

THAPBI FINAL REPORT FORM

1. Project title

Global threats from *Phytophthora* spp.; understanding drivers of emergence and opportunities for mitigation through nursery best practice

2. Name and address of lead Principal Investigator

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3. Project duration

April 1 2016 – December 31 2019. The original project completion date was March 31st 2019. Two extensions were approved; one to July 31st 2019 due to delay in hiring a PDRA for workpackage 4 and a further extension until December 31 2019 due to the maternity leave of the PDRA on workpackage 3.

Project team

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4. Summary project report.

The multidisciplinary 'Phyto-threats' project was initiated in 2016 to address the increasing risks to UK forest and woodland ecosystems from trade-disseminated *Phytophthora* as implicated in the recent upsurge of *Phytophthora* diseases in the UK and the establishment and spread of these pathogens in the wider UK environment in diseased propagation material. The project focused on understanding the drivers of emergence of *Phytophthora* species and opportunities for mitigation by; i) examining the distribution and diversity of *Phytophthoras* in different UK plant nursery management systems to identify high risk nursery practices, ii) conducting feasibility assessments with nursery managers, consumers and other stakeholders to identify economic and social opportunities and barriers to implementation of best practice, iii) identifying future global *Phytophthora* threats through modelling biological traits and environmental profiles, and iv) analysing genome sequences of *Phytophthora* species to gain genetic insights into what makes a species virulent.

Diversity of *Phytophthora* in UK plant nurseries

A major component of the Phyto-threats project was to address the risk of *Phytophthora* spread through nursery practices. Over the last three years, the project team together with Plant Health inspectors collected close to 4000 water and root samples from plant nurseries located across Britain. These included fifteen partner nurseries intensively sampled in a 'fine-scale' survey and a further 118 nurseries sampled as part of a 'broad-scale' survey. Samples were tested for the presence of *Phytophthora* DNA using a state-of-the-art metabarcoding approach. Approximately 50% of all samples were positive for *Phytophthora*, with over 50 *Phytophthora* species identified across all samples including quarantine regulated pathogens and species not previously reported in the UK. Some of the most diverse *Phytophthora* assemblages were found in river water used to irrigate plants and in open reservoir irrigation sources, as well as in puddles which formed around plant stock, confirming that effective water treatment and good drainage are essential components of *Phytophthora* management. *Phytophthoras* were also detected in diseased shelterbelt or landscape trees present at some nurseries, acting as additional inoculum reservoirs. Trees growing in the immediate environment of each nursery should therefore be included in routine Statutory Plant Health inspections. Another issue highlighted by the survey is that, in some cases, native plant stock being raised from seed collected from ecologically sensitive sites and destined for planting back out at these sites were found to be harbouring damaging *Phytophthora* species. These findings have considerable implications for the proposed massive expansion of UK woodland and have led to discussions on new guidance documentation for woodland restoration.

Feasibility of accreditation and introduction of nursery best practice to mitigate disease risks

Data on *Phytophthora* diversity are being related to nursery management practice and this evidence will inform the Plant Health Management Standard being developed as part of the new 'Plant Healthy' accreditation scheme which targets producers and consumers across the UK plant supply chain. Social and economic scientists have generated information on the feasibility of accreditation and what it should look like, based on a survey of 1500 plant buying members of the general public and collated perspectives from nurseries, retailers (including garden centres), landscape architects and designers following 153 survey responses and 37 interviews. One of the key findings was the need for engagement on plant health risks to secure collaboration from all sectors. The public are driven by quality rather than biosecurity practices so quality should be emphasised to promote an accreditation scheme. Many nurseries will have to improve their biosecurity practices to become accredited, however, few nurseries are willing/able to incur substantial cost to become accredited. A requirement for large landscape contracts to use stock from accredited growers would increase demand (and therefore suppliers' interest). Importantly, large retailers are willing to work with science and

policy to improve practices and could serve as important players in raising awareness of plant health.

A cost-benefit analysis of introducing best practice from a nursery perspective highlighted the difficulties of obtaining good data in order to estimate costs, as the overall number of quantitative responses from nursery managers on costs of implementing best practice and costs of avoiding outbreaks of *Phytophthora* was too low to enable confidence in the analysis. However, an exploratory cost-benefit analysis indicated that the predicted benefits of introducing nursery best practice outweigh the costs when a wider range of pests and diseases (for example, *Xylella*) are considered.

Global *Phytophthora* risks to the UK

In order to model *Phytophthora* risks, a global *Phytophthora* distribution database containing close to 40,000 country-level records from garden/amenity, forest, nursery and agricultural sectors was collated in addition to a large dataset of UK *Phytophthora* records. A database listing the biological traits of all 179 described *Phytophthora* species was also developed. These databases have been made available to researchers and other end-users. Applying ecological modelling approaches to these data, global *Phytophthora* introductions were found to be strongly correlated with the level of connectivity to source regions by the live plant trade, with cold-adapted *Phytophthora* species better able to exploit trade pathways than others. The extent to which a species can disperse geographically was also linked to cold tolerance, the ability to infect roots and the ability to cause foliar symptoms. Wider host ranges were strongly linked to optimum growth rate, thick resting-spore walls (facilitating long-term survival) and ability to cause both root and foliar symptoms. In summary, thermal traits and cold tolerance are strongly linked to invasion success. These factors could be used in horizon scanning when looking to predict the potential impact of newly discovered species.

Tools to help policy and practice were developed from this study. These included a *Phytophthora* importation tool which focusses on potential trade pathways; users can visualise the *Phytophthora* diversity in exporting countries and the volume of imports from those locations to assess risk. An interactive database of *Phytophthora* pathogens and associated hosts was also developed, which is searchable by host and by pathogen and links to risk maps of UK suitability for the pathogens. A third tool allows users to interact with maps of *Phytophthora* disease records in the UK. The maps show which species are predominantly nursery-associated and which are common in the wider environment.

Predicting risk via analysis of *Phytophthora* genome content

A better understanding is required of what makes a particular *Phytophthora* species more virulent than others. This knowledge gap was addressed by identifying genetic differences between closely related highly damaging and less-damaging *Phytophthora* species, the rationale being that by comparing the genes present in less damaging species with those of highly damaging species, it might be possible to find genes linked to virulence. To do this, targeted genome sequencing was carried out for three *Phytophthora* species not known to be damaging but which are closely related to aggressive pathogens. Using the latest sequencing and assembly technology, this project produced three of the most complete *Phytophthora* genomes currently available for a *Phytophthora* species (at this time 30 *Phytophthora* species have had their genomes sequenced). Each of the three genomes sequenced in this project have ~19,500 predicted genes and the analyses looked at how many genes are shared and how many are unique to each group of species. Initial findings revealed genes present in highly damaging species which are not present in the less damaging species and which might be linked to virulence. The next steps are to analyse the function of these genes in order to start to unravel what makes a *Phytophthora* virulent. Gene content can then be used to predict which newly discovered *Phytophthora* species have the potential to be most damaging.

5. Scientific report

Work package 1: *Phytophthora* distribution, diversity and management in UK nursery systems

The overall aim of this work package was to analyse the distribution and diversity of *Phytophthora* species in UK nursery systems and identify high risk management practices that could be targeted in an accreditation scheme.

