

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

Using metabarcoding to analyse *Phytophthora* community structure in nurseries and associated ecosystems:

In the first year of the project the focus has been on the fine-scale sampling of nurseries by project staff to establish a baseline of sampling methods for the broad-scale survey to be conducted during statutory plant health inspections of nurseries conducted by APHA and SASA. To this end a key task completed in year one was to make contact with a range of UK nurseries requesting access to sample. A shortlist of premises producing trees and hardy ornamental plants was compiled covering a range of scales and production systems and over thirty nurseries were contacted by email with an outline of the project. A project flier was also mailed to nursery managers which directed them to the project website (<https://www.forestry.gov.uk/fr/phytothreats>) for more information. Nurseries were also asked to complete a short survey detailing their main business focus and providing information on growing medium, water source and market for their products. These responses helped identify those to follow up. Overall the responses were positive with only four nursery managers declining to be sampled.

Over the course of the first year 15 of these nurseries were sampled by project staff; 7 in England and Wales and 8 in Scotland. The nurseries include a range of scales and production systems from smaller-scale producers of native trees to large-scale producers and wholesalers of imported trees, shrubs and hardy ornamental plants from UK and European sources. On each nursery visit we met with the manager, explained the background to the project and asked follow-up questions on the key factors influencing risk of *Phytophthora* importation and spread (e.g. plant imports, water sources, composting, biosecurity protocols). Samples from water sources and plant roots (3 replicates) were collected by the team of 3-4 researchers over the course of a full day at each nursery. Sampling of plant roots was focussed on specific plant species known to be hosts of *Phytophthora*, any symptomatic plants and any batches of plants highlighted as problematic by the nursery managers. Water was also filtered on-site using knapsack sprayers and in-line filter holders and included irrigation water, run-off collection ponds, water passed through batches of pots of growing plants and surface water (puddles). For each nursery and environmental sample, metadata (e.g. host diversity, propagation and trading practices, irrigation and disease management, recent planting etc) has been collected for downstream analysis that will inform best practice. In total over 1000 samples have been returned to the laboratory for PCR-testing with *Phytophthora*-specific primers and metabarcoding. Metabarcoding combines technological advances in PCR-detection with high-throughput sequencing technology to analyse complex communities of *Phytophthora* species in unprecedented detail. The findings of their first sampling will be passed to each nursery along with comment on their significance in the context of disease management. Each of the nurseries will be re-sampled in 2017.

The initial focus of the testing has been on the filters and the stabilisation buffer into which filters are placed on-site. Fewer root samples have been tested to date but most remain freeze-dried for PCR-testing at a later date once the findings of the water are known. Results are available for samples from 10 of the 15 nurseries from which we have tested 395 samples. Of the 93 root samples tested just over 70% were positive for *Phytophthora*. A slightly lower rate of just over 60% positive was found for the 302 filter and filter-buffer samples. The slightly lower rate may reflect the irrigation water and blank control samples which would be expected to be negative. There are now sufficient positive samples to complete an Illumina metabarcoding run with appropriate control

samples. The first batches of samples will be run in May 2017 and will reveal the *Phytophthora* species present in these samples.

In parallel to the sampling and PCR testing conducted in the first year of the project, progress has been made on the computational biology pipeline required to analyse the massively parallel data generated via Illumina DNA sequencing. Each run generates 15 million reads of a 250bp barcode of ribosomal DNA that is used to identify the *Phytophthora* species. The DNA barcode data from the nursery samples must be compared to a database of reference sequences of known species found in international sequence databases. A reference ITS sequence database for the 177 known *Phytophthora* species has been generated in collaboration with the work in WP3. This will be updated as new species are described over the course of the project. Given the amount of data to be generated in this project it was critical to streamline an analysis pipeline that completes the following steps; quality control and trim the raw data, assemble the sequence reads, cluster the reads based on similarity and compare to known species. A pipeline has been written in the programming language Python and will be released on the development platform github (www.github.com) in 2017.

A review of the fine-scale sampling trips and discussions with APHA and SASA staff have highlighted the need to focus on plant material only during the statutory plant health inspections of nurseries conducted by APHA and SASA. It was considered that water sampling would be too time-consuming for the plant health inspectors to undertake in addition to their other duties. Sample packs and protocols are being distributed in May 2017 to commence this sampling from a target of 75 nurseries across the UK. This is being supplemented by a community sampling of water from natural ecosystems associated with recent tree or horticultural planting via the OPAL network. Contact with the OPAL water sampling team has been made and water sampling protocols and equipment will be sent to the OPAL teams for sampling ponds and lakes in four regions in June 2017.

