Phyto-threats Research Summaries June 2018

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

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

In the second year of the project the team have continued the fine-scale sampling of nurseries, completed the first year of the broad-scale survey with the support of APHA and SASA and worked with the Open Air Laboratory team (OPAL) on community engagement. Sample processing has continued and the first batch of nursery samples has been sequenced and data passed to nursery owners.

Most of the 15 nurseries in the fine-scale survey have now been sampled 4 times over the course of 57 sampling trips. Each nursery was sampled once in 2016, twice in 2017 and once in 2018. Replicated samples from water sources and plant roots were collected by the team of 3-4 researchers over a full day at each nursery. Almost 1500 samples of roots from around 150 different plant species have been collected. The focus has been on plants known to be hosts of *Phytophthora*, symptomatic plants or any plants highlighted as problematic by nursery managers. Water was also filtered on-site using a stirrup pump and in-line filter holders; this included irrigation water, run-off collection ponds, water passed through batches of pots of growing plants and surface water (puddles). The team have collected filters from almost 1000 water samples. 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. Sample processing has three stages; DNA extraction, PCR-testing with *Phytophthora*-specific primers and metabarcoding. The rates of positive *Phytophthora* detection for the 1660 samples tested to date are around 40% for water samples and 60% for root samples. Differences between production systems have been observed and further data analysis is underway.

Metabarcoding combines technological advances in PCR-detection with high-throughput sequencing technology to analyse complex communities of *Phytophthora* in unprecedented detail. The first batch of metabarcoding on 78 samples from 12 nurseries was processed in Nov 2017 and the preliminary findings were passed to nursery managers with our comments on their significance in the context of disease management. Synthetic control samples were included in this first batch to assess sequence error rates and to guide detection thresholds in future analyses. The high throughput sequencing generated almost 15 million DNA sequence barcodes which were analysed using the custom-built computational pipeline built in this project. A comparison of the nursery sequences against the reference database of 170 *Phytophthora* species revealed the DNA signatures of at least 41 known *Phytophthora* species. Other related pathogens such as 17 species of downy mildews were also detected. The data suggested the presence of quarantine pathogens in a few cases and these were reported to APHA and SASA. Each nursery manager was provided with a report on the findings on their premises. Further analysis of the findings in relation to the type of nursery and their production and plant health systems is underway to inform ‘best practice’ in the context of future accreditation guidelines. The key findings are also being reported to the Plant Health Risk Group.

In addition to the fine-scale sampling of fifteen nurseries, a broader approach has been taken in collaboration with the inspection teams co-ordinated by APHA and SASA. One hundred sampling packs were sent out in summer 2017 and over 400 samples of plant roots from almost 70 nurseries have been returned to The James Hutton Institute. The roots have been freeze-dried for processing in the final year of the project and an additional 100 sample packs were sent to inspectors in 2018. Lastly, this work is being supplemented by community sampling of water from natural ecosystems associated with recent tree or

horticultural planting via the OPAL network. Instructions and the sampling equipment were sent to the OPAL teams in June 2017. Sampling by community teams and OPAL co-ordinators has helped inform the public about the risks posed by plant pathogens such as Phytophthoras. Filters have been returned from 26 sampling points across Scotland and in north and south Wales. These samples will be processed in 2018.

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

Nursery practices

Semi-structured interviews have been conducted with 13 nurseries with another 5 interviews planned for June 2018. Nursery interviews have largely been conducted at the same time as WP1 sampling days so that the interviewer is able to spend time at the nursery and understand practices better.

We have agreed a list of specific best practice measures to focus on in the project, and are also collaborating with Fera economists who are dealing with similar issues (funded through the Future Proofing Plant Health FPPH programme) relating to responsibility and cost sharing between the nursery sector and government on biosecurity measures. However, it is proving more difficult than envisaged at the outset to collect economic data from the nurseries for the cost-benefit analysis because nurseries often have little information readily available on the costs of measures already introduced and do not know what other measures would cost to put in place, and they find it difficult to estimate the benefits of introducing biosecurity measures. We are exploring different options to proceed with the economics component.

In addition, we will be interviewing the nurseries that took part in the HTA pilot Plant Health Assurance Scheme to understand how they engaged with the audit criteria, any changes in biosecurity practices and lessons learned from the pilot. Some nurseries involved in the HTA scheme have also been approached directly to enquire about costs and benefits of introducing best practice measures in collaboration with Fera.