Nursery sampling methodology

The incidence and diversity of *Phytophthora* species was examined in water and plant samples collected from nurseries located in England, Wales and Scotland over a three-year period. The nursery survey was conducted on two scales; a fine scale survey which involved the detailed sampling by the project team of fifteen partner nurseries operating a range of management practices including the production of bare root and containerised forest trees, wholesale horticultural plants, specialist native trees, stock for botanic garden collections and retail garden centres, and a 'broad scale' survey involving 118 nurseries and garden centres sampled systematically during annual statutory plant health inspections.

Since roots and water are the major transmission pathways of *Phytophthora*, both substrates were targeted for the fine scale survey. The sampling method was not random but biased to finding *Phytophthora*. Roots were sampled from symptomatic plants as well as a range of asymptomatic plants that are known to be *Phytophthora* hosts. Irrigation water, run-off from potted plants, puddles and collection ponds were also sampled on-site by filtration methods that capture *Phytophthora* propagules. For the broad scale survey, root samples were collected. For each nursery sample, data were collected on associated host species, symptoms present at time of sampling, sample origin and location on nursery. This was supplemented with wider data on propagation and trading practices, irrigation sources and disease management practice for downstream analysis to inform best practice. The nursery sampling was supplemented by a community sampling of water from natural ecosystems associated with recent tree or horticultural planting via the Open Air Laboratories network (OPAL) to investigate the broader range of nursery-associated *Phytophthoras*.

Development of the *Phytophthora* classification tool

To try and capture the highest diversity of *Phytophthora* species as possible in each sample a DNA detection tool known as metabarcoding was used. This method uses Illumina sequencing technology to detect all *Phytophthora* species present within an environmental sample, including species as-yet undescribed. A bioinformatics pipeline was developed as part of this project and this has now been published online <https://pypi.org/project/thapbi-pict/>. Much of the development work focused on technical variation in metabarcoding, determined through the use of four synthetic DNA control sequences alongside real samples when sequencing. Analyses revealed that Illumina produces 1000s of sequence variants generated in very low abundance. These 1bp variants are most likely PCR artefacts as single base changes during PCR can happen and this needs to be accounted for in metabarcoding diagnostics. Many variants come through at low level and these variants were used to set a minimum threshold, which was 100. Thus, any unique sequence needs to be present at least 100 times before going through the pipeline. Sequence thresholds are then set for each plate for reporting species in a sample based on the level of contamination of synthetic control sequences in environmental samples. This is a plate-by-plate threshold set above the highest level of known sequence contamination.

Sequence data are prepared by quality trimming FASTQ reads, merging the overlapping FASTQ reads into single sequences, discarding reads without both primers, converting reads into a non-redundant FASTA file and then filtering reads with hidden Markov models of ITS1 and synthetic control sequences so that non-matching sequences are discarded. Edit graphs

are used to illustrate the number of ITS1 variants observed for each species. Many at low sample abundance are likely to be PCR variants, but those occurring at higher sample abundance and with higher read numbers could be true ITS1 variants within the genomes, for example in *P. austrocedri* and *P. nicotianae*. Complex ITS1 clusters with up to 3 bp edits are observed for *P. rubi* and *P. cambivora* (the latter which is likely to be a hybrid complex) but the most complex cluster occurs with *P. gonapodyides*, *P. megasperma*, *P. chlamydospora*, *P. lacustris*. All of these species are hard to distinguish by ITS1.

Species classification starts with a 100% match to a database sequence. If there is no perfect match, then the classifier looks for a 1bp difference. The database has ~170 individual sequences curated to species by members of the project team. This custom database includes sequences from verified voucher specimens plus sequences generated ‘in house’ from an Illumina ‘control’ plate in which DNA from pure cultures of known *Phytophthora* species were processed for metabarcoding and sequenced to gauge within-genome ITS1 variability. In addition to the custom curated set of sequences, the database includes a much larger set of sequences downloaded from the NCBI database. Any sequences not matching the curated database, but which match the NCBI database based on 2bp difference, are reported to genus only. If it doesn’t match anything within a 2 bp difference then it is marked as ‘unknown’. The pipeline produces Excel spreadsheets of results plus ‘human readable’ text files which are used for reporting to nurseries. The classification tool has been tested on other *Phytophthora* datasets as well as a nematode ITS1 dataset and was found to work well.

Outcomes from nursery sampling

Overall, the project collected close to 4000 samples from 163 host genera; the top five most frequently sampled species being *Juniperus*, *Taxus*, *Viburnum*, *Pinus* and *Rhododendron* (Fig. 1). The sample analysis was a two-stage process, with the first stage being a PCR test to determine whether *Phytophthora* is present in the sample or not. All *Phytophthora*-positive samples were then progressed to the second stage of Illumina sequencing to determine which *Phytophthora* species are present. Overall, ~50% of all nursery samples collected were positive for *Phytophthora* or closely related oomycetes. This varied according to host genus, with *Chamaecyparis*, *Pinus* and *Fagus* yielding some of the highest proportions of positive samples. In terms of *Phytophthora* test results in relation to nursery practices, the percentage of positive samples ranged from 20-70% across the fifteen partner nurseries, and this reflected the plant health status and nursery management practices observed at sampling, which varied greatly across the nurseries.

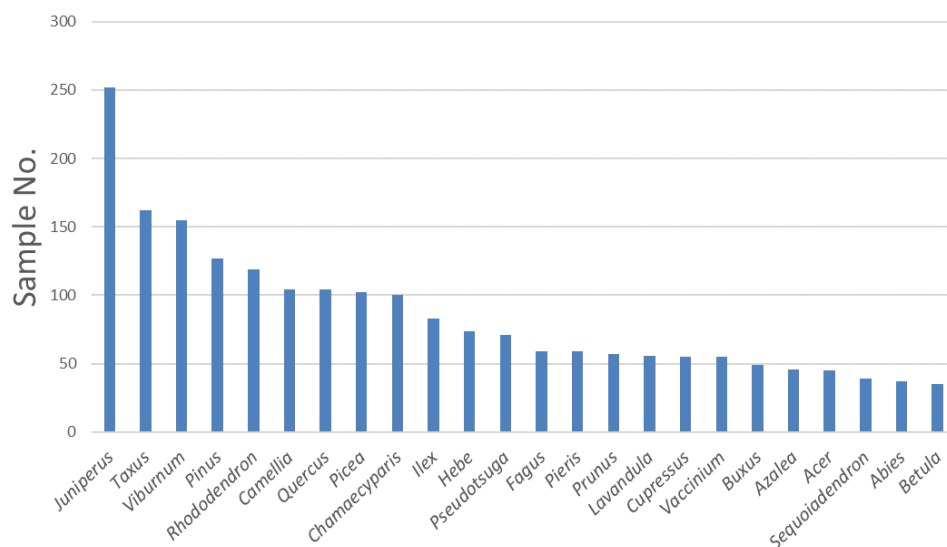


Fig. 1. Top 25 host genera sampled in the fine scale nursery survey.

