rapidly advancing technology that has the potential to detect all species of a target genus present within an environmental sample, including species as yet undescribed, by PCR amplification of each species' unique DNA 'barcode' region that is then matched to a database of known sequences (Cock et al., 2023). The potential of metabarcoding was demonstrated by Prigigallo et al. (2015) who used the genus-specific PCR primers of Scibetta et al. (2012) to detect 15 *Phytophthora* taxa in soil and root samples across nine nurseries in southern Italy. The application of metabarcoding, combined with optimized sampling and sample processing methods, may facilitate the necessary understanding of *Phytophthora* infestations in British nurseries, providing the evidence base to support effective mitigation based on a raised awareness of the most at-risk hosts and nursery environments.

In this study we apply a standardized nursery sampling method combined with a refined metabarcoding detection method to capture the diversity of *Phytophthora* species at 134 British plant nurseries representing a range of biosecurity and trading practices over multiple sampling years between 2016 and 2022. Specifically, we report variation in pathogen detection across nurseries, identify prevalent and novel host-*Phytophthora* associations and reveal *Phytophthora* nursery niche preferences for water or roots. We discuss the implications of these analyses in terms of pathogen diversity and abundance, high risk hosts, our information dissemination approach and resulting risk reduction in nursery practices. Our analyses will aid the prioritization of statutory plant health surveillance to increase the chances of early detection of emerging *Phytophthora* species in nurseries and the wider environment.

## 2 | MATERIALS AND METHODS

The incidence and diversity of *Phytophthora* species in water and root samples was examined in British nurseries from May 2016 to October 2021 as part of two projects, Phyto-threats (Green et al., 2021) and ID-PHYT (Green et al., 2023). The nursery survey was aimed at maximizing the detection of *Phytophthora* and was conducted on two scales; a fine-scale survey that involved the detailed sampling by the project team of 17 partner plant nurseries twice a year (spring/summer and autumn/winter) over 2–3 years between 2016 and 2021, and a broad-scale survey involving 118 nurseries and garden centres sampled systematically during annual statutory plant health inspections conducted over a 3 year period (2017–2019).

### 2.1 | Fine-scale survey

The 17 partner nurseries were located in Scotland (10), England (6) and Wales (1), and spanned a geographical range of approximately 50° to 57° latitude N and 0.9° to –4.8° longitude W. Partner nurseries encompassed a range of management practices and business types including the production of bare root and/or containerized

forest trees (5), wholesale horticultural plants including herbaceous perennials, shrubs and trees (10), specialist native trees (1) and stock for botanic garden collections (1). Five of the wholesale horticultural nurseries had on-site retail garden centres from which plants were also sampled. Water was sampled from various points around each nursery and roots were sampled from stock plants as well as from plants discarded in waste piles, as described below.

#### 2.1.1 | Water sampling

Prior to each nursery visit, laboratory mains water was sampled as the laboratory blank control, which acted as a check for *Phytophthora* carry-over between nurseries in the sampling kit. On arrival at each nursery, mains tap water was sampled as the field blank control, which acted as a check for contamination in the on-site water used for water flow-through sampling (as described below) and equipment cleaning. Water was sampled for environmental DNA (eDNA) extraction from various locations on each nursery including the irrigation water at source (e.g., mains taps, storage tanks, boreholes, irrigation ponds, rivers, rainwater tanks) as well as water collection and run-off points (e.g., puddles, drainage ditches, run-off ponds and adjacent streams). Three 5-L replicate subsamples of water from each source were pumped through a 47 mm diameter mixed cellulose ester filter (Millipore Sigma) of 1.2 µm pore size held in a 47 mm polycarbonate in-line filter holder (Pall Corporation) using either an adapted knapsack sprayer (CP15 2000 Series Knapsack Sprayer 15 L; Figure 1a) or custom-designed pumping system with disposable water reservoirs (2 L). The latter set-up used 2-L plastic bottles for sampling that were previously unused and contained drinking water. Each bottle was connected to a stirrup bike pump and silicone tubing to a set of three filter holders before the water was pumped through the three filters simultaneously. The drinking water contents were filtered on site as a blank field sample before filtering the nursery water samples.

For nursery water samples rich in soil or compost particulates, for example those scooped from puddles (Figure 1b) or from water flow-through testing of plant stock (see below), each was prefiltered through multiple layers of clean muslin cloth. Filters were replaced if they became blocked with particulate material (e.g., Figure 1c), up to a maximum of three filters per replicate subsample, so that for samples that blocked filters quickly less than 5 L was filtered per subsample. All filters from each replicate subsample were placed in the same 15 mL tube containing 8 mL of Longmire lysis buffer (100 mM Tris, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) and stored at 5°C. As soon as possible after returning to the laboratory, the tubes were agitated on a rotating wheel for 10 min at room temperature, the filters removed, cut in half, placed into 2 mL Eppendorf tubes and frozen at –20°C. Additionally, 1.5 mL of buffer solution in which the filters had been stored was placed in a separate 2 mL Eppendorf tube. All samples, including the original 15 mL tube, were stored at –20°C before DNA extraction.



**FIGURE 1** Photographs of nursery sampling to illustrate methods and symptoms. (a) Adapted knapsack sprayer for water sampling, (b) sampling from a puddle, (c) particulates captured on a cellulose filter after pumping through a water sample, (d) *Pinus sylvestris* seedlings in trays being sampled using the water flow-through method, (e) *Chamaecyparis lawsoniana* root sampling, (f) *Rhododendron* with foliage lesions and wilting due to *Phytophthora ramorum* infection, (g) recently imported *Cupressus × leylandii* with foliage bronzing due to *P. austrocedri* infection.

Water flow-through sampling was also carried out on batches of plants showing typical *Phytophthora* symptoms or which are known *Phytophthora* hosts. This included stock plants and, in some cases, plants in waste piles. Plants were placed in three replicate 63×63×10cm trays, watered from above to field capacity and the water allowed to percolate through the pot compost into the bottom of the tray (e.g., Figure 1d). The number of plants sampled per replicate tray varied according to the size of the batch of plants being tested but was usually a minimum of three. Plants were left for at least 30min before filtering about 5L of flow-through water in each replicate tray as described above.

Equipment was rinsed and soaked for at least 30min in chlorine solution to denature DNA between each water sample. The chlorine solution initially used was 10% household bleach (sodium hypochlorite), but due to foaming was changed to the use of one Instachlor PR-150 rapid-release chlorine tablet (Palintest Ltd) per 5L of mains tap water. The chlorine solution was pumped through the filter system after soaking. After chlorination, all equipment was rinsed several times in mains tap water.

### 2.1.2 | Root sampling

Roots were sampled from batches of plants showing typical *Phytophthora* symptoms or which are known *Phytophthora* hosts, including plants subjected to water flow-through sampling. Small plants were knocked out of their pots (Figure 1e) and the roots pinched off. Where plants were larger and could not be removed from pots, the roots were collected from close to the compost surface near the top of the pots. Up to 10g of fine roots were sampled from each plant, targeting discoloured or water-soaked roots where possible, and placed in a small, perforated seed envelope that was in turn placed in a plastic bag. Roots were pooled from at least three plants per replicate subsample into a single envelope, with three replicate subsamples per batch of plants. Where only one plant was present in a batch, that plant was sampled alone. On a few occasions (<10), foliage and/or phloem tissue was sampled from stock plants, shelterbelt or landscaping trees within the nursery perimeter if these plants exhibited symptoms typical of *Phytophthora* infection, such as foliage lesions or bronzing (e.g., Figure 1f,g) and/or stem bleeds.

All plant-associated samples were stored at  $-20^{\circ}\text{C}$ , freeze-dried for 5–7 days (Model Delta 1–24 LSC; Martin Christ, GmbH) and stored with silica at room temperature until DNA extraction.

### 2.1.3 | Data on sample attributes, nursery management practices and location

For all fine-scale survey samples, data were collected for sample type (water versus plant, the latter encompassing both root and foliage samples), location of nursery, host species, any symptoms observed at sampling, plant age (height), pot volume if containerized, approximate number of plants in the whole batch from which samples were taken, nursery stock identifier code, and whether propagated on site or bought in. If plants were bought in then arrival date, supplier and plant passport number were recorded where this information was available. To allow future analyses of *Phytophthora* communities in relation to nursery attributes and management practice (not reported here), data were also collected on the water source and irrigation system, growing system (i.e., indoor or outdoor, whether grown on bare ground, Mypex fabric or raised benches). Data were also collected on practices such as use of a quarantine holding area for imported plants, method(s) of disposal of plant waste, range of water sources used for irrigation and any treatments applied, growing system (containerized or bare root), growing medium, reuse and disinfestation of pots, use of raised benches for growing stock, installation of drainage and use of disinfestation stations for equipment, boots and vehicles/machinery.

## 2.2 | Broad-scale survey

For the broad-scale survey, Plant Health inspectors collected root samples from 117 plant nurseries across Britain between June 2017 and July 2019 as part of routine statutory Plant Health inspections. This survey involved the collection of 10 root samples from individual or small batches of symptomatic plants per nursery with each host species recorded. Root samples, which were not collected in triplicate but rather as a single batch from any one host, were placed in prelabelled perforated seed envelopes and mailed to the laboratory for processing in the same way as for the fine-scale survey.

## 2.3 | DNA extraction

All replicate subsamples were treated individually for downstream processing and metabarcoding analysis. Root samples (40 mg) were ground in a PM400 ball mill (Retsch) for 2 min at 25 Hz, changing orientation after 1 min. DNA was extracted from roots using the DNeasy Plant Pro kit (QIAGEN) according to the manufacturer's instructions except that step 1 used  $450\mu\text{L}$  CD1 and  $50\mu\text{L}$  PS (up

to  $350\mu\text{L}$  additional CD1 was added if very absorbent), step 5 used  $250\mu\text{L}$  CD2 and the elution process in step 14 used  $50\mu\text{L}$  EB, which was passed through the membrane twice.