Consumer plant purchasing behaviours

The consumer survey results have been analysed and a summary report was produced in time for THAPBI dissemination event in February 2018. Headline results suggest that:

- There is a greater tendency for older generations to buy plants. In fact, 75% of those qualifying for the study (i.e. purchased outdoor plants within the last 5 years) were at least 45 years old, and 57% were at least 55 years old.
- Garden centres are the most commonly relied upon source for plants, followed by DIY stores, supermarkets, from seed, and nurseries. The most important factors influencing the choice of source are quality of stock, cost, and range of plants. Factors relating to biosecurity and plant pests and diseases (such as site cleanliness, presence of biosecurity measures, and plant provenance i.e. origin of individual plant) are relatively unimportant when choosing where to buy plants.
- Individual plant buying choices are driven by their appearance, suitability to one's planting site, and cost. Plant provenance emerged as the least important factor.

- Of the various sources of advice and guidance available when buying plants, 'friends, family and neighbours' is the most important, followed by the internet and thirdly, the plant seller's advice.
- Plant buyers' awareness of pests and diseases is low, both in terms of the general threat to British trees and woodlands from pests and diseases, and of the individual pathogens posing a threat.
- Perceptions of risk from sellers in relation to plant pests and diseases differ substantially, with non-specialist retailers without public access being regarded as the riskiest sources (international and domestic online retailers, and mail order sources). In contrast, self-grown plants and those from specialist suppliers and nurseries were seen to carry the lowest levels of pest and disease risk.
- There are established markets for many accredited/certified products, with over half of the sample reporting to buy such products at least some of the time. This decision is influenced by their favourable outlook on the ideals of such schemes, but also perceptions of a higher quality product. Added expense emerged as the key reason why respondents sometimes choose not to buy accredited products.
- Respondents reacted favourably to a hypothetical accreditation scheme for the plant trade on the basis that it would help to safeguard the wider landscape and ensure high quality products. However, a substantial proportion (25%) noted that despite agreeing with such a scheme in principle, they are concerned about higher prices resulting for consumers.
- In general, the more someone spends on plants per annum, the further they would likely travel to obtain plants from an accredited source. This signifies an opportunity for early adopters of a would-be scheme to attract new and valuable customers.

A journal article is now in preparation based on this survey.

The team also developed online surveys for (i) nurseries/garden centres, (ii) Landscaping sector, (iii) the general public. These surveys were advertised through various networks and the RHS magazine. Take up has been relatively low so we plan to do 10 semi-structured phone interviews with each of three sectors: Garden centres, local authorities/large gardens, retailers.

Ethics

An ethics committee meeting was convened in late 2017 for oversight of the social research agenda but also led to the development of a decision-framework to communicate the implications for discoveries of notifiable phytophthoras at nurseries. A diagram illustrating the courses of action to the undertaken in case of the finding of a notifiable *Phytophthora* was posted on the Phyto-threats project website.

WP3: Global *Phytophthora* risks to the UK

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.

The biological traits database completed last year from literature review and expertise of project pathologists at FR has been combined with a parallel traits database from a research team in Australia and New Zealand. It includes all 179 *Phytophthora* species or sub-species described so far from forestry, agricultural and horticultural settings. Traits included are related to survival and persistence, reproduction, dispersal, host range, disease symptoms and taxonomy.

A conceptual framework has been developed for how these traits may link to success through different invasion stages of arrival, establishment and spread. Phylogenetic analyses of traits and trait syndromes across *Phytophthora* species have been carried out to understand whether closely-related species share similar trait values and whether some traits are more labile across the phylogeny indicating that they may be under stronger selection. For example, thermal tolerance range and minimum growth temperatures were shown to be less phylogenetically conserved across the phylogeny than some of the reproductive traits and to be more labile (greater within-clade disparity in recent evolutionary history), which may indicate that they are under stronger selection during invasion. We will test this in year 3, combining our global occurrence database with climate data, to test whether species with higher cold tolerance have invaded further north worldwide.
The global impact (extent and known host range) of *Phytophthora* species has also been modelled against species traits indicating that the highest impact species are those that cause root disease, are heterothallic, have multiple survival structures, broad thermal tolerances and rapid growth at optimum temperature. The strength of these relationships will determine whether traits can be used for horizon scanning, to identify the potential impacts of new species, as they are discovered, on the basis of their trait similarity to species that have already had high impact. We expect to submit two manuscripts on these sets of results by October 2018.