WP2: Feasibility analyses and development of 'best practice' criteria

Nursery practices

Preliminary work has been undertaken to learn more about plant trade and nursery issues including interviews with the Expert Advisory Panel members on biggest threats to plant biosecurity, policies and perceived role of nurseries. We have also joined WP1 scientists during their sampling trips at 5 nurseries with detailed notes written up from conversations at the nurseries. Plans are now being made to conduct interviews and participant observation (e.g. spend a day) with a number of nurseries.

Attitudes towards an accreditation scheme

The WP leader joined a meeting of nurseries organised by HTA to discuss piloting a nursery assurance scheme and will now include pilot nurseries in a joint research effort with HTA to understand perspectives, opportunities and challenges associated with development and operation of an accreditation scheme. Questions on attitudes towards accreditation were also included in the consumer survey

Consumer plant purchasing behaviours

A consumer survey was planned to identify plant buying habits (what, where, how many); knowledge and awareness of tree pests and diseases; perceived benefits (or not) of buying accredited plants; willingness to pay extra or travel further for accredited plants. We commissioned an online panel company to run the survey and received 1500 responses. These data are now being analysed.

The advice from the Expert Advisory Panel was that we should consider a number of consumers and not just the 'public.' We are currently identifying ways to access different consumers by adapting the questionnaire and looking for network sites to recruit respondents. For example, we will distribute a survey for landscapers and for nurseries via the HTA in conjunction with attendance at the National Plant Show in June this year.

Ethics

On the advice of funders we convened an ethics committee to review social research methods and progress. To date, the committee has met twice and has contributed to overall methodological development, questionnaire survey design and addressing issues of stakeholder anonymity across the project.

WP3: Global *Phytophthora* risks to the UK

Developing biological trait and global occurrence databases for *Phytophthora* species

This WP aims to identify and rank *Phytophthora* risks to the UK, both of individual species and pathways, by modelling introduction, establishment and spread of different *Phytophthora* species in relation to their biological characteristics or traits but also in relation to environmental and social factors like trade flows and climatic conditions. These analyses required us to collate information on traits and on global occurrence of different species at both national and local (site) scales.

A biological traits database has been completed from literature review and expertise of project pathologists at FR and includes all 177 *Phytophthora* species or sub-species described so far from forestry, agricultural and horticultural settings. This milestone was scheduled for year 2 but has been completed early. Traits included are related to survival and persistence (survival structures, substrates, oospore wall index), reproduction (thermal requirements for growth, heterothallism/homothallism), dispersal (caduceus sporangia), host range (number of host plant families), disease symptoms and taxonomy (clade, genome size, hybrid). Literature alerts for new species have been set up so that the database can be updated during the lifetime of the project. This database is invaluable for analysing how traits of pathogens mediate their spread and impact (this project), but also constitutes a valuable global resource for pathologists, ecologists and risk managers. We are in discussion with a research team in Australia and New Zealand that have developed a parallel traits database on how best to combine these databases into a publicly available global resource.

Data on country- and site- level occurrence of *Phytophthoras* have been collated from a wide range of sources including plant health diagnostic labs, culture collections, literature records, citizen science initiatives, government organisations, global distributional databases like GBIF. The database includes records from the forestry, agricultural and nursery sectors as well as from natural/semi-natural forests, urban spaces, parks, and private and public gardens. The 8034 site-level records collated so far span 78 *Phytophthora* species, 38 countries, and are distributed as indicated in Fig. 1 and vary in the spatial precision of locations and the methods used to identify species. Though initially scheduled to be done by the end of Year 1, this data collation process is being extended into the second year since further substantial datasets are expected from the US, Europe and Africa from our global network of pathologists and will expand the range of environments and species that can be included in and predicted by our models. During year two, we will also explore methods of estimating patterns of recording effort at both the national and sub-national level.