DNA was extracted from the aliquoted 1.5 mL Longmires buffer samples, in which the filters from the water sampling had been stored, using the DNeasy Blood & Tissue Kit (QIAGEN). For filter extraction the protocol was slightly modified in that  $400\mu\text{L}$  buffer AP1 and  $4\mu\text{L}$  RNase from step 7 were added to a half filter before the bead beating step. After bead beating, samples were centrifuged for 5 min at  $16,627g$  and the supernatant transferred to a fresh 2 mL tube without the filter pieces. In the final step, DNA was eluted with  $50\mu\text{L}$  of buffer AE. More detail on the sample processing protocols used in this study are available in Randall et al. (2024).

## 2.4 | Amplicon PCR, Illumina sequencing library preparation and sequencing

An approximately 262 bp region of the ribosomal DNA (rDNA) internal transcribed spacer 1 (ITS1) was amplified from each DNA sample using nested PCR with primer pairs 18Ph2F (5'-GGATAGACTGTTGC AATTTTCAGT-3') and 5.8S-1R (5'-GCARRGACTTTCGTCCTCCYRC-3') in the first round and ITS6F (5'-GAAGGTGAAGTCGTAACAAGG-3') and 5.8S-1R in the second round following the protocol of Scibetta et al. (2012) except that proof-reading enzyme KAPA HiFi HotStart ReadyMix (KAPA Biosystems) and a reaction volume of  $12.5\mu\text{L}$  was used in both rounds, with each reaction containing  $4.5\mu\text{L}$  PCR-grade water,  $6.25\mu\text{L}$  Kapa HiFi ReadyMix,  $0.375\mu\text{L}$  ( $10\mu\text{M}$ ) of each forward and reverse primer and  $1\mu\text{L}$  DNA or  $1\mu\text{L}$  round 1 reaction product. Amplification conditions were also modified from the Scibetta et al. (2012) protocol with initial denaturation at  $95^{\circ}\text{C}$  for 3 min (first and second round), followed by 30 cycles of  $98^{\circ}\text{C}$  for 20s,  $61^{\circ}\text{C}$  for 25s and  $72^{\circ}\text{C}$  for 40s, with a final cycle of  $72^{\circ}\text{C}$  for 1 min (first round) and 25 cycles of  $98^{\circ}\text{C}$  for 20s,  $61^{\circ}\text{C}$  for 25s and  $72^{\circ}\text{C}$  for 25s, with a final cycle of  $72^{\circ}\text{C}$  for 1 min (second round). Samples with the expected approximately 262 bp product were identified using gel electrophoresis following round 2, and the round 1 product was reamplified in round 2 using the PCR primers amended with overhang adapters to ensure compatibility with the Illumina index and sequencing adapters. These were forward overhang: 5'-TCGT CGGCAGCGTCAGATGTGTATAAGAGACAG-[ITS6] and reverse overhang: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[5.8S-1R] (Illumina, 2013). The extension time was slightly reduced during the repeated cycles to 20s at  $72^{\circ}\text{C}$ . Six synthetic sequence control samples (Cock et al., 2023) per 96-well plate were also amplified using the second round primers. Control samples comprised four synthetic 268 bp long DNA oligonucleotides (Integrated DNA Technologies) of randomly generated base composition that include the amplification primer sequences (ITS-6 or 5.8S-1R) at each end (GenBank accession numbers PP407413 to PP407416). A preprepared mix of the four synthetic DNA fragments, each diluted to a different concentration (10 ag, 100 ag, 1000 ag and 10 fg per reaction)

were used. These synthetic sequence controls acted as a check for cross contamination in the plate preparation process and were used to set read abundance thresholds as described below.

Samples were prepared for sequencing following the protocols described for 16S Metagenomic Sequencing Library Preparation (Illumina, 2013). In brief, this involved clean-up of amplicon PCR using Agencourt Ampure XP beads (Agencourt Bioscience), or SPRI select beads (Beckman Coulter) followed by index PCR in KAPA HiFi HotStart ReadyMix (KAPA Biosystems) to attach dual indices and Illumina sequencing adapters to each sample using the Nextera XT Index Kit (Epicentre). This step ensured that each sample could be uniquely identified during the sequencing run. A second PCR clean-up (as above) was then carried out and DNA of each sample visualized on a 2200 TapeStation (Agilent Technologies) or on a 2% agarose gel. Libraries were quantified using a fluorimetric method based on double-stranded DNA binding dyes Qubit assay (Qubit2 fluorimeter; Invitrogen) or Quant-iT PicoGreen assay with samples read with a standard fluorescent microplate reader (Fluoroskan FL; Thermo Fisher Scientific). For each sequencing run, 96 or 192 samples were pooled for paired-end (2×250bp) sequencing on a single flow cell of an Illumina MiSeq sequencer using the MiSeq v. 2500bp standard kit (Illumina) at the James Hutton Institute, Dundee, UK. Following quality control and demultiplexing, FASTQ files containing reads for each sample were exported for bioinformatics analysis.

## 2.5 | Bioinformatics analysis

Sequence data were analysed with the THAPBI PICT pipeline, which was developed in part for this project (Cock et al., 2023), with the results here generated using v. 1.0.3 using the included reference database and default settings save use of the 1s3g classifier, which applies a species level to a perfect match or 1bp difference (deletion, insertion or substitution) and a highly conservative genus level match with up to 3bp difference. Beyond 3bp difference reads were defined as being of an unknown genus, though it is accepted that those closest to the threshold will probably be taxa of the genus *Phytophthora*. The default sample read abundance thresholds of 0.1% and 100 reads were used (relatively stringent with MiSeq data volumes), increased to match the most abundant biological sequence detected in the synthetic control samples on the same plate. The reports were generated using sample metadata cross referenced by the sequenced sample filenames, using a largely automated mapping with occasional manual overrides to deal with rare data entry corrections as needed.

## 2.6 | Data processing

Sample replicates were pooled if they shared the same host and substrate by summing the number of reads for each *Phytophthora* species using the Python script (pooling.py) included with the THAPBI PICT pipeline (Cock et al., 2023). A subset of samples comprised

replicates from different hosts; these replicates were treated as distinct samples. The read data for each sample were converted to a binary format by assigning 1 to species with any reads above our thresholds, and 0 to all other species (non-detections). The total number of samples was tabulated by nursery, host and sample substrate in order to account for sampling effort across nurseries, hosts and substrate when modelling positive samples.

## 2.7 | Rarefaction of *Phytophthora* assemblages

To assess how comprehensively the *Phytophthora* communities within nurseries were sampled, we used interpolation (rarefaction) and extrapolation (prediction) of curves relating number of samples to *Phytophthora* diversity detected. Curves were calculated using the R package iNEXT, which uses Hill numbers (effective number of species) to quantify diversity within a community and facilitate comparisons between *Phytophthora* assemblages at different nurseries, while standardizing for sample size (Hysiehet al., 2016).

## 2.8 | Modelling framework

We fitted two hierarchical Bayesian models to estimate the probability of a sample being positive for *Phytophthora* (and related oomycetes) in relation to (a) host genus to estimate the overall probability of detection of *Phytophthora* across host genera and (b) sample type, to estimate *Phytophthora* species communities in water and plant substrates. Hierarchical models allow these effects to be estimated while accounting for the non-independence of observations within host genera, nurseries and within *Phytophthora* species. For example, we might expect greater similarity in *Phytophthora* diversity in samples taken within the same nursery than between samples taken from different nurseries, due to spatial proximity, sampling effort and other unmeasured, environmental or management covariates. We may also expect the number of positive samples to be non-independent among *Phytophthora* species if, for example, some species are inherently more detectable than others due to the metabarcoding methodology or their ability to cause disease in hosts. Non-independence among samples effectively reduces the sample size available for inference and it is important to account for this so that the precision of the effect sizes is not overestimated (or conversely the uncertainty is not underestimated); this can lead to effects appearing significant when they are not.

Bayesian models estimate a posterior distribution of parameters, rather than a point estimate. Parameters with a posterior distribution where the estimated 95% credible interval does not overlap zero were interpreted as having statistically significant effects on detection probability. We report effect sizes and variance explained (a measure of model performance) for each model. These estimates are reported with the associated 95% credible intervals in brackets (lower quantile = 0.025, upper quantile = 0.975), which reflects the uncertainty in the estimate and underlying variability in the data.

### 2.8.1 | Modelling prevalence of *Phytophthora* across host genera

To compare *Phytophthora* risks associated with different nursery stock, we modelled the number of positive samples for each host–pathogen association across the nurseries sampled to estimate the probability of detection on different host plant genera as our metric of prevalence. The model predictions were used to identify host plant genera with higher predicted *Phytophthora* pathogen burdens, broad host range *Phytophthora* species and the overall prevalence of different host–*Phytophthora* interactions across nurseries. For this analysis, we selected 35 well-sampled host plant genera defined as those that were sampled in at least two nurseries and with at least 10 samples in total across all fine- and broad-scale sampled nurseries. We included 53 known *Phytophthora* species identified to species level with at least two detections across the well-sampled host genera. The samples for these hosts and *Phytophthora* species spanned 131 of the 134 fine- and broad-scale sampled nurseries. Non-interactions (where a host is not associated with a particular *Phytophthora* species) were only included if the host genera had been sampled from the nursery, yielding 34,662 unique nursery–host–*Phytophthora* combinations (654 unique host–nursery samples  $\times$  53 *Phytophthora* species). We modelled the number of positive samples for each host–*Phytophthora* sp. association as a binomial response, with the number of trials specified as the number of times each host was sampled within a nursery, to account for unbalanced sampling across host genera and nurseries.

We allowed the models to estimate unique intercepts for each nursery, host genus and *Phytophthora* species to account for non-independence of samples from the same nursery and host genus and differences in overall prevalence among *Phytophthora* species. Using the predictions, we ranked the 35 well-sampled host genera from those with the heaviest predicted *Phytophthora* burdens (in terms of number of *Phytophthora* species the host interacts with) to the least affected. We also ranked the 53 *Phytophthora* species from those with the broadest predicted number of host species to those predicted to have the narrowest range of host species within the nursery dataset and estimated the most prevalent host genus–*Phytophthora* interactions across the sampled nurseries, adjusted for sampling effort and non-independence of samples from the same nursery, host or *Phytophthora* species.