We have also modelled the rate of arrival of *Phytophthora* species per country this century against that level of plant pathogen recording (number of IPPC pest reports) and trade connectivity (rate of imports of live plants) with *Phytophthora* source countries. We found that the number of *Phytophthora* species arriving per country increased with the rate of live plant imports and with plant pathogen recording effort (together explaining 60% of the variability in arrivals) (Fig. 1). This work will be extended over the next six months to integrate other trade pathways, climate similarity between source and sink regions and species traits.

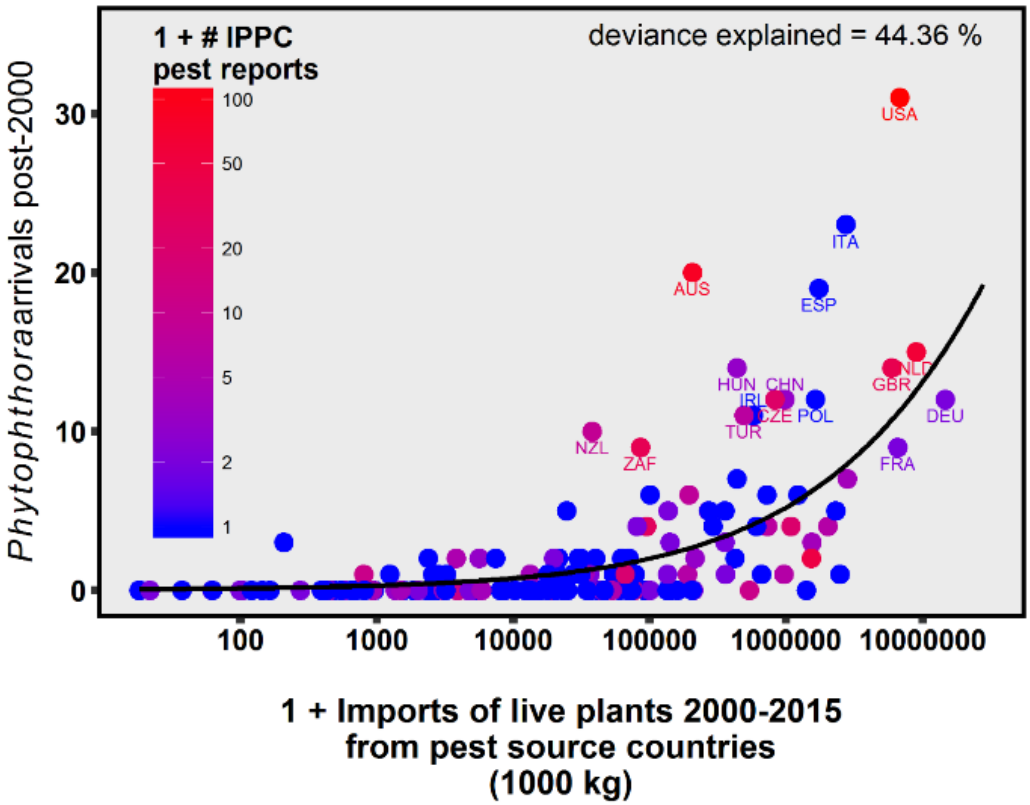

Fig. 1. Rate of arrival per country since 2000 versus rate of imports of live plants from source countries and country recording effort.