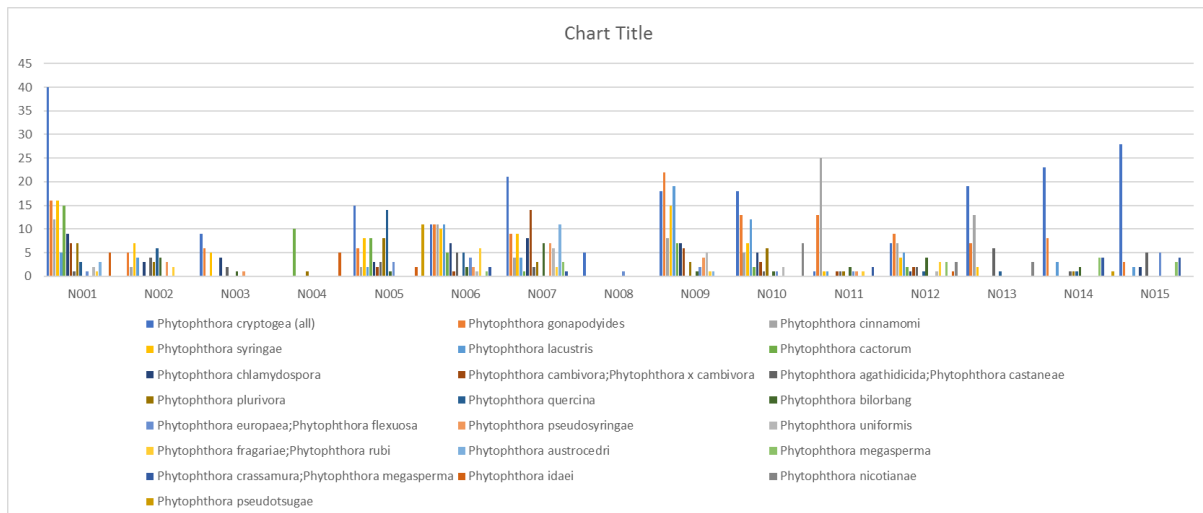


Fig. 3. Abundance (by number of samples) of the top 22 *Phytophthora* species found in the fifteen partner nurseries in the 'fine scale' survey.

Best management practices can affect pathogen arrival (source and health of plant material coming in, growing media, water source, mud on vehicles/boots), pathogen spread on site (including water management and hygiene) and pathogen dispersal off site (quality control at sale, water run-off, plant disposal). Some of the most diverse *Phytophthora* assemblages, including highly damaging species, were found in river water used to irrigate plants (up to eight known species in a single sample) and in open reservoir irrigation sources, as well as in puddles (up to twelve known species in a single sample) which formed around plant stock, confirming that effective water treatment and good drainage are essential components of *Phytophthora* management. Plant disposal was clearly an issue across nurseries, with most disposal areas or 'dumps' containing *Phytophthora*-infected material which could be a source of inoculum. *Phytophthoras* were also detected in diseased shelterbelt or landscape trees present in and around some nurseries, acting as additional inoculum reservoirs. One outcome of this project was the recommendation that trees growing in the immediate environment of each nursery should be included in routine Statutory Plant Health inspections. Another issue highlighted by the survey is that, in some cases, native plant stock being raised from seed collected from ecologically sensitive sites and destined for planting back out at these sites were found to be harbouring damaging *Phytophthora* species. Also found was a quarantine regulated *Phytophthora* infecting 'off-colour' plants offered for sale at discount price in the retail area of one nursery. Thus, this study identified a number of key practices that should be targeted in an accreditation scheme or in guidance documentation for woodland restoration.

Direct engagement with a range of nurseries operating different practices enabled the science team to understand the challenges faced by the industry and some of the key reasons leading to poor biosecurity practice. Following the reporting of *Phytophthora* findings to each nursery, feedback and advice on improving practice was given using a 'non-blaming' approach to facilitate dialogue. Often there was a genuine lack of awareness of the risks and routes of *Phytophthora* contamination and spread, for example through apparently healthy imported plants, unclean water sources and inadequate drainage. There was also a frequently held assumption that symptoms of poor health in plants was caused by other factors such as frost or drying out when the subsequent sampling results confirmed infection by *Phytophthora*.

As the project progressed there was evidence of partner nurseries improving practice based on advice delivered by the science team as a result of *Phytophthora* findings, for example raising plants off the ground, improving drainage and taking decisions not to trade in high-risk hosts. Nurseries also started asking questions of growing media suppliers – what are the processes of sterilisation and can they guarantee pathogen-free components?, and seed

suppliers – has this seed come from disease-free orchards? In relation to raising from locally collected seed native plant stock destined for restoration planting at ecologically vulnerable sites, one nursery manager is now considering offering dedicated growing of stock to high biosecurity specification close to each restoration site and well away from the main commercial premises. Another partner nursery has now employed a full time Plant Health specialist to train staff on plant health issues, audit existing plant health processes within the nursery, and to update plant health standards.

Informing accreditation and broader Plant Health surveillance

Parallel to the Phyto-threats project a joint accreditation initiative led by the HTA and industry has been developing. This is the new Plant Healthy Certification Scheme (<https://planthealthy.org.uk/>) which is based on the Plant Health Management Standard currently consisting of a check-list of 23 requirements that demonstrate that a business is operating responsibly. The PHMS is not prescriptive at this early stage in the development of the scheme but rather highlights problem areas on a nursery and offers advice on how this can be resolved. It is expected that as more information on biosecurity risk becomes available, more prescriptive measures will be incorporated. The Phyto-threats science team are liaising with those leading on PHAS to provide the scientific basis for more prescriptive measures around water source and usage, plant disposal, growing media and raising plants off the ground to be referenced in the scheme guidance since the project's main outcomes strongly suggest that a set of priority prescriptive measures will be necessary for accreditation to be effective. Additionally, the project's findings of widespread *Phytophthora* contamination have led to the recommendation that the PHAS audit process includes a component of targeted testing for pests and pathogens. To this end the various strands of engagement with the Statutory Plant Health teams in England/Wales and Scotland has facilitated a greater awareness of what the metabarcoding technology can offer and generated discussion of its potential for routine nursery testing, as part of regular statutory surveillance or for incorporation into an accreditation scheme.

Work package 2: Feasibility analyses and development of 'best practice' criteria

i) Socio-economics analysis to assess applicability of best practice criteria

The key objective of the social research was to provide evidence of existing management practices and the feasibility and appetite for improving practices through a UK-wide 'best practice' nursery accreditation scheme. Several areas of work were conducted that broadened the scope beyond nurseries to include other key stakeholder groups along a supply chain. Stakeholder and consumer attitudes to implementation of an accreditation scheme and what this should look like were identified through surveys, interviews and workshops (see WP5) involving a range of key consumer, nursery, landscape and retail stakeholders.

Nursery and Garden Centre stakeholders: Nurseries that were involved in the Phyto-threats sampling programme were visited in person by a social scientist and interviewed. Key areas explored included current pest and disease management; how management relates to existing values, beliefs and practices; the governance frameworks and decision-making processes that direct their nursery practices; factors that influence uptake of advice; level of support for accreditation and the form accreditation might take, including existing frameworks (e.g. HTA BOPP scheme); perceived social, political and economic barriers/benefits of accreditation and/or improving management practices. In most cases, the interview was followed up by telephone call with economic questions related to the feasibility of applying best practice (see ii below). Interviews with 20 nurseries highlighted a concern about the knowledge and practices of others, particularly from the landscape and retail sector. Nurseries highlighted measures they were taking to reduce the risks of introduction and spread of pests and diseases. Not all nurseries were able to take up all or any of the measures, which encompassed using trusted suppliers, not importing, regular inspections, ensuring clean water

supplies and basic husbandry (removing waste, keeping sites tidy and free of puddling etc.). Important factors influencing decision-making amongst nurseries included contact with a trusted source of information (such as an official plant health inspector), relationships with suppliers, risks to reputation, consumer demand and costs. There were differences in perspectives as to whether there should be stricter legislation on high risk plants and general agreement that an accreditation scheme could provide a number of benefits such as training, traceability, a potential safety net and reassurance for the customer that the plants are pest and disease free. However, there was scepticism over current consumer awareness of pests and diseases and whether there would be a demand for accredited products. There was also concern that a scheme would be largely superficial and a 'tick-box' exercise or that it will demand changes in management practices that are costly and/or not feasible. Interviewees questioned how the scheme would be policed stressing that it would "need to have teeth".