### 2.8.2 | Modelling *Phytophthora* communities in relation to sample substrate

To test if *Phytophthora* communities showed preferences for an aquatic niche (water samples) or a plant niche (mainly root, plus a few foliage or phloem samples and water flow-through samples that had been passed through plants and growing media), we modelled the number of *Phytophthora*-positive samples in relation to sample

substrate. The number of positive samples was modelled as a binomial response where the number of trials was specified as the total number of water or plant samples within each nursery. There were 68 identified *Phytophthora* species (excluding unknown species, but including complexes) sampled in two or more of the 17 nurseries, where the sample substrate was known to be either water or plant material. The broad-scale sampled nurseries are excluded from this analysis as no water samples were taken from these nurseries. We pooled positive samples within these two substrates, yielding 2312 ( $2 \times 17 \times 68$ ) observations for unique combinations of sample substrate, nursery and species. A species-level intercept was specified to account for variation in overall prevalence of *Phytophthora* species and a species-level slope allowed the effect of sample substrate to vary across *Phytophthora* species, capturing whether some species were more common in water samples and others more common in samples from plants. We accounted for unmeasured nursery-level differences (e.g., environmental effects) by specifying a nursery-level random intercept.

## 3 | RESULTS

Data on the number of replicate samples of each type (excluding the few foliage and stem samples) collected in the fine-scale and broad-scale surveys and the number and proportion of those samples that were positive for *Phytophthora* and related taxa, are shown in [Table 1a](#). Overall, approximately 40% of root and water samples were PCR-positive in the fine-scale survey, with a lower positivity rate (32%) for broad-scale survey root samples ([Table 1a](#)). Of the 121 laboratory and 87 field blank control water samples collected to test for cross-contamination, 15% were PCR-positive for *Phytophthora* (and related oomycetes) ([Table 1a](#)). [Table 1b](#) presents the broader taxonomic groups identified in the study by the number of unique amplified sequence variants (ASVs) and number of reads of each ASV detected per taxon. The number of reads is presented as it provides a measure of the depth of the barcode sequencing. Similarly, the proportion of reads and ASVs number gives an indication of the success of the assay at detecting the specific target and non-target taxa.

A total of 59.4 million barcode reads that passed quality control were generated for the nursery samples across 21 MiSeq flow cells, which resulted in 2489 unique ASVs ([Table 1b](#)). The majority of ASVs (61%) and 77% of the reads matched the classifier's definition of the genus *Phytophthora* (known or unknown species), with 20% and 11% of the reads beyond the formal 3bp threshold to a known genus match and called as unknown genera. ASV barcodes of seven downy mildew genera were detected comprising 13% of ASVs and 10% of all reads, with the other anticipated target genus of *Nothophytophthora* comprising 1.6% of ASVs and 0.04% of reads. Other non-target oomycete genera comprising *Pythium*, *Phytophythium*, *Elongisporangium* and *Globisporangium* ([Table 1b](#)) combined were a minor component with 4% of ASVs and 1% of reads and

**TABLE 1** Summary of sample results from the survey of British plant nurseries excluding the few foliage or stem samples. (a) Sample numbers (including replicates) and those that were positive for *Phytophthora* and related taxa shown according to the type of sample. (b) The number of barcode reads and amplified sequence variants (ASVs) from different taxonomic groups.

(a)					
Sample type	Number of collected samples	Number of tested samples	PCR +ve samples	PCR -ve samples	Proportion of tested samples +ve
Fine-scale total	3158	3079	1252	1827	0.41
Fine-scale laboratory blank controls	121	98	15	83	0.15
Fine-scale field blank controls	87	86	13	74	0.15
Fine-scale water samples	1110	1100	453	647	0.41
Fine-scale root samples	1794	1748	737	1011	0.42
Broad-scale root samples	797	757	245	512	0.32
(b)					
	Number of ASVs	Number of reads	Proportion of ASVs	Proportion of reads	
Total	2489	54,924,414			
Unknown genera	500	5,933,234	0.19976	0.10803	
Unknown <i>Phytophthora</i>	408	1,709,143	0.16300	0.03112	
Known <i>Phytophthora</i>	1120	40,796,574	0.44746	0.74278	
<i>Nothophytophthora</i> (all)	40	221,181	0.01598	0.00403	
<i>Peronospora</i>	225	3,223,682	0.08989	0.05869	
<i>Hyaloperonospora</i>	66	2,041,431	0.02637	0.03717	
<i>Bremia</i>	12	105,720	0.00479	0.00192	
<i>Plasmopara</i>	9	126,574	0.00360	0.00230	
<i>Basidiophora</i>	4	40,979	0.00160	0.00075	
<i>Paraperonospora</i>	4	31,066	0.00160	0.00057	
<i>Pseudoperonospora</i>	2	71,749	0.00080	0.00131	
<i>Pythium</i>	10	76,821	0.00400	0.00140	
<i>Phytophthium</i>	72	377,570	0.02877	0.00687	
<i>Elongisporangium</i>	4	27,307	0.00160	0.00050	
<i>Globisporangium</i>	11	132,062	0.00439	0.00240	
Synthetic sequence controls (all)	2	9141	0.00080	0.00017	

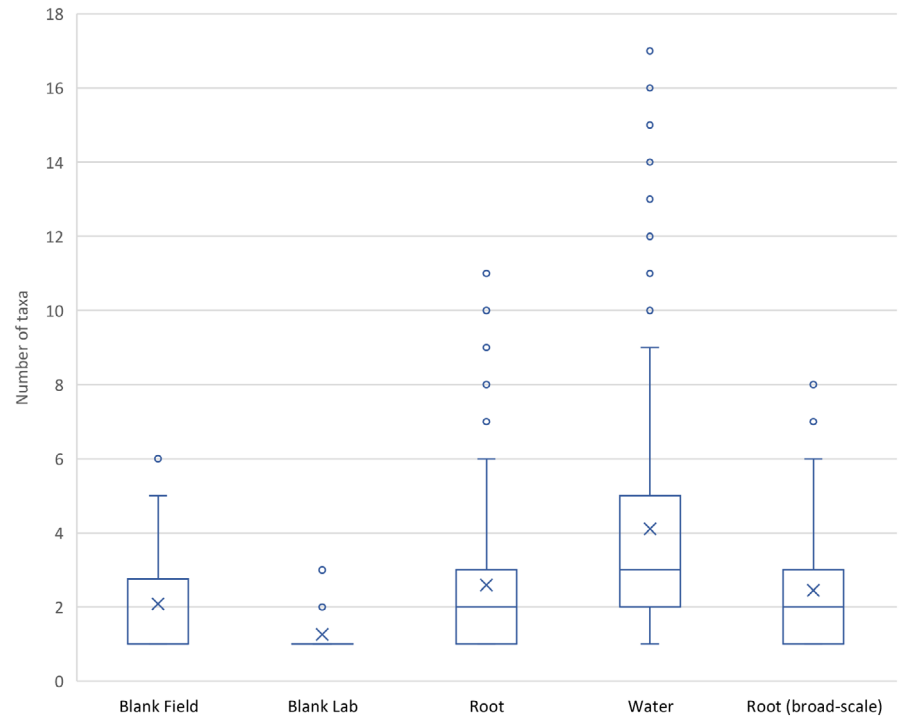
were almost exclusively detected in samples with low or no detected *Phytophthora* or downy mildew barcodes. Water samples hosted the greatest number of taxa and laboratory blank control samples the least, with similar numbers of taxa detected in root samples and field blank control samples (Figure 2).

Analyses were based on data from 1894 samples (pooled replicates within samples from the same host or substrate) taken across the 134 nurseries (17 fine-scale sampled and 117 broad-scale sampled), with 1674 from plants or associated soils and 220 from water sources (from fine-scale sampled nurseries only). Across all 134 nurseries, 85 unique *Phytophthora* species or complexes were detected, which could be identified using the THAPBI PICT ITS1 classification tool and reference database (Figure 3a). Reviewing the

unknown sequences, we labelled a further 11 unique sequences as novel *Phytophthora* taxa that were detected in multiple samples over multiple sites, which were added to our reference database. There were 266 detections of sequences with no assigned *Phytophthora* species due to their not having a match to the reference database (Figure 3a). The number of detections per identifiable *Phytophthora* species ranged from 1 to 200 with a median of six, with *P. cryptogea*/*P. pseudocryptogea*, *P. gonapodyides*, *P. cinnamomi*, *P. syringae* and *P. cactorum* representing the top five species detected across the study (Figure 3a). Samples were taken from hosts in 177 different plant genera (Figure 3b). Sampling intensity per host genus ranged from 1 to 133 samples with median of two samples per host genus. The relationships between



**FIGURE 2** Box and whisker plot of the number of taxa (by genus and species) in each category of sample collected during the survey of British nurseries. All sample types relate to the fine-scale survey except root (broad-scale). Bar shows median, × mean and box lower and upper quartiles.



*Phytophthora* species detected and host, nursery and sample substrate are explored further below.

### 3.1 | *Phytophthora* detections in relation to nursery

Sampling was insufficient in broad-scale sampled nurseries to estimate total species richness. Rarefaction within fine-scale sampled nurseries indicated that *Phytophthora* communities in some nurseries were more completely sampled than others. *Phytophthora* diversity in nurseries N002, N005, N007, N009, N011 and N013 is approaching an asymptote, suggesting the sampling was close to capturing the complete *Phytophthora* community (Figure 4a). In nurseries N001, N015, N016 and N017, the observed diversity is still accumulating steeply at the total sample size ( $n = 119, 51, 50$  and  $58$ , respectively) and a large proportion of *Phytophthora* diversity is predicted to be undetected (Figure 4a). Extrapolating the sampling curves to the asymptote, the total predicted *Phytophthora* species richness in the fine-scale sampled nurseries ranged from 22.2 to 74.1 with a median of 48.8. Comparing rarefaction curves for broad- and fine-scale sampling protocols, total *Phytophthora* species richness across the fine- and broad-scale sampled nurseries was predicted to be 119 and 49, respectively, indicating that fine-scale sampling protocols are predicted to detect 2.4 times more *Phytophthora* diversity within nurseries (Figure 4b). There was considerable variability among the 17 fine-scale sampled nurseries in terms of recorded detections of the 20 most abundant *Phytophthora* species encountered in the study as a whole (Figure 5). Nurseries N002, N003, N004 and N008 had notably fewer recorded detections of these *Phytophthora* species whereas nurseries N001, N009 and N017 had a particularly high number of detections (Figure 5).