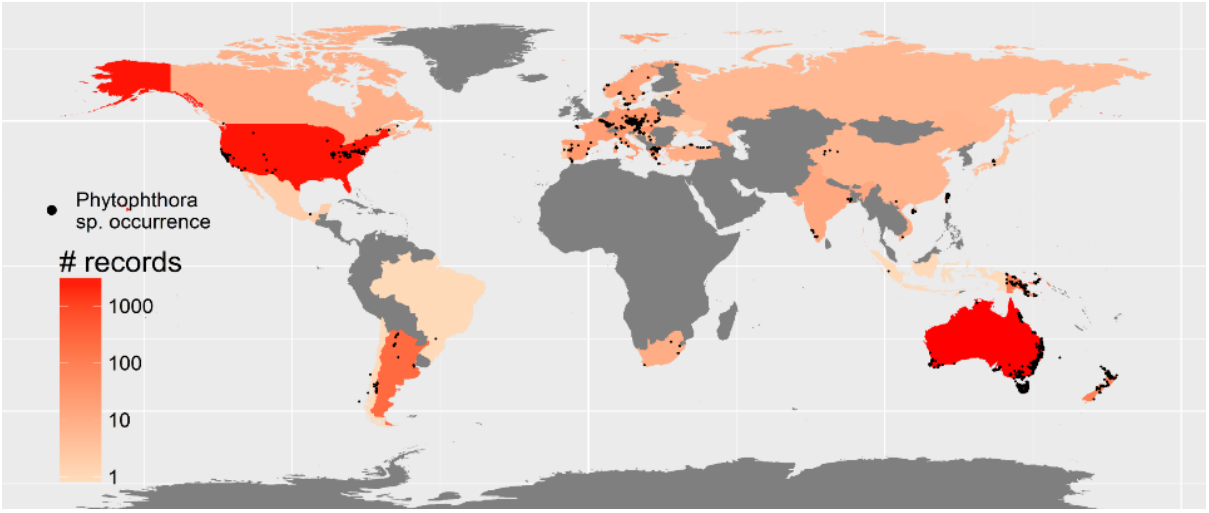

Fig. 2. Number of site level *Phytophthora* records per country in the Phytothreats database,

We have continued to collate data on country- and site- level occurrence of phytophthoras from a wide range of sources (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 11407 site-level records collated so far span 82 *Phytophthora* species, 38 countries, and are distributed as indicated in Fig. 2. At national level, our database contains 17371 records, equating to 1417 species x country combinations. The spatial mapping of

plant health recording effort and development of global niche models is scheduled for year 3. We have conducted a participatory study of important environmental risk factors with the project team and advisory board experts to understand which factors might determine *Phytophthora* distributions and should be mapped and included in niche models. We are hoping to co-develop tools from all models above with stakeholders in year 3.

Scoping of knowledge gaps for horizon scanning of emerging pathogens

A strategy for assessing knowledge gaps in pathways of *Phytophthora* arrival and spread has been developed between FR and CEH. In line with the steering group's advice, activities have focussed on assessing the potential for tourists to act as a pathway for *Phytophthora*. A search of the literature has sought to verify the precise mechanisms for spread as well as the conditions which impact viability (e.g. means of arrival, environmental conditions, activities pursued by tourists etc.). In addition, data from an international consortium of plant pathologists has been collected via a survey. Their responses are being used to contextualise the threat posed by tourists relative to other pathways, and to highlight instances of tourist-related introductions and spread which may not have been publicised. Thirdly, we are continuing to pursue visitor data (number, origin and season) to supplement a VisitBritain dataset by engaging with a number of parks and gardens demonstrably popular with both international and domestic tourists. These include Kew Gardens, Royal Botanic Gardens Edinburgh, Royal Horticultural Society, Chatsworth House, Alnwick gardens, The Eden Project, Westonbirt Arboretum and National Trust properties). Although of varying availability and comprehensiveness, data such as visitor origin and numbers could be compared with the aforementioned *Phytophthora* distribution maps to illustrate the level of risk posed by tourists from different countries or regions. In addition, data on the seasonality of tourist visits could be considered in combination with what is known about how environmental conditions alter viability, allowing staff at parks and gardens to consider when risk is highest and thus when biosecurity measures are most important. A written output of the work will be produced following further analysis and discussion with the project partners.

WP4: Predicting risk via analysis of *Phytophthora* genome evolution (WP started in August 2017)

Here we aim to study genes and sources of variation between *Phytophthora* species that may be linked to key disease traits such as host range, virulence, etc. To investigate this, a number of approaches are considered:

1. Comparison of genes from available sequenced *Phytophthora* genomes to identify a core set of genes common to all *Phytophthora* species and then to identify species-specific genes or variants and potential instances of hybridisation or horizontal gene transfer between species
2. Sequence genomes from three less damaging species, which are closely related to highly damaging species with a view to improve understanding of genes involved in virulence
3. Comparative study of target genes and gene families known to be important for pathogen virulence and to examine how variations between these can influence the pathogen

Comparison of sequenced *Phytophthora* genomes

Genome sequences for 26 different species of *Phytophthora* have been obtained. In most cases these assemblies also included sequences for predicted genes and proteins and,

where available, these published gene/protein sequences have been used in this study. In cases where gene/protein sequences were not supplied *ab initio* gene prediction with Augustus was carried out in-house. Numbers of predicted genes ranged from around 10,000 to over 75,000 with the highest numbers most likely indicating over-prediction by the prediction algorithm.