To expand the sample size, an online survey was conducted with 100 nurseries and garden centres (see Dunn and Marzano 2019 for a summary). Only a few nurseries and garden centres surveyed had received any recent training or undertaken educational courses on plant/tree pests and diseases. Out of twelve biosecurity practices listed in the survey, only four were used by the majority of nurseries. Having a quarantine/holding facility, water treatment and reliance on UK suppliers were all practices perceived to be most costly, whereas boot washing, disinfecting stations and vehicle washing stations were perceived to be least costly. The general public and landscaping businesses were considered to be the most important clients. There was strong agreement from both nurseries and garden centres that an accreditation scheme would ensure better quality trees and plants and safeguard the wider environment from the spread of pests and diseases. However, there was a mixed response in terms of interest from nurseries and garden centres in joining a hypothetical scheme. Both sectors were slightly more negative than positive, but the largest response category was in the middle (50/50 whether they would join a scheme). This might be because they were being asked to consider a hypothetical scheme with no detail on how it would work and what it would cost. There was some scepticism among nurseries and garden centres about insufficient interest amongst growers and consumers needed for accreditation to be successful, as well as the costs of such a scheme.

Consumers of plants (members of the public): We conducted a UK wide survey of 1500 members of the public (who have purchased an outdoor tree/plant in the last 5 years) to assess their plant purchasing behaviours. The underlying premise for the consumer survey was that the uptake and success of an accreditation scheme would likely depend on the willingness of consumers to acknowledge and support such a scheme, particularly through their purchasing behaviour. The survey asked a series of questions around plant buying habits (e.g. where do consumers buy their plants?); the perceived benefits and dis-benefits of accreditation; willingness to pay extra or travel further to buy accredited products; and, for those willing to pay more for accredited stock, their current spend and what percentage increase in price they would be willing to pay. Key findings from the consumer survey show that awareness of pests and diseases is generally low. The general public obtain plants from multiple sources (most frequently garden centres, DIY stores, supermarkets, friends and nurseries) so there is a need to consider the whole supply chain, not just nurseries, when developing an accreditation scheme (Dunn et al. 2019). When making decisions about which plants to buy and where to buy from, factors such as quality, range of products offered, and cost are the main drivers. Existing purchases of non-horticultural products (fair trade coffee, red tractor produce etc) is driven by ideals and quality. Thus, promoting accredited products on the grounds of high-quality healthy plants is required. When asked about which sources they feel are riskiest in terms of pests and diseases, it was clear that the public are using sources they themselves would consider to be of higher risk, for example non-specialists such as supermarkets and

DIY stores (Dunn et al. 2019). The largest interest in a accreditation comes from the biggest spenders, who are prepared to spend more and travel further for accredited plant products (Dunn et al. 2019).

Landscape sector and large retailers: Several interviews were conducted with large retailers and a survey of 38 landscape architects or garden designers (NB there were a few landscape contractors in the sample). While nurseries and garden centres have similar pest and disease concerns (i.e. *Xylella*, *Phytophthora*), the greatest proportion of landscape architects rated ash dieback as their greatest concern. Although the best practices aimed at growers would be inapplicable to landscape architects and designers, over 90% of the sample said that biosecurity and pest and disease issues influence their choice of plants when preparing planting specifications. Landscape architects and designers may also include pest and disease precautions in the specifications. However, a limitation of the role of landscape architects and designers in biosecurity is that there is no guarantee that the trees and plants they specify will actually be planted in the way they intended or outlined. If an accreditation scheme became established in future, landscape architects and designers may advise that plants are obtained from an accredited grower.

We interviewed 5 multi-store retailers. Retailers are influential consumers and could help drive good practice if they demand transparency in their supply chain, including biosecurity beyond the border. Large multi-store retailers are particularly influential due to the volumes of plants they receive and influence they can have on both suppliers and consumers. Retailers are willing to work with science and policy to improve practices and could serve as an important player in raising awareness of plant health. Support of the accreditation scheme would be an important component but there are still questions around who or what will be accredited, how good is good enough with biosecurity (is it subjective?) and who will bear the burden of costs?

Real change could require those procuring contracts to put biosecurity concerns ahead of other factors, such as how timely or cheaply a site can be planted. If this were the case, demand for accredited products would increase, and interest in becoming accredited or stocking accredited tree or plant products would also likely increase. The overall findings in terms of appetite for accreditation can be summarised:

- Accreditation must cover most businesses in the plant supply chain, or at least their plant stocks
- Many nurseries will have to improve their biosecurity practices to become accredited
- Few nurseries are willing/able to incur substantial cost to become accredited
- What accreditation 'looks' like (teeth, costs and benefits) will be influential
- More evidence is needed of how regulated pests and diseases could impact growers, and how biosecurity practices avert such risks
- Public are driven by quality rather than biosecurity practices. Thus, quality could be emphasised to promote an accreditation scheme
- A requirement for large landscape contracts to use stock from accredited growers would increase demand (and therefore suppliers' interest).

Follow on research with a broad range of stakeholders along the supply chain including online retailers and large-scale consumers such as construction companies is being carried out through new projects. Efforts are continuing to engage with difficult-to-reach stakeholders such as landscape contractors and large retailers.

ii) Cost-benefit analysis of introducing 'best practice'

Nursery perspective

To investigate the economics of introducing best practices from a nursery perspective, managers at 75 plant nurseries were interviewed to gather information on costs and benefits of introducing best practices. The nurseries included 9 contacted in an initial survey by Forest

Research, 11 during joint visits with FERA and 55 from a subsequent telephone survey of nurseries and garden centres.

Responses highlighted difficulties in obtaining robust quantitative estimates of the costs and benefits of introducing the best practices, with only a minority of the nursery managers providing a quantitative response on costs of implementing specific best practices, or of the expected benefits, and a wide range of estimates partly reflecting uncertainties. Nonetheless, the results provide a useful indication of the rough balance of the costs and perceived benefits.

Aggregating quantitative responses, the results indicated a mean set-up cost for implementing all of the 12 best practices of £0.25 million (median cost of around £35,000) and a mean annual cost of £39,000 (median (£4,000)). These can be compared with an estimated mean benefit of avoiding the cost of an outbreak of *Phytophthora* of £47,000 (median £15,000). However, some of the best practices could be considered to constitute common practice. (Five out of the 12 were reported to have already been implemented by a majority of the nurseries who indicated whether a measure was currently used or not).

Focusing on measures not currently common practice, the mean cost for implementing 7 best practices not already implemented as common practice was £135,000 (median cost £8,000), with a mean annual cost of £33,000 (median (£3,000)). The average estimated avoided cost of a *Phytophthora* outbreak (mean £47,000; median £15,000) was far lower than the average cost for set-up and maintenance of the best practices (estimated present value for mean costs over a 10-year investment time horizon of £386,000 and £31,000 for median costs).