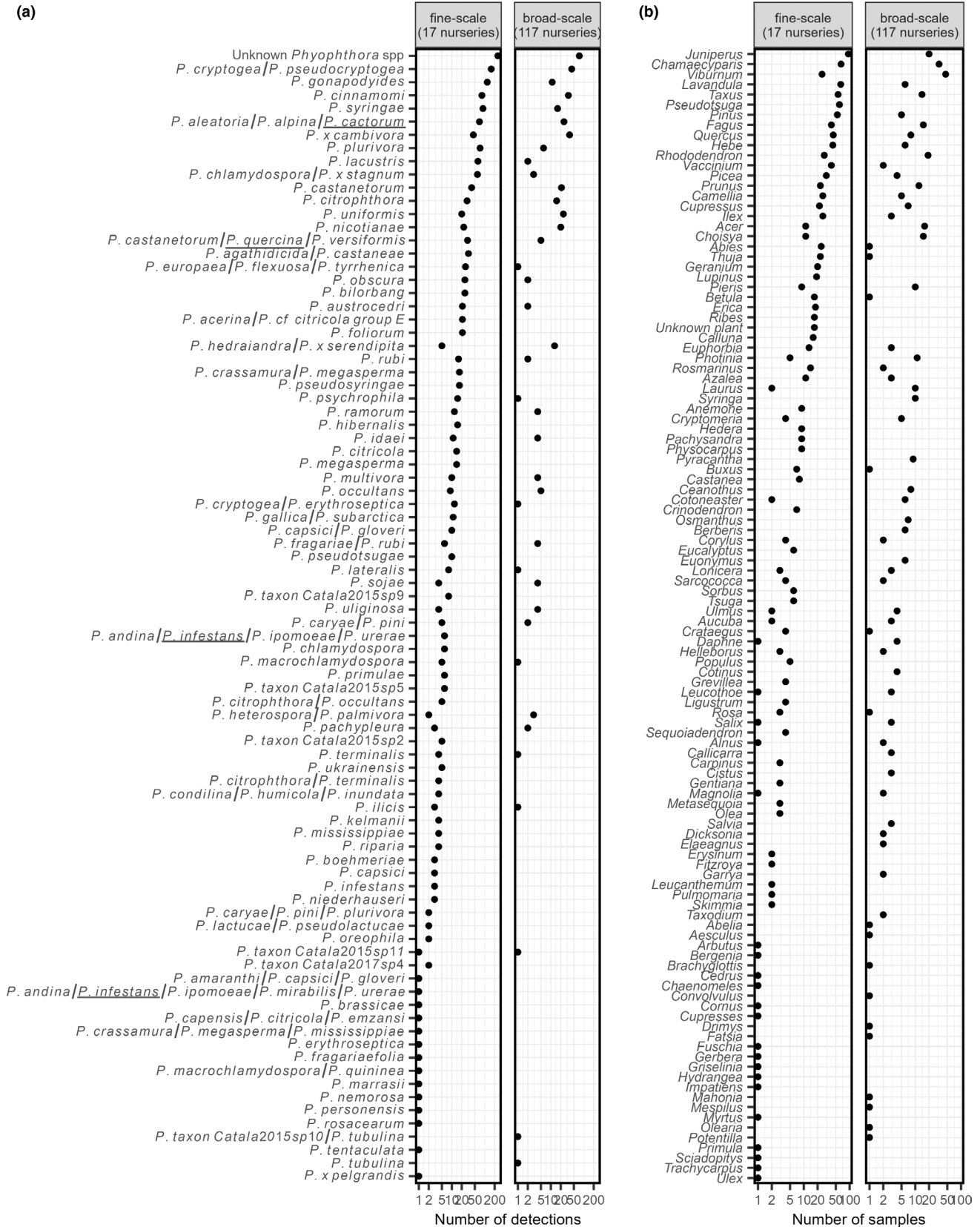
### 3.2 | Prevalent host-*Phytophthora* associations in nurseries

There were 1656 samples associated with a host plant. We calculated the proportions of samples positive for each *Phytophthora* species on 35 host genera that were sampled in at least two nurseries and with at least 10 samples in total across all 134 nurseries (Figure 6). There were 1290 samples and 57 *Phytophthora* species associated with these well-sampled hosts. Table 2 presents the 20 most frequently detected host-*Phytophthora* associations across the well-sampled hosts.

*Phytophthora austrocedri*, a regulated pathogen listed on the UK Plant Health Risk Register and linked to recent declines of native juniper populations in Britain, and three *Phytophthora* species on the EPPO A2 list ([https://www.eppo.int/ACTIVITIES/plant\\_quarantine/A2\\_list](https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list)) (*P. fragariae*/*P. rubi*, *P. lateralis* and *P. ramorum*) were detected in this study. Table 3 identifies the five host genera most often associated with these regulated pathogens in this study and the predicted prevalence of these interactions within the 134 nurseries sampled.

We modelled the number of samples positive for *Phytophthora*, adjusting for the number of samples from each unique host genus-nursery combination, and the non-independence of detections within the same *Phytophthora* species and host genus. The total variance in detection probability across hosts and nurseries was partitioned into nursery-level, *Phytophthora* species-level and host genus-level, which explained 10.5% (4.8, 22.2), 10.3% (4.8, 21.5) and 1.3% (0.5, 3.7), respectively, of variance in the probability of detection. On average, across all nurseries and well-sampled host genera, the *P. cryptogea*/*P. pseudocryptogea* complex was estimated to be most prevalent, with detection probability of 0.04 (0.00, 0.35).

Adjusting for unbalanced sampling effort across hosts and nurseries, the host genera predicted to have the greatest associated



**FIGURE 3** Summary of (a) *Phytophthora* species detections and (b) host plants sampled across the 17 nurseries with fine-scale sampling and 117 nurseries with broad-scale sampling. Species and hosts are ordered from most frequently sampled (top) to least sampled (bottom). Underlined species names indicate the most likely identity of a detection that cannot be resolved to species-level, using the known geographic distribution or host associations of the species within that cluster.

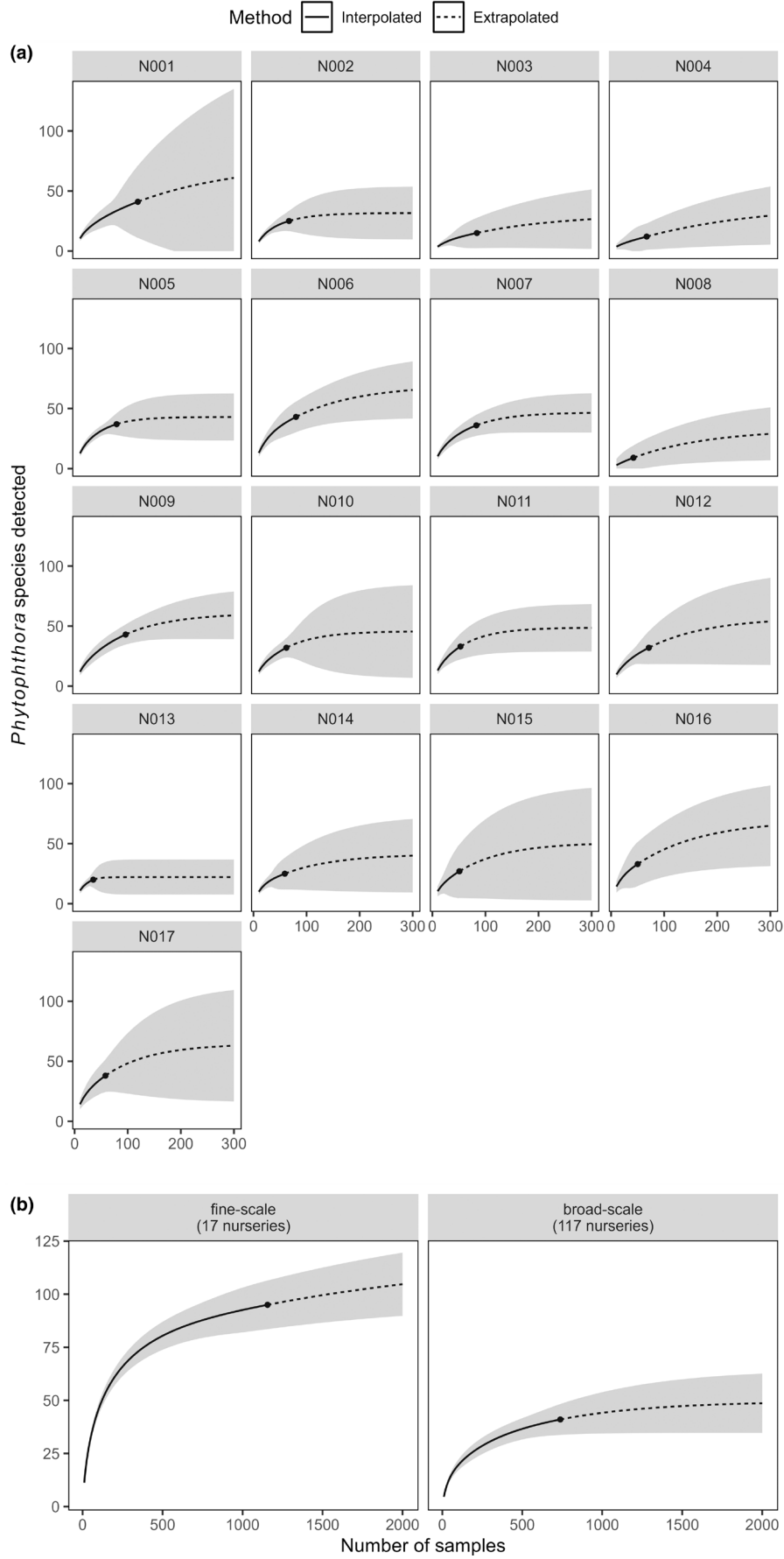


FIGURE 4 Legend on next page

**FIGURE 4** Rarefaction to assess completeness of sampling using Hill numbers (Hysie et al., 2016). (a) Accumulation of the number of detected *Phytophthora* species with number of samples (solid lines) and extrapolation to predicted asymptotic species richness (dashed lines) in each nursery and (b) compared between fine- and broad-scale nursery samples. Points represent the total observed number of *Phytophthora* species in each nursery. Shaded areas around lines are the 95% confidence intervals.