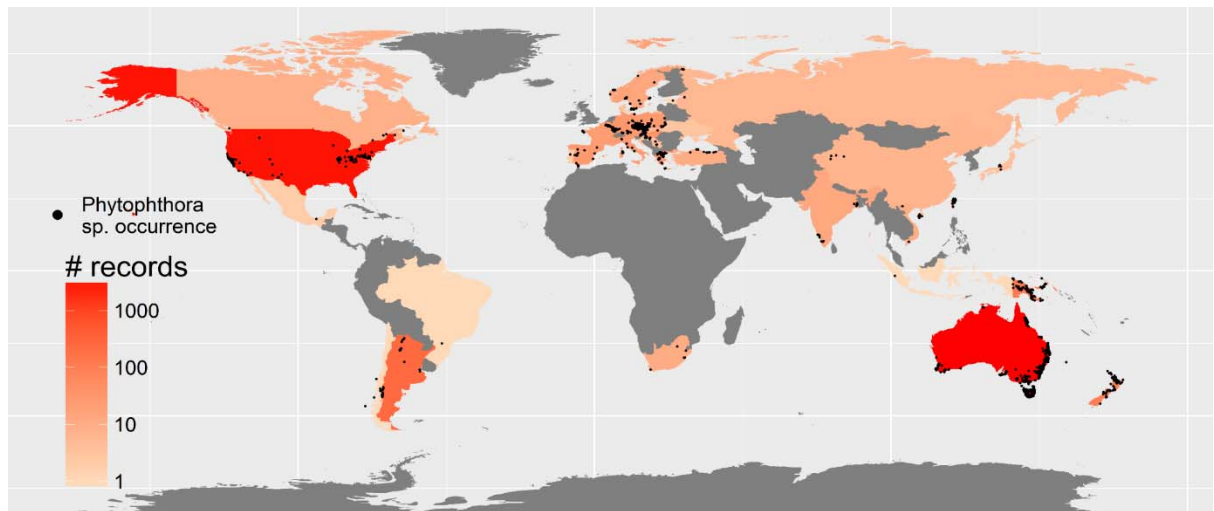


Fig. 1. Distribution of *Phytophthora* records collated to date in WP3 across individual sites (black dots) and countries (shading from white to red, where red indicates countries in which *Phytophthoras* have been recorded more intensively): Note that *Phytophthora* occurrence data for the UK are being compiled separately.

Risk of introduction

Models relating patterns in *Phytophthora* arrival to trade connectivity, pathogen traits and other environmental and social factors will be developed in year 2 of the project using a new method from CEH already applied to pests and pathogens for the EPPO region in a parallel NERC National Capability project (Chapman et al. *Global trade networks determine the distribution of invasive non-native species*, Global Ecology and Biogeography, in press). This year, our data collation activities (above) have produced a rich global dataset of 5838 country-level records for parameterising these models, including year of first record and invasion stage information for 1208 unique species x country combinations. Novel historical trade network data sources (from USDA, Chatham House and the UN FAO) are also being explored spanning agricultural, forestry and horticultural commodities. This analysis is thus well on track for completion by the end of year 2.

Risk of establishment and spread

WP3 aims to develop models of the global environmental niches of 10-15 *Phytophthora* species present in the UK, as proof-of-concept that distribution of suitable habitat for pathogens in the UK is predictable from such models, before rolling the methodology out to 25-40 global species not yet present in the UK. Using the completed traits database, we have defined a set of focal UK and non-UK *Phytophthora* species that fulfil key criteria, for example that they are able to infect woody hosts, particularly forestry species found in the UK, and are not only recorded from agricultural settings or from water or soil with no clear host association. The compilation of the global, fine-scale occurrence data will now focus in on these species. Key environmental data sources for the niche models have also been identified and modelling methodologies will be tested out on well recorded species early in year 2.

Predicting global impact from species traits

By building on the collation of national scale global occurrence data in WP3, we were able to develop broad-scale metrics of global impact of *Phytophthora* species such as the number of countries in which each species is reported worldwide (related to

distribution/spread), the number of countries in which species are regulated (related to disease impacts) and the number of reports of new hosts and geographic regions in the recent past (related to recent spread/emergence). In year two we will explore a further impact metric capturing disease severity at the individual or population level. We are interested in whether the impact of species depends on their ecological traits, either individual traits or combinations of traits (trait syndromes) that reflect a particular survival strategy. Our preliminary analysis of the relationships between traits and global impact metrics indicate for example that species with wider host ranges are more widely distributed and suggests *Phytophthoras* may have more than one survival strategy to achieve high impact globally. These analyses will be finalised and submitted to a peer-reviewed journal in Year 2.

Scoping of knowledge gaps for horizon scanning of emerging pathogens

A preliminary strategy for assessing knowledge gaps in pathways of *Phytophthora* arrival and spread has been developed between FR and CEH and will be developed further through discussion at a Phyto-threats all project-team meeting in May. This will focus on large garden and park premises which represent a key attraction for millions of UK and overseas tourists and have been substantially impacted by and involved in spread of past *Phytophthora* outbreaks. We will aim to link patterns in from where and when tourists visit key large garden and park premises with pathogen diversity and impacts in these premises (controlling for plant import behaviour) and also interview site managers to understand their concerns and needs around biosecurity and spread pathways. To date, we have obtained annual visitor number data for premises owned by Kew, Royal Botanic Gardens Edinburgh, Royal Horticultural Society, Chatsworth House, Alnwick gardens, and Westonbirt Arboretum and we are in the process of identifying key contacts in each organisation (including the National Trust) that hold more detailed data on visitor origins and seasonality.

WP4: Predicting risk via analysis of *Phytophthora* genome evolution

Not yet started (this WP is scheduled to commence in 2017/18). However, all 21 currently available *Phytophthora* genomes have been collated at JHI. The DNA of three more *Phytophthora* species are being prepared for targeted genome sequencing currently, with a view to sequence data being ready for analysis in summer 2017.