The frequency of *Phytophthora* outbreaks that would be avoided by implementing the 7 best practices is unclear. However, responses from the nurseries indicate that outbreaks have been relatively infrequent in the past. Hence, in general from a nursery perspective the results suggest that the introduction of the best practices cannot be justified purely on the basis of avoided *Phytophthora* outbreaks alone. Around one *Phytophthora* outbreak per annum would need to be avoided at least in order for the benefits of introducing the best practices to exceed the mean costs, or one outbreak every 4 years avoided for median costs.

The introduction of the best practices could be expected to reduce the risks of other pest and disease outbreaks too. *Xylella* is of especial concern. It is viewed as an existential threat by many nurseries who consider an outbreak could put them out of business. Introduction of one of the best practices in particular (sourcing plant materials from UK suppliers) would be expected to significantly reduce the risk of a *Xylella* outbreak if applied across the plant trade.

An additional question inserted in the survey of nurseries and garden centres explored the benefits of avoiding an outbreak. The nurseries' responses indicated an estimated mean benefit of avoiding an outbreak of £416,000 (median £25,000). On average, the results imply that from a nursery perspective around one *Xylella* outbreak in 9 years would need to be avoided at least for the benefits of introducing the best practices to exceed the mean costs, or one outbreak every 7 years avoided for median costs. (Significant distributional issues are involved as nurseries sourcing plant material in the UK can consider those obtaining plants abroad as putting their business at risk, whereas those sourcing plant material abroad can view the prospect of sourcing plant material entirely from UK suppliers as a threat to their business).

Societal perspective

From society's viewpoint, the value of phytosanitary measures in nurseries, in addition to saved nursery costs and maintained nursery benefits, is the expected reduction in the losses that would be entailed if infections spread into forests. The cost to the country of avoiding an outbreak that caused widespread death of the existing trees is the difference between the value of business-as-usual (no infection) minus the value of the trees if the infection arises

and spreads. This cost is calculated for two species, Sitka spruce and oak, for the areas under each species and the current yield class and age class distributions.

For Sitka spruce (SS) the yield classes used range from 6 m³ to 24 m³ per ha per year. Unthinned SS of YC6 and 8 is assumed to be left unreplanted after infection. Unthinned SS of YC10 is replaced by thinned noble fir of YC 10, as is thinned SS of YC12. Thinned SS of YC14–24 is replaced by Douglas fir of one YC lower (i.e. YC12-22). Thinned oak of YC4, 6 and 8 is replaced by commercial broadleaves (sycamore or birch) of one YC higher (i.e. YC6-10). The rotation period for YC6–12 Sitka is assumed to be 50 years (wind constraint) and for YC14–24 is 80 (a compromise between “normal” rotation and rotation designed for carbon benefits). Replacements are assumed to have the same rotation. The assumed rotation length for oak is 120 years, and that of replacements is 100 years.

Areas of each yield and age class are derived from information from Forest Research. Extent of spread, up to 25 years in future, is derived by consideration of disease outbreaks on other tree species.

Economic calculations use the CARBBROD model, created to explore impacts of *Dothistroma*. Forestry costs and revenues are based upon Forestry Commission figures. Land management costs are assumed to be the same for infected and uninfected areas.

Discounting of future costs and benefits is undertaken using the Treasury’s advised schedule (H.M. Treasury, 2018). A “representative” social value of £100 per tonne CO₂ is used. (Use of BEIS carbon prices was initially considered, but decided against as its use can lead to results that appear nonsensical from a tree health policy perspective if carbon released is valued less than subsequent carbon sequestration by trees that are replanted). No account is taken of carbon in soil: the effects are too varied between soil types to be encompassed in an overview evaluation.

For sensitivity analysis, carbon prices were ranged for one central case, Sitka spruce YC14, current age 40 years. The estimated per hectare cost of an outbreak is £30,529 using the £100/tCO₂ price; £17,084 using a £50/tCO₂ price; £57,420 using a £200/tCO₂ price. The result for the whole GB Sitka spruce resource is a loss of about £11,436 million. For the oak resource – less extensive, less extensively affected and involving much lower productivity of carbon and timber – the loss is about £483 million.

The results do not give a full estimate of the effect on society of an outbreak, as they do not include other effects, e.g. on biodiversity, landscape, recreation, water quality and quantity and influence on floods. For replacement of oak by other broadleaved species, these further effects might be judged to be negative. For Sitka spruce the net result is not evident. Unlike carbon in wood, these further effects are highly variable with location, and so are problematical to include in an overview. There is still much dispute about methods of valuation and the results produced.

In cost–benefit analysis, the value of an action is often taken as proportional to value of achieving the action’s goals [preventing a *Phytophthora* outbreak] multiplied by the probability of the action’s achieving its goals [reduction in probability of the outbreak]. Thus for an outbreak followed by spread, actions which reduced the probability of an outbreak from p to $p-0.01$ (i.e. 1% reduction in probability) would have value about £114 million for Sitka spruce and £5 million for oak (or around £120 million if nursery best practice measures reduced the risks of outbreaks on both Sitka and on oak by 1%).

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Work package 3: 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 (traits) but also in relation to environmental and social factors like trade flows and climatic conditions. To facilitate these analyses, we first collated global databases of traits and national and local-scale occurrence of different species.

Global occurrence of *Phytophthoras*

Country- and site- level occurrence data of *Phytophthora* species were 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. Importantly, our database integrates records from the forestry, agricultural and nursery sectors from natural/semi-natural forests, urban spaces, parks, and private and public gardens (Fig. 1). Where available, it also includes the host, substrate and disease symptom for each record, and thus indicates potential behaviour of individual species across a range of environments.

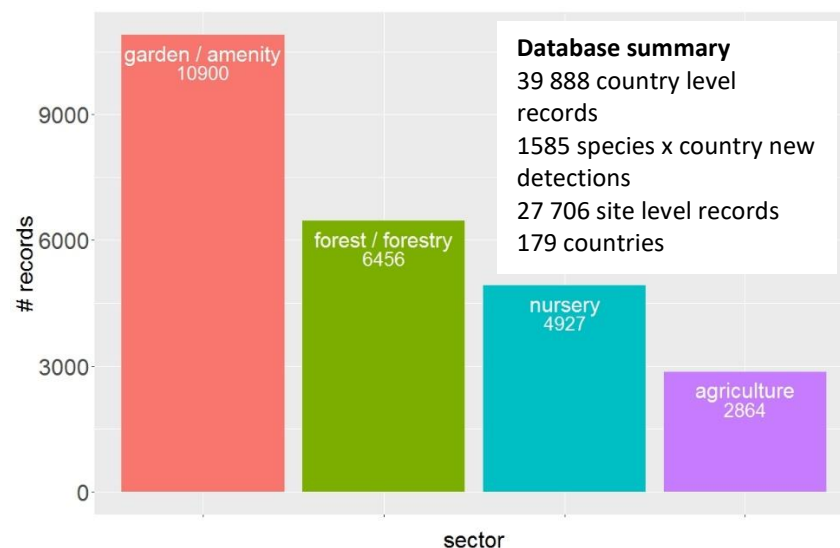


Fig. 1. Number of records in our global occurrence database arising from different sectors

The 27,706 site-level records collated so far span 173 *Phytophthora* species and 179 countries, globally distributed as indicated in Fig. 2. It can be seen that much of the recording effort for *Phytophthora* pathogens has been concentrated in the US, Australia, New Zealand and Europe. At national level, our database contains 39,888 records, equating to 1585 species x country combinations and the year of the earliest record in each country. Despite the effort to accumulate this data, the number of site level data points yielded for individual *Phytophthora* species was low, exceeding 30 records for only 17/179 species and 50 records for only 13/197 species. Data are therefore insufficient for most species to model the environmental niches or landscape-scale drivers of spread. This highlights the disproportionately low global recording effort for pathogen taxa (versus plant host taxa or taxa of conservation concern) and the importance of initiatives to develop centralised, cross-sectoral databases for plant pathogens.