*Phytophthora* species diversity per 100 samples were *Pseudotsuga* (0.58 [0.03, 16.79] *Phytophthora* species), *Chamaecyparis* (0.47 [0.02, 13.30]), *Erica* (0.40 [0.02, 13.30]), *Fagus* (0.40 [0.02, 10.98]) and *Lavandula* (0.37 [0.02, 10.44]). The *Phytophthora* species predicted to be associated with the greatest number of host genera per 100 samples were *P. cryptogea*/*P. pseudocryptogea* (3.99 [0.34, 34.4]), *P. cinnamomi* (3.09 [0.28, 29.9]) and *P. aleatoria*/*P. alpina*/*P. cactorum* (2.00 [0.17, 19.4]). This latter ITS1 species complex is assumed in this study to be *P. cactorum* because neither *P. aleatoria* (Scott et al., 2019) nor *P. alpina* (Bregant et al., 2020) have yet been recorded as present in the UK. Other ubiquitous species on our sampled host genera included *P. × cambivora* (1.68 [0.12, 19.1]), *P. gonapodyides* (1.61 [0.12, 15.4]), *P. syringae* (1.52 [0.10, 13.7]), *P. castanetorum* (1.49 [0.100, 14.00]) and *P. plurivora* (1.41 [0.09, 14.00]).

### 3.3 | *Phytophthora* communities in water and plant-associated samples

There was no significant effect of sample substrate on the overall probability of detection, on average, across all *Phytophthora* species (Figure 7). However, the effect of sample substrate varied in direction and magnitude among different *Phytophthora* species. Probability of detection in water samples was significantly greater than in host plant-associated material (mostly root or water flow-through samples) for 22 *Phytophthora* species including *P. taxon Catalá 2015 sp. 9* (Català et al., 2015), *P. bilorbang*, *P. lacustris*, *P. mississippiiae*, *P. riparia*, *P. pseudosyringae*, *P. gonapodyides*, *P. chlamydozpora*, *P. megasperma* and *P. syringae* (Figure 7). The strongest association with water samples was predicted for *P. taxon Catalá 2015 sp. 9*, for which the predicted probability that a detection of the species was from a water sample was 0.97 (0.88, 0.99). Within samples from host plants, the probability of detection was significantly higher than in water for *P. castanetorum*, the ITS1 species complexes *P. castanetorum*/*P. quercina*/*P. versiformis* (assumed to be *P. quercina* due to a strong association with *Quercus*) and *P. europaea*/*P. flexuosa*/*P. tyrrhenica* as well as *P. nicotianae*, *P. foliorum*, the complex *P. aleatoria*/*P. alpina*/*P. cactorum*, *P. cinnamomi* and the *P. cryptogea*/*P. pseudocryptogea* complex. The strongest association with plant samples was for *P. castanetorum*, for which the predicted probability that a detection of the species was from a plant sample was 0.05 (0.003, 0.22) (Figure 7). There was a large number of species for which the predicted probability of detection in water and host samples were approximately equal, including *P. pseudotsugae*, *P. lateralis*, *P. ramorum*, *P. pachypleura*, *P. × cambivora*, *P. occultans*, *P. plurivora*, *P. citrophthora*, *P. multivora* and *P. austrocedri* (no significant association: Figure 7).

## 4 | DISCUSSION

We report the first comprehensive analysis of *Phytophthora* communities in British plant nurseries using a novel sampling for metabarcoding approach and present our results in relation to variation across nurseries, host plant genera, sample type and advice for practitioners.

*Phytophthora* diversity within plant nurseries has been previously linked to management practices, with implications for plant health and associated biosecurity risks (Parke & Grünwald, 2012). The metabarcoding method applied here combined with multiyear sampling of water and roots from a large number of nurseries enabled detection of ITS1 barcodes corresponding to a very broad range of *Phytophthora* taxa compared with those previously reported for nursery environments in other countries by direct isolation from symptomatic tissues or baiting (e.g., Moralejo et al., 2009; Parke et al., 2014; Rooney-Latham et al., 2019; Sims et al., 2019). Although it is recognized that detection of an ITS1 barcode consistent with a species is distinct from detection of that species, for simplicity in this discussion we refer directly to species detection rather than adding the proviso in each case. For six of the 17 fine-scale nurseries our sampling methodology came close to capturing their complete *Phytophthora* communities, whereas 11 fine-scale nurseries are predicted to harbour a larger number of undetected species. The fine-scale nurseries also varied greatly in terms of recorded detections of the 20 most abundant *Phytophthora* species encountered in the study as a whole. The fine-scale nurseries encompassed a broad range of business types, genera of host plants grown, growing practices and geographical locations within Britain. The extent to which *Phytophthora* communities are influenced by nursery type and management practice and the identification of highest risk practices and business types that might be targeted in certification schemes aimed at reducing biosecurity risk, will be the subject of a subsequent publication. Here, we discuss the influence of host genera and sample substrate on *Phytophthora* detections and how our results might be used to inform and reduce risk more generally.

High-risk hosts are those most likely to harbour invasive, highly aggressive or multiple broad host-range *Phytophthora* pathogens. Because our nursery sampling strategy was not random but aimed at maximizing the likelihood of *Phytophthora* detections, the most sampled hosts reflected those species previously regarded as having strong associations with a particular *Phytophthora* species. These included invasive pathogen species (e.g., juniper and *P. austrocedri*; *Chamaecyparis* and *P. lateralis*; *Taxus* and *P. cinnamomi*; *Rhododendron*, *Viburnum* and *P. ramorum*). Our analyses also identified other previously reported *Phytophthora* associations with ornamental hosts, such as *Chamaecyparis* and both *P. austrocedri* and *P. castanetorum*, *Choisya* and *P. nicotianae*, *Camellia* and *P. cinnamomi* as well as a

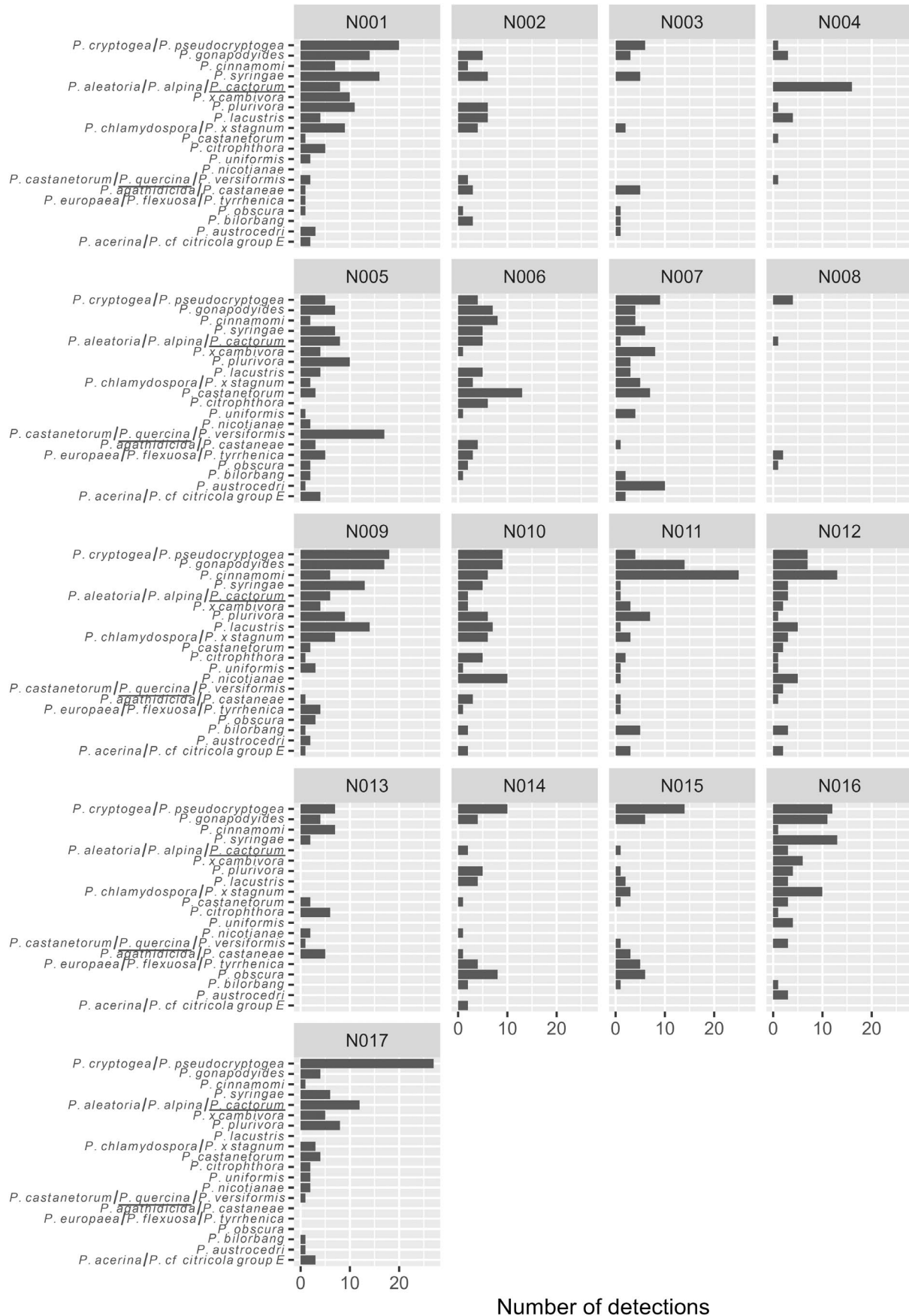


FIGURE 5 Number of positive samples per nursery for the 20 most frequently detected *Phytophthora* species across the 17 fine-scale sampled nurseries. The species underlined is the one most likely to be present in that species complex.