An initial estimate of completeness for all 26 genomes was carried out using BUSCO (Benchmarking Universal Single-Copy Orthologs), based on the presence of 234 conserved genes considered to be present in all stramenopiles. In total, assemblies for 22 of the 26 species were indicated to be over 90% "complete" based on the presence of these genes, 3 were classed as being over 70% complete and one assembly, *P. alni*, appeared to be only 37% complete, possibly arising from a highly fragmented genome assembly.

Repetitive DNA content in each genome was then quantified using RepeatModeler and RepeatMasker software to indicate the relationship between genome size and sequence composition, with larger *Phytophthora* genomes tending to be more repetitive, suggesting that increased genome size is often driven by expansion of repetitive sequence, although hybrid species such as *P. alni* (and possibly *P. cambivora*) have larger genomes due to the presence of sequence from their two parent species. Predicted gene content tended to be a poor indicator of genome size. Table 1 summarises genome size, repeat content, predicted number of proteins over 30 amino acids in length and number of complete BUSCO genes for each of the 26 species.

Initial steps towards identifying a "core gene set" for *Phytophthora* were taken by carrying out an all-by-all comparison of the set of proteins of at least 30 amino acids in length from each species using Orthofinder. This identified 55,134 clusters of proteins of which 4,156 contained sequences from at least 20 of the species and 2,107 contained sequences from all 26 species. Although given the apparently low completeness of *P. alni*, *P. cambivora*, *P. lateralis* and *P. palmivora* indicated by BUSCO it may be advisable to omit these from this component of the analysis at present and focus on the 22 species suggested to be over 90% complete.

Table 1. Summary statistics for 26 sequenced *Phytophthora* species

Species	Clade	Assembled genome size (Mbp)	% Repeat masked	No. proteins over 30 aa in length	Complete BUSCO genes (out of 234)
P. agathidicida	5	37.3	22.6	13,363	230
P. litchii	4	38.2	21.2	13,441	231
P. multivora	2	40.0	27.2	14,299	231
P. plurivora	2	40.5	25.5	16,023	226
P. kernoviae	10	43.2	15.4	10,051	225
P. lateralis	8	44.0	38.3	11,279	181
P. pluvialis	3	53.6	34.5	16,219	229
P. parasitica	1	55.2	34.8	28,117	229
P. taxon totara	3	55.6	32.0	15,718	230
P. colocasiae	2	56.6	41.0	17,954	226
P. pisi	7	58.9	36.4	19,363	227
P. cryptogea	8	61.2	37.4	21,778	224
P. capsici	2	64.0	38.8	19,805	213
P. ramorum	8	66.7	36.1	15,605	227

P. cactorum	1	67.8	42.4	22,577	228
P. fragariae	7	73.7	45.6	18,876	218
P. rubi	7	74.9	52.2	19,255	227
P. cinnamomi	7	78.0	35.1	26,131	219
P. sojae	7	82.6	42.7	26,489	229
P. pinifolia	6	89.9	54.7	19,687	227
P. megakarya	4	101.5	53.8	34,798	218
P. palmivora	4	107.8	45.0	24,658	165
P. austrocedri	8	114.4	49.0	31,326	219
P. alni	7	185.5	55.0	39,673	88
P. cambivora	7	216.4	48.8	75,671	186
P. infestans	1	228.5	68.2	17,785	224

Sequencing of additional *Phytophthora* species

Sequencing of *P. europaea*, *P. foliorum* and *P. obscurum* is still in progress due to difficulty in obtaining sufficient quantities of high molecular weight DNA for long-read PacBio sequencing.

Target gene family study: xylanase enzymes

A family of four xylanase enzymes was chosen as an exemplar of a gene family with potential importance for virulence. These enzymes degrade the plant cell wall by breaking down hemicellulose and so facilitate entry of the pathogen into the host's tissues.

The 26 sequenced *Phytophthora* genomes were searched for these four sequences (using sequences from *P. parasitica* as references). Family members xyn1 and xyn2 were present in almost all species, this is consistent with reports that suggest these two are the most important of the four for virulence. However, much more variability is seen with family members xyn3 and xyn4 and Clade 8 *Phytophthora* species appear to possess a single sequence instead of the pair, suggesting that the duplication event which gave rise to the xyn3/xyn4 pair occurred after the speciation of the Clade 8 species.