Phytophthora trait database

The biological traits database was compiled through literature review and expertise of project pathologists (Forest Research - Ana Perez Sierra, Anna Harris, Sarah Green, Beatrice Henricot, JHI – David Cooke) together with pathologists in Australia (Murdoch University – Treena Burgess and Giles Hardy), New Zealand (Scion – Peter Scott and Nari Williams). It includes all 179 *Phytophthora* species or sub-species known from forestry, agricultural and horticultural settings as of January 2018. Of these species, 166 are formally described, 13 are provisionally named and 8 are known hybrids. Traits included are related to survival and persistence, reproduction, dispersal, host range, disease symptoms and taxonomy. Most of these traits have historically been collected for use in morphological identification of species; their functional significance is less well understood.

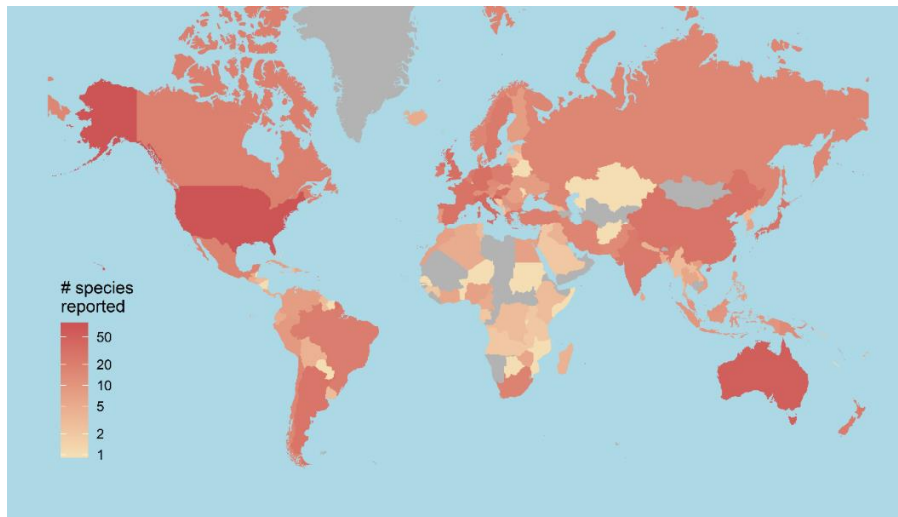


Fig. 2. Number of *Phytophthora* species reported by country.

Functional significance of *Phytophthora* traits: Are some traits under stronger selection during invasion?

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, minimum growth temperatures and oospore wall index were shown to be less phylogenetically conserved across the phylogeny than some of the reproductive traits and to be more labile: they showed greater disparity through time in trait values within clades. This pattern was especially apparent in more recent evolutionary history, which may indicate that these traits are under stronger selection during invasion.

Functional significance of *Phytophthora* traits: Do biological traits explain differences in global extent and host range between *Phytophthora* species?

The global impact (extent and known host range) of *Phytophthora* species has also been modelled against ecological traits (those commonly collected when species are described) and time known to science, accounting for phylogenetic relatedness between species. The ability to cause disease symptoms (in roots or foliage), primary ecological traits and phylogenetic position could partially predict geographical spread and host ranges. Different primary ecological traits increased host ranges (increased by a higher oospore wall index, higher optimum temperature for growth and higher growth rates) versus geographic extent (increased by cold-tolerance) reflecting different mechanisms underpinning *Phytophthora* impacts. Higher oospore wall index could allow greater persistence in the soil which might increase encounter rates with novel hosts. These relationships may help to rank the risks from emerging pathogens, as they are discovered, on the basis of their trait similarity to species

that have already had high impact. A large amount of species-level variation in global impacts was not explained by the traits included in models, highlighting knowledge gaps surrounding the functional traits of plant pathogens which have adaptive value during invasions. We suggest that quantifying sporangial production, oospore production and chlamyospore production at different temperatures may be important for understanding the role of propagule pressure in invasion success. Incorporating genomic traits associated with virulence and host range may also be of value for understanding the evolutionary potential of pathogens during invasions. Addressing these knowledge gaps may improve the predictive power of such horizon scanning frameworks.

What is the role of trade pathways, biological traits and climate matching in driving arrival of *Phytophthora* species in new countries? We aimed to understand (i) how *Phytophthoras* are introduced into new areas, from which key source areas and by which trade pathways and (ii) whether particular traits favour species arrival via such transport pathways and subsequent establishment. We modelled the rate of arrival of *Phytophthora* species per country this century (for 76 countries with at least one documented arrival since 2000 and 66 species with a known source distribution prior to 2000) in relation to (i) live plant trade volumes between source countries for *Phytophthoras* and sink countries (ii) ecological traits and (iii) similarity of climate between source and sink countries (climate matching) accounting for national levels of effort in (iv) pest reporting and (iv) biosecurity. We found that *Phytophthora* arrivals are more likely when sink countries are more connected to source countries via the live plant trade. More-over, species with a broad thermal tolerance range are significantly more likely to arrive with live plants from source countries, possibly due to their ability to tolerate extreme and variable conditions during transport and establishment. Greater climatic distance between exporting and importing countries also reduced the probability of new *Phytophthora* species arrivals. These factors together explained 31.8% of the recorded species arrivals in countries since 2000. Major source countries posing a risk of contributing to UK *Phytophthora* arrivals, due to their connectedness and similar climate included Germany, France, Canada, Netherlands and the United States. Species most likely to arrive from these source countries and known impacts elsewhere in their invaded range are ranked by arrival risk in Table 1. Input data and predictions from these models, were integrated into a prototype Shiny App tool for use by decision makers in nurseries, the landscape sector and plant health (Fig. 3). We plan to seek translation funding to further co-develop this tool with end-users.

Table 1. *Phytophthora* species with the greatest predicted risk of arrival in GB, based on trade connectivity to source countries, climate similarity and species traits

Species	Probability of arrival (lower, upper 95% credible intervals)	Impacts elsewhere
<i>Phytophthora medicaginis</i>	0.36 (0.10, 0.73)	Root rot of alfalfa and chickpea where-ever hosts are grown (reported in 16 countries)
<i>Phytophthora pseudotsugae</i>	0.26 (0.06, 0.60)	Root rot of Douglas Fir in forest nurseries in USA and Argentina
<i>Phytophthora pisi</i>	0.22 (0.05, 0.54)	Root rot of pea and faba bean in Sweden.
<i>Phytophthora chrysanthemi</i>	0.20 (0.04, 0.50)	Root and stem rot on ornamental Chrysanthemum species in Germany and United States.