**FIGURE 6** Relative frequency of associations between well-sampled host plant genera and *Phytophthora* pathogens. The species underlined is the one most likely to be present in that species complex. Deeper red indicates higher proportions of positive samples. Grey indicates that the host–*Phytophthora* association was not detected. *Phytophthora* species are ranked from top to bottom based on the overall proportion of detections across all host genera. Host genera are ranked from left to right based on the overall proportions of positive *Phytophthora* samples associated with them.

particularly consistent association between *Quercus* and *P. quercina*, a species first described from necrotic roots of declining *Quercus* spp. in central and southern Europe (Jung et al., 1999). The distribution of this pathogen on *Quercus* spp. in the wider environment in Britain is not well documented but our study has demonstrated its ubiquity on this host in the nursery trade (Green et al., 2021). This is of particular concern for restoration plantings at existing *Quercus* woodland sites where local seed is raised by commercial plant nurseries for planting back on site. We also found that certain hosts are more likely than others to harbour multiple *Phytophthora* species in a nursery environment, and that some of the most frequently detected species (*P. cryptogea*/*P. pseudocryptogea*, *P. cinnamomi*, *P. syringae*, *P. cactorum*, *P. plurivora* and *P. cambivora*/*P. × cambivora*) are damaging, cosmopolitan pathogens able to cause disease on a broad range of host species including woody hosts.

*P. cryptogea*/*P. pseudocryptogea* was estimated in this study to have the greatest probably of detection in the sampled plant nurseries. *P. cryptogea* was first described in 1919 (Pethybridge & Lafferty, 1919) and is reported (as either *P. cryptogea* or *P. pseudocryptogea*) as a causal agent of root disease in over 150 plant genera in 55 countries worldwide. More recently, *P. cryptogea* has been redefined as a species complex, involving the new description of *P.*

*pseudocryptogea* (Safaiefarhani et al., 2015). In many samples processed in this study, these two species could not be separated by their ITS1 sequences and so are grouped together as a species complex. *P. cinnamomi*, another species prevalent across British nurseries and on high-risk hosts, is of particular concern as this is an invasive and highly destructive pathogen impacting forest ecosystems on several continents (Dobrowolski et al., 2003). It is reported to be most damaging in Mediterranean-like climates with its range checked by cold winters. Its distribution in the UK wider environment is thus predicted to expand in a warming climate (Brasier & Scott, 1994; Burgess et al., 2017) and this could be exacerbated by northerly movement of plants grown in the south of the country. *P. plurivora*, found in this study to be associated with 11 plant genera, is an example of a pathogen that has undergone recent global expansion due to its international spread in traded diseased plant material, enabling the pathogen to colonize new environments and hosts (Schoebel et al., 2014). This species was formerly considered as part of the *P. citricola* species complex and is thought to be involved in widespread declines of beech (*Fagus sylvatica*) and oak species (*Quercus* spp.) in continental Europe (Jung, 2009; Jung et al., 2000).

Many of the *Phytophthora* species found to be most prevalent in our surveys of British nurseries were also commonly detected in

TABLE 2 Top 20 most frequently detected host-*Phytophthora* associations in a survey of British plant nurseries.

<i>Phytophthora</i> species	Host genus	Proportion of positive samples	Detections	Samples per genus	Nurseries where host was sampled	Predicted prevalence
<i>P. nicotianae</i>	<i>Choisya</i>	0.53	10	19	13	0.01 (0.00, 0.07)
<i>P. castanetorum</i> / <u><i>P. quercina</i></u> / <i>P. versiformis</i>	<i>Quercus</i>	0.42	22	52	19	0.01 (0.00, 0.08)
<i>P. cinnamomi</i>	<i>Erica</i>	0.40	4	10	7	0.05 (0.00, 0.30)
<i>P. cinnamomi</i>	<i>Laurus</i>	0.40	6	15	13	0.02 (0.00, 0.18)
<i>P. castanetorum</i>	<i>Chamaecyparis</i>	0.38	24	63	28	0.03 (0.00, 0.21)
<i>P. cryptogea</i> / <i>P. pseudocryptogea</i>	<i>Lavandula</i>	0.38	14	37	15	0.06 (0.00, 0.34)
<i>P. cinnamomi</i>	<i>Camellia</i>	0.31	17	55	28	0.02 (0.00, 0.16)
<i>P. × cambivora</i>	<i>Prunus</i>	0.29	10	35	26	0.02 (0.00, 0.19)
<i>P. aleatoria</i> / <i>P. alpina</i> / <u><i>P. cactorum</i></u>	<i>Lavandula</i>	0.27	10	37	15	0.03 (0.00, 0.20)
<i>P. aleatoria</i> / <i>P. alpina</i> / <u><i>P. cactorum</i></u>	<i>Betula</i>	0.27	4	15	7	0.02 (0.00, 0.18)
<i>P. aleatoria</i> / <i>P. alpina</i> / <u><i>P. cactorum</i></u>	<i>Abies</i>	0.25	4	16	8	0.03 (0.00, 0.20)
<i>P. × cambivora</i>	<i>Photinia</i>	0.25	5	20	18	0.02 (0.00, 0.18)
<i>P. plurivora</i>	<i>Pseudotsuga</i>	0.24	6	25	6	0.03 (0.00, 0.24)
<i>P. gonapodyoides</i>	<i>Vaccinium</i>	0.23	6	26	11	0.02 (0.00, 0.18)
<i>P. plurivora</i>	<i>Vaccinium</i>	0.23	6	26	11	0.02 (0.00, 0.17)
<i>P. uniformis</i>	<i>Prunus</i>	0.23	8	35	26	0.01 (0.00, 0.10)
<i>P. cryptogea</i> / <i>P. pseudocryptogea</i>	<i>Hebe</i>	0.22	8	36	18	0.05 (0.00, 0.33)
<i>P. cinnamomi</i>	<i>Azalea</i>	0.21	5	24	12	0.02 (0.00, 0.18)
<i>P. aleatoria</i> / <i>P. alpina</i> / <i>P. cactorum</i>	<i>Photinia</i>	0.20	4	20	18	0.03 (0.00, 0.18)
<i>P. cryptogea</i> / <i>P. pseudocryptogea</i>	<i>Erica</i>	0.20	2	10	7	0.06 (0.00, 0.38)

Note: The *Phytophthora*-host associations are ranked by the proportion of detections of a *Phytophthora* species given the number of times a host genus was sampled. Underlined species names indicate the most likely identity of a detection that cannot be resolved to species-level, using the known geographic distribution or host associations of the species within that cluster. We also present the predicted prevalence (median, and upper and lower 95% credible interval) of these associations (probability of detection of a particular *Phytophthora* species on a host genus, on average, across all 134 nurseries sampled), adjusting for uncertainty arising from differences in sampling effort across hosts and nurseries and other unmeasured covariates at the host, nursery and pathogen level.

TABLE 3 Host genera most frequently associated with *Phytophthora austrocedri* (a regulated non-quarantine pest in the UK) and three *Phytophthora* species on the EPPO A2 list ([https://www.eppo.int/ACTIVITIES/plant\\_quarantine/A2\\_list](https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list)).

<i>Phytophthora</i> species	Host genus	Detections	Host genus samples	Proportion of positive samples	Nurseries where host sampled	Predicted prevalence
<i>P. austrocedri</i>	<i>Chamaecyparis</i>	6	63	0.10	28	0.009 (0.001, 0.096)
	<i>Juniperus</i>	8	133	0.06	33	0.004 (0.000, 0.047)
	<i>Cupressus</i>	2	37	0.05	20	0.004 (0.000, 0.044)
	<i>Prunus</i>	1	35	0.03	26	0.007 (0.001, 0.079)
	<i>Fagus</i>	1	36	0.03	25	0.008 (0.001, 0.085)
<i>P. lateralis</i>	<i>Chamaecyparis</i>	6	63	0.10	28	0.004 (0.000, 0.049)
	<i>Thuja</i>	1	24	0.04	17	0.002 (0.000, 0.028)
	<i>Rhododendron</i>	1	80	0.01	45	0.002 (0.000, 0.026)
<i>P. ramorum</i>	<i>Vaccinium</i>	2	26	0.08	11	0.005 (0.000, 0.061)
	<i>Rhododendron</i>	4	80	0.05	45	0.004 (0.000, 0.047)
	<i>Camellia</i>	2	55	0.04	28	0.002 (0.000, 0.035)
	<i>Viburnum</i>	4	115	0.03	68	0.003 (0.000, 0.040)
	<i>Prunus</i>	1	35	0.03	26	0.005 (0.001, 0.063)
<i>P. fragariae/P. rubi</i>	<i>Cotoneaster</i>	1	13	0.08	11	0.002 (0.000, 0.024)
<i>P. rubi</i>	<i>Lavandula</i>	2	37	0.05	15	0.004 (0.000, 0.054)
<i>P. fragariae/P. rubi</i>	<i>Photinia</i>	1	20	0.05	18	0.002 (0.000, 0.028)
<i>P. rubi</i>	<i>Vaccinium</i>	1	26	0.04	11	0.004 (0.000, 0.053)
<i>P. fragariae/P. rubi</i>	<i>Acer</i>	1	28	0.04	19	0.003 (0.000, 0.032)

Note: *P. kernoviae* is on the A2 list but there were no detections across the nurseries sampled. Up to five host genera are ranked by the proportion of positive samples on each host genus. We also present the predicted prevalence (median, and upper and lower 95% credible interval) of these associations.

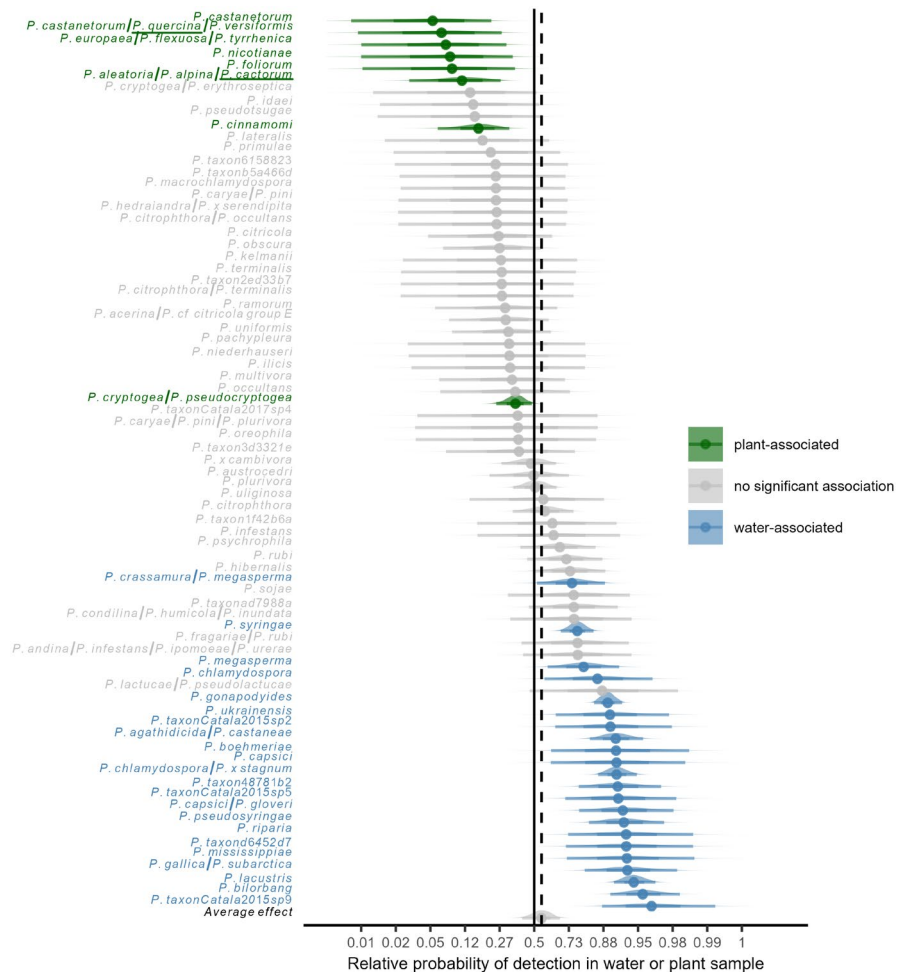
plant nurseries surveyed in the United States (Parke et al., 2014), Italy (Prigigallo et al., 2015) and more widely across Europe (Bačova et al., 2024; Jung et al., 2016), reflecting a general ubiquity in the plant trade. Although all these species cause visible disease symptoms on a range of hosts, their detection by visual inspection may be complicated by a potential to persist asymptotically in plant tissues, including the tissues of non-hosts. For example, Crone, McComb, and O'Brien (2013) and Crone, McComb, O'Brien, and Hardy (2013) demonstrated the persistence of *P. cinnamomi* resting structures in the roots of asymptomatic non-hosts and it is likely that other species can do the same. Thus, plant health surveillance that relies on visual symptoms alone will inherently have a high rate of false negative detections. This could be addressed at the national level through the incorporation of metabarcoding technology into the downstream processing of samples collected during statutory nursery and plant import surveillance programmes as this method will allow identification of cryptic infections.

High-risk hosts likely to harbour multiple broad host range *Phytophthora* pathogens include a range of important ornamental and forestry species, notably *Pseudotsuga* (Douglas fir), a commercial forestry species considered to be of increasing future importance to the UK timber industry (Ennos et al., 2019). Of particular concern is the detection of DNA of *P. pseudotsugae*, predominantly on this host but also on other conifer species, at three of the sampled nurseries. *P. pseudotsugae* was first described as a pathogen causing root disease of nursery-grown Douglas fir seedlings in the

United States (Hamm & Hansen, 1983) and has not been previously reported in the UK. Further surveillance is required for this pathogen in UK forest tree nurseries. It is unclear why Douglas fir supports such a high number of *Phytophthora* species in the nursery setting. Douglas fir is native to North America where it covers a very wide geographical range from sea level to 1500m. At least two of the nurseries surveyed in this study propagated Douglas fir from seed collected from seed orchards in France and one of these nurseries has reported persistent poor health in young stock planted out in the UK, requiring further investigation into the cause, and indeed whether seed transmission of *Phytophthora* can occur. Certainly, more research effort needs to be focused on understanding and reducing the prevalence of *Phytophthora* species on these high-risk hosts, which also include *Lavandula*, *Erica*, *Vaccinium*, *Abies* and *Fagus*, particularly those hosts intended for extensive planting in the wider environment. On the basis of our study's findings, a two-sided flier (Forest Research, 2023a) was produced for nursery managers to raise awareness of those hosts that pose elevated risk from *Phytophthora* infections. This will inform decisions about continuing to trade in such hosts (Green et al., 2021) or applying higher levels of biosecurity practice, for example ensuring that suppliers have documented plant health procedures in place, inspecting high-risk stock carefully upon arrival, quarantining new arrivals and maintaining stock away from other species on a clean, well-drained surface, preferably elevated off the ground. The flier and its recommendations for management of particularly risky hosts are also available on the UK's Plant



**FIGURE 7** Posterior distributions of estimates for the effect of sample substrate (water or plant) on detection probabilities of *Phytophthora* species across the 17 fine-scale nurseries. The species underlined is the one most likely to be present in that species complex. Species are ranked from top to bottom by their probability of detection in water samples relative to plant samples. Probabilities significantly greater than 0.5 (blue) indicate species that are more likely to be detected in water samples, while probabilities less than 0.5 (green) indicate species that are more likely to be detected in samples from plants (including root plus foliage or stem samples). Species with no significant association with either water or plants (grey) are those for which the probability of detection in water and plant samples are not significantly different.



Healthy Certification Scheme website (<https://planthealthy.org.uk/certification>). This Certification Scheme was initiated in 2020 and aims to improve plant health and biosecurity practice across the national nursery sector.

Other prevalent *Phytophthora* species detected across nurseries include common clade 6 species (e.g., *P. gonapodyides*, *P. chlamydospora*, *P. lacustris*), which flourish in aquatic habitats and may be regarded as weak pathogens (Jung et al., 2011). However, it should be noted that *P. gonapodyides*, which is thought to play a role in breakdown of plant debris (Brasier et al., 2003), has been isolated from lesions on diseased trees of various species in Britain (Forest Research Tree Health Diagnostic and Advisory Service database records) (<https://www.forestresearch.gov.uk/services/tree-health-diagnostic-and-advisory-service/>) although its role as a pathogen in these cases is unclear (Riddell et al., 2019).

Among the *Phytophthora* species detected in this study were several new records for the UK. Some of these species are new descriptions, for example a barcode matching *P. oreophila*, a clade 6a species originally described from an alpine herb field in Tasmania (Khaliq et al., 2019), was detected on roots of noble fir (*Abies procera*) and Sitka spruce (*Picea sitchensis*) at two geographically distant forest tree nurseries in this study. In the Sitka spruce sample, a barcode 2 bp different from *P. oreophila* that matched the closely related *P. rosacearum* was also detected. Although reported mainly on rosaceous tree hosts

in North America, there has been a recent report of *P. rosacearum* associated with declining alder in Europe (Bregant et al., 2023). Also *P. ukrainensis* (Jung et al., 2022), a clade 10 species isolated from forest streams in Ukraine and Sweden, was detected in six samples in our study, mainly in irrigation and ditch water at two forest nurseries. The clade 1 species *P. tentaculata*, detected in this study in roots of a discarded *Chamaecyparis* plant at an ornamental nursery, was originally described in 1993 from ornamental plants in Germany (Kröber & Marwitz, 1993). At one nursery sampled here, roots of dwarf mountain pine (*Pinus mugo*) yielded DNA matching clade 9 species *P. macrochlamydospora*/*P. quininea*, which are very closely related and cannot be separated by their ITS1 sequences. *P. macrochlamydospora* was first isolated from soybean (*Glycine max*) in Australia in 1974 (Irwin, 1991) and *P. quininea* was first described from cinchona (*Cinchona officinalis*) in Peru in 1947 (Crandall, 1947). However, of most intrigue was the finding of often abundant ASV reads matching *P. agathidicida*/*P. castanae* in 48 samples collected from 12 of the fine-scale nurseries in this study over multiple years. Both of these very closely related species are aggressive, tropical clade 5 tree-infecting pathogens, with *P. agathidicida* responsible for widespread dieback and killing of native kauri (*Agathis australis*) in northern New Zealand (Weir et al., 2015). Our metabarcoding method also picked up unknown *Phytophthora* sequences in a large number of samples that could not be identified to species level using the THAPBI-PICT

classifier. Subsequent studies incorporating both traditional baiting methods and metabarcoding at nurseries from which samples have yielded abundant sequences from novel *Phytophthora* species have failed, to date, to obtain live cultures of these species (Green et al., 2023; Schiffer-Forsyth et al., 2023). It is possible that in some cases the DNA detected by metabarcoding did not originate from live propagules of the source organism. Schiffer-Forsyth et al. (2023) detected twice as many *Phytophthora* taxa by metabarcoding (16) compared with baiting (8) in a study of *Phytophthora* diversity in green waste piles at three nurseries in Scotland. The authors concluded that both methods were complementary and should be used in combination, with the metabarcoding method able to enhance early detection and the use of baiting or direct isolation to confirm species identity and role in disease.