Which species have established in the wider environment in the UK and does this depend on traits?

We compiled data on where *Phytophthoras* occurred in the UK from several key sources (THDAS, SASA, eDOMERO, SOD inspections, RHS), distinguishing between nursery premises versus establishment in the wider environment. Fig. 4 shows the number of times each species in the UK has been recorded in the UK, identifying whether the records arose from ports of entry or the nursery, garden, forest or agricultural sectors. Comparing the latitudinal extent reached by individual species, we showed that species with lower minimum temperatures for growth established at higher altitudes, consistent with field studies of establishment at higher latitudes elsewhere in Europe. This further highlights the importance of improving measurements of cold-tolerance of transmission and growth processes in *Phytophthora* pathogens and understanding the biological basis for this trait.

International trade flows of live plants species

Pathways for global movement of *Phytophthora*

Trade in live plants is considered the main pathway for the introduction of *Phytophthora* species to new geographic regions. By selecting a country from the menu on the right of the map you can view that country's import volumes of live plants from different source countries. The *Phytophthora* species present in each country can be viewed by clicking on the country. For recently discovered *Phytophthora* species (described after 2014), you can link to trait-based predictions for future global spread from the pop-up species lists for each country.

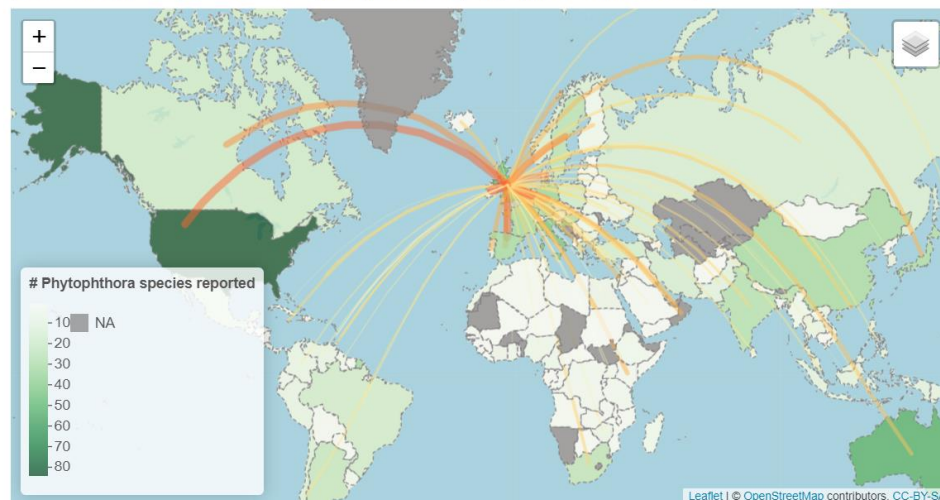


Fig. 3. Example screen shot from the WP3 trade model tool.

For *Phytophthora* species with sufficient data, global niche models can be used to understand which areas of the UK are at risk from establishment.

Models and maps of species environmental niches, indicating their potential distribution, can be developed by matching patterns in species occurrence with patterns in key environmental drivers. All nine species for which there were sufficient global records to make a niche model are already present in the UK. By reserving the UK data from the global niche models for each species and using the wider environment data points as an independent validation dataset, we determine whether environmental niche models can predict the occurrence of species in invaded areas accurately. Models additionally tried to account for unsuitable habitat, dispersal distances and spatial biases in recording effort and used expert-elicitation to identify key environmental drivers, following Chapman et al. 2017.

The fitted models predicted both the global (cross-validated Area Under the Curve [AUC] values exceeding 0.972 for all species) and UK distributions with reasonable accuracy (AUC values for presences in natural and managed outdoor environments across the whole UK exceeding 0.65 for all species except *P. gonapodyides*). Fig. 5 illustrates how the UK distribution of *P. ramorum* in the wider environment was predicted accurately from the global niche model. However, this assessment was hampered by low numbers of UK records for most species (AUC increased in accuracy to approximately 0.75 for species with >100 UK records). Climatic variables had a greater influence than land cover on global distributions,

with the strongest effect being from winter temperature and slightly weaker effects of summer temperature and humidity. Climatic limits inferred from global occurrence data were correlated with species thermal performance documented in the trait database (e.g. highest summer temperature with occurrence correlated to maximum growing temperature with $r = 0.601$), suggesting the models estimated realistic niche tolerances. Our overall conclusion is that global environmental niche models could provide useful risk maps for *Phytophthora* outbreaks in outdoor environments in the UK, but that incomplete and extremely biased recording of *Phytophthora* species globally and in the UK pose major challenges for the modelling.

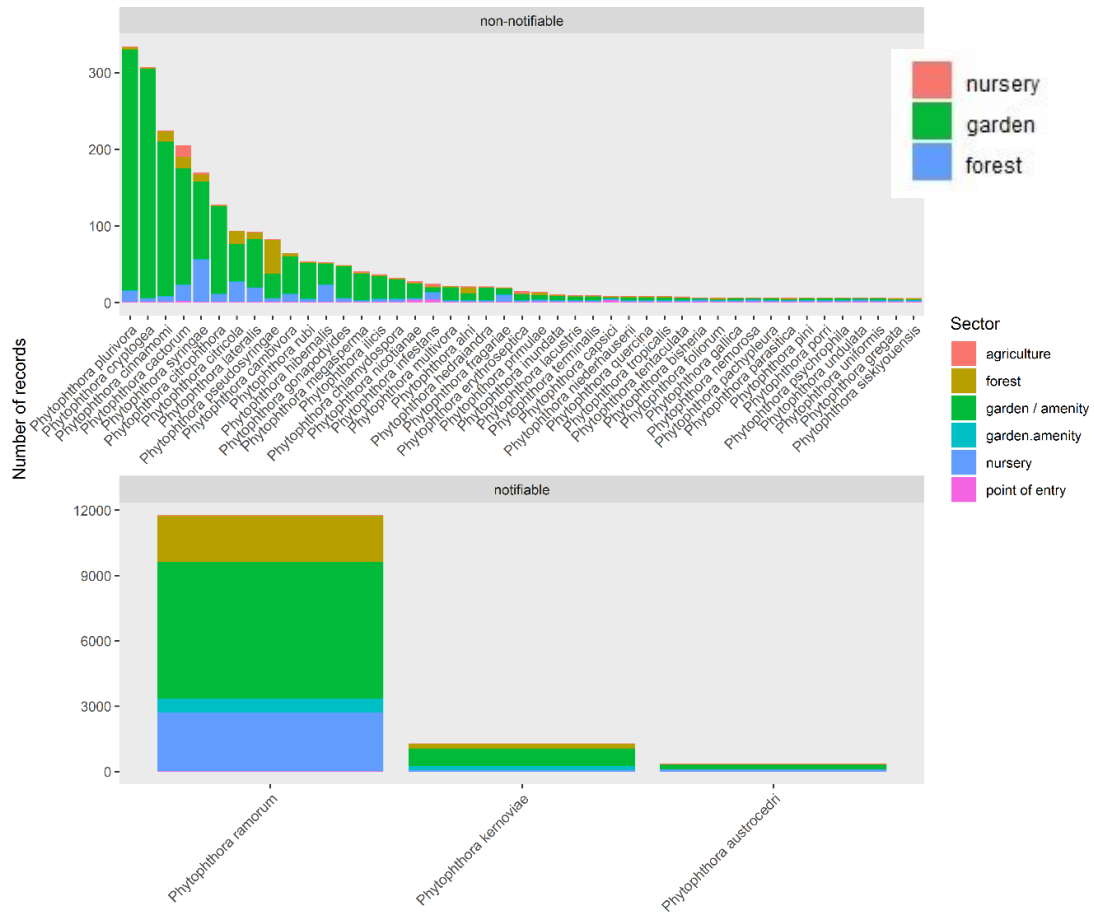


Fig. 4. Number of times each species in the UK has been recorded in the UK, in ports of entry, the nursery, garden, forest or agricultural sectors for notifiable and non-notifiable species.

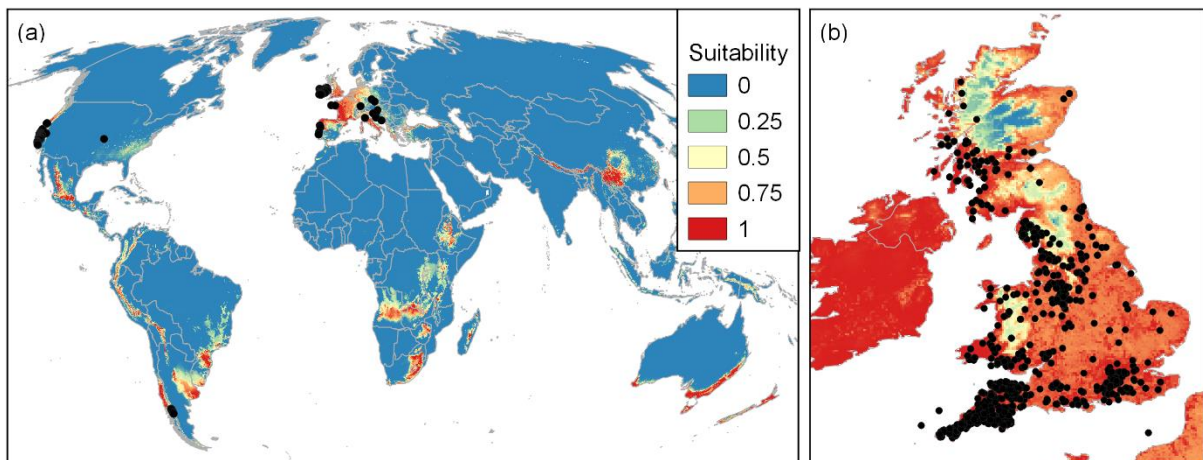


Fig. 5. Map (a) showing the training dataset occurrences and global model predictions and (b) showing the UK prediction and validation occurrences from natural and outdoor managed environments (AUC = 0.747, vs all UK background).

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.

Work package 4: Predicting risk via analysis of *Phytophthora* genome content

Rationale

The aim of this work package was to sequence the genomes of three species of *Phytophthora* that are considered to be less damaging than their close relatives, in order to compare the gene complements of these with more pathogenic species to identify possible differences (e.g. presence/absence of genes or variations in gene sequences) that may be of interest with respect to virulence. The following steps were carried out:

- Genome sequencing of the three species
- Genome assembly, scaffolding and error-correction
- Estimating completeness of genome coverage
- Top-level annotation to identify repetitive elements and gene model prediction
- Comparison of protein sequences translated from gene models with protein sequences from published *Phytophthora* genomes

Selection of target species

P. europaea (a phylogenetic clade 7 species) is closely related to *P. alni*, a cause of lethal disease in *Alnus* species, and *P. cambivora*, a serious pathogen of a range of forest tree species. The strain of *P. europaea* selected for sequencing in this study was originally isolated from the rhizosphere of declining *Quercus* species in France in 1998. Although found associated with declining *Quercus*, *P. europaea* was found to be only weakly aggressive to oak seedlings.

P. foliorum (clade 8) is closely related to *P. lateralis*, a lethal pathogen of Lawson cypress, and *P. ramorum*, a lethal pathogen of larch and some *Quercus* species. The isolate of *P. foliorum* characterised in this study was isolated from Rhododendron in North-West Scotland in 2016.

P. obscura (clade 8) is closely related to *P. austrocedri*, a lethal pathogen of Cupressaceae species. The strain selected for sequencing in this study was isolated in 1994 from soil under *Aesculus hippocastanum*, in Germany.

Genome sequencing and assembly

DNA extracted from all three species was sent to the Exeter Sequencing Service for PacBio long-read sequencing, 2 SMRT cells per species. The use of PacBio for this study, rather than short-read based Illumina sequencing offered two major advantages: the long sequence reads can bridge repetitive regions much more effectively than short reads, leading to an overall much more complete genome assembly, and the error rate, although overall higher than that of Illumina, is unbiased meaning that sequencing errors can often be corrected by increased coverage.

Outcome of sequencing

We obtained a total yield of 13.9 Gbp (giga-base pairs) sequence for *P. europaea*, 12.0 Gbp for *P. foliorum* and 13.0 Gbp for *P. obscura*. Expanded results for the raw sequencing yield are shown in Table 1.

Table 1. Raw PacBio sequencing yield

	<i>P. europaea</i>	<i>P. foliorum</i>	<i>P. obscura</i>
No. reads	1,471,617	1,048,533	1,048,533
Maximum read length (bp)	85,983	80,795	80,795
Average read length (bp)	9,421	11,437	11,437
Total yield (Gbp)	13.9	12.0	13.0

Test assemblies were generated from SMRT cell 1 of each species and these were used to estimate genome sizes prior to full assembly. Sizes obtained were 77 – 95 Mbp (mega-base pairs) for *P. europaea*, 61 – 62 Mbp for *P. foliorum* and 60 – 69 Mbp for *P. obscura*, indicating three moderately sized *Phytophthora* genomes, comparable to *P. ramorum* and *P. sojae* at 65 Mbp and 95 Mbp, respectively.

Following assembly, scaffolding and error-correction, the statistics for the three genomes are shown in Table 2.

Table 2. Genome assembly statistics

	<i>P. europaea</i>	<i>P. foliorum</i>	<i>P. obscura</i>
Estimated genome size (Mbp)	77 – 95	61 – 62	60 – 69
Total assembly length (Mbp)	76.9	59.1	62.2
Scaffolds	39	22	28
N50 (Mbp)	11.0	7.5	6.4
Max scaffold (Mbp)	15.7	12.4	12.7
Mean scaffold (Mbp)	2.0	2.7	2.2
Inserted Ns	44,056	6,799	34,145
% GC content	53.6	53.4	51.9
% Repeat content	35.5	29.0	28.7
Predicted gene models	19,658	19,484	19,441

The small number of scaffolds (sequences making up the genome assembly) and high N50 values (50% of the assembly's sequence is contained within scaffolds of length N or greater) indicate that all three assemblies are highly contiguous, much more so than any of the 27 currently published genome assemblies for other *Phytophthora* species. Of the published genomes, the closest in terms of contiguity is *P. sojae*, containing 82 scaffolds and with N50

of 7.6 Mbp, with many of the published genomes being highly fragmented (e.g. *P. cambivora* contains over 136,000 scaffolds and has N50 of only 2.7 Kbp).

Estimating completeness of coverage

Following genome