In addition to the amplification of all species of *Phytophthora*, the metabarcoding assay based on the primers of Scibetta et al. (2012) is known to amplify related plant-pathogenic downy mildews and species of the genus *Nothophytophthora*. In this study a range of downy mildew species from seven genera were detected but as anticipated from the project objectives, samples of roots and water were dominated by *Phytophthora*. The downy mildew diversity will be the subject of later publications. Barcodes of *Nothophytophthora* species were almost exclusively detected in water samples from streams adjacent to nurseries rather than being associated with plants in the nurseries.

The metabarcoding assay proved an effective tool for monitoring *Phytophthora* diversity and distribution for this application and can be assessed in relation to a systematic validation scale proposed by Thalinger et al. (2021). The assay has moved beyond basic levels of in silico analysis (Level 1), testing against non-target species (Level 2) and has demonstrated robust methods for extraction and detection in eDNA samples (Level 3). Given its extensive field testing in numerous studies at an international scale, we consider it to fit between 4 and 5 on Thalinger's 1–5 eDNA assay readiness scale. Although Thalinger et al. (2021) describe this scale for single species assays, we consider it also appropriate and relevant to specific taxonomic groups at higher taxonomic levels than species.

*Phytophthora* species significantly associated with water samples included, as expected, several aquatic clade 6 species (*P. bilorbang*, *P. lacustris*, *P. riparia*, *P. mississippiiae*, *P. chlamydospora*, *P. gonapodyides* and *P. megasperma*) as well as the clade 10 species *P. gallica*/*P. subarctica* and *P. ukrainensis* that probably have the water-associated lifestyles as saprotrophs and opportunistic pathogens of riparian plants typical of species in this clade (Corcobado et al., 2023). *P. gallica* is assumed to have a limited distribution in the UK and has previously been detected in a water sample in Scotland (data not shown). However, these species known for their water-associated niches also co-occur with some species regarded as terrestrial pathogens such as *P. pseudosyringae*, *P. capsici*, *P. boehmeriae* and *P. syringae*. *P. pseudosyringae* is of particular interest as this aerially disseminated species was first reported in Britain in 2009 causing disease on *Nothofagus* spp. (Scanu et al., 2012) and was assumed to be invasive. However, since then the pathogen has been detected on an increasing number

of hosts, including woody hosts such as *Fagus* and *Larix* spp. on which it causes cankers, and in different environments and substrates including soils and stream water (Riddell et al., 2019, 2020). Thus, it is now speculated that this species may have been present in the country for longer than previously thought. *P. syringae*, a ubiquitous pathogen of many hosts in the UK, is non-caducous (so not aerially dispersing) and its prevalence in water samples is surprising. The presence of these pathogens with higher disease impacts, particularly on woody hosts across the UK, in water samples has implications in terms of elevated risks for nurseries using open or untreated irrigation water sources. Also worth noting is the strong water-association of *P. agathidicida*/*P. castanae* and *P. taxon Catalá2015sp9*, *P. taxon Catalá2015sp5* and *P. taxon Catalá2015sp2*, the latter three being unidentified DNA sequences originally reported from wider environment samples in northern Spain (Català et al., 2015). These species await formal description, and their implications for plant health have yet to be confirmed.

Our analyses revealed *Phytophthora* species that were more strongly associated with plant samples (predominantly roots) than with water samples. For example, the ITS1 complex identified as *P. castanetorum*/*P. quercina*/*P. versiformis* was detected on 65 root or root wash samples from 13 nurseries. Considering that 45 of the 65 samples were of the genus *Quercus*, we consider this to be *P. quercina*. Genetically similar barcodes identified as *P. castanetorum* alone (i.e., not as part of complex above) were also exclusively detected in 66 samples of plant roots or root wash from 18 nurseries. These samples showed no overlap to those in the complex described above and remarkably were never detected on *Quercus* but rather on conifers such as *Chamaecyparis* (30), *Juniperus* (8), *Cupressus* sp. (4), *Taxus* (4) and *Thuja* (3). The species *P. castanetorum* was recently described from the rhizosphere of sweet chestnut (*Castanea sativa*) forests in Portugal and Italy and regarded as a weak pathogen (Jung et al., 2017) and this case thus warranted closer analysis. A single discrete single-nucleotide polymorphism (SNP) and a shortening of a run of nucleotide A from 7 to 6 accounted for the discrimination of this ASV from the *P. castanetorum*/*P. quercina*/*P.versiformis* complex. One reference database sequence of *P. castanetorum* also had six A's that brought this novel ASV within the permitted 1bp threshold and made it a match to *P. castanetorum*. However, no other sequences of the complex of three species currently in the databases have the C/T SNP and it is therefore possible that it is a new species or variant of *P. castanetorum*. Other plant-associated species were *P. europaea*, another weak pathogen described from the rhizosphere of oak forests in Europe (Jung et al., 2002), *P. nicotianae* and *P. cactorum*, both cosmopolitan pathogens with a very broad host range, with *P. cactorum* also able to cause infections on many woody hosts, and *P. cryptogea*/*P. pseudocryptogea* and *P. cinnamomi*. Interestingly, *P. foliorum* was also strongly plant-associated and found on a range of hosts across eight nurseries. *P. foliorum* is very closely related to *P. ramorum* and has only been recently recorded in the UK (Riddell et al., 2020). Other species are estimated to be equally likely to be detected in water samples as in host samples. These include regulated pathogens such as *P. ramorum*, *P. austrocedri* and *P. rubi*, as well as *P. occultans*,

an invasive pathogen of ornamental plants in Europe, particularly *Buxus* (Man in't Veld et al., 2015) and *P. pseudotsugae*, the Douglas fir pathogen previously unreported in the UK. These species could be regarded as higher risk in the plant trade due to their ability to spread readily in plants as well as in water.

It should be acknowledged that 15% of laboratory and field blank control water samples contained traces of a limited number of oomycete taxa including *Phytophthora*. The source of the DNA in the laboratory blank samples may have been inoculum carried over in the water pumping equipment from the previous nursery sampling visit, or, less likely, contamination from the laboratory. The presence of oomycete taxa in the field blank controls used as a check for the water-flow-through testing may be due to contamination of nursery mains or borehole water. In some cases, the source of this contamination could have been soiled hoses and hose-ends stored on the ground that may act as a potential pathway of spread of *Phytophthora*, as discussed with nursery managers. Our study has illustrated the importance of having blank control samples, allowing us to accurately gauge and monitor contamination and review nursery data accordingly. Another factor that can confound findings in this type of study is the association of a *Phytophthora* species on a particular host due to inoculum contamination rather than infection. The prevalence of *P. rubi* and *P. fragariae*, for example, on non-rosaceous hosts was unexpected but could be due to inoculum spread from rosaceous hosts nearby or from legacy inoculum on contaminated matting or soil. In busy nurseries with many hundreds or thousands of batches of plants, stock turnover and movement is a necessity but good hygiene practices such as cleaning or replacing anti-weed plastic membranes underneath plant pots do not always occur.

Overall, our study illustrates the promise of metabarcoding approaches for gaining a fuller picture of the distribution, host interactions and impacts, nursery and wider environment niches and pathways for oomycete pathogen taxa. Collating and interpreting these data across countries within centralized databases and networks, holds considerable promise for predicting emergence and impact of different oomycete species across hosts, countries and trade and wider environment contexts. Although eDNA sampling does not prove live pathogen presence, multiple findings across more than one sampling visit across multiple years suggests that many of these *Phytophthora* species are established and persistent at the nurseries studied.

Throughout this study, results were reported back to nursery managers along with the implications of the *Phytophthora* species found and recommended practices that might help to reduce risk. Because water samples hosted the highest diversity of *Phytophthora* and in many cases were contaminating the water used to irrigate plants, recommendations included use of clean covered (mains/borehole) water sources or treated open water sources, controlling watering to minimize run-off and puddling, improving drainage and raising plants off the ground. The sampling teams also observed that symptoms of disease often went unnoticed by nursery staff, there being an assumption that these were caused by abiotic factors such as frost

or lack of watering. Therefore, a flier focused on recognizing and acting on symptoms of ill health in plants (Forest Research, 2023b) was produced and disseminated through various industry channels, along with the flier on high-risk hosts (Forest Research, 2023a) in addition to a general best practice flier (Forest Research, 2023c). Identifying a need for training, another outcome of this study was two 'Plant Health for Plant Nurseries' events held for nursery sector staff in October 2023, one in England and one in Scotland. These two training days presented the outcomes of the Phyto-threats and ID-PHYT projects along with practical advice and solutions to improving plant health in the sector.

Insights into the role of general attitudes to plant health in determining the extent to which nurseries improved during the sampling period could be gained from linking the sampling data and changes in practice to the interviews carried out with those nurseries during the Phyto-threats project (Green et al., 2021). These interviews addressed a range of questions on what influences decision-making, where nurseries are least and most able to change, and their perspectives on accreditation (Marzano et al., 2021). At least one observed major change in the phytosanitary practice within a nursery was directly attributable to project feedback: a borehole was installed following feedback on positive *Phytophthora* samples within the open water sources previously used for irrigation. Other changes that occurred as a result of project feedback to nurseries included raising plants off the ground, improving drainage, making decisions not to trade in high-risk hosts and employing plant health specialists on the nursery (Green et al., 2021). Nursery N003 had a particularly notable decline in the number of positive samples over the three-year sampling period. This nursery was a non-commercial specialist grower greatly concerned about introducing diseases into public gardens. Anecdotally, this nursery was the most proactive in terms of introducing the above-mentioned biosecurity measures, including disinfestation stations. Six of the 15 partner nurseries that participated in the Phyto-threats project are now members of the Plant Healthy Certification Scheme, thus illustrating the value of cooperation between research and industry enabling the translation of science into better practice.

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#### DATA AVAILABILITY STATEMENT

The raw Illumina MiSeq FASTQ reads of data that support the findings of this study are available from the European Nucleotide Archive/NCBI Short Read Archive at <https://www.ncbi.nlm.nih.gov/sra> under project accession PRJEB76241 or ERP160800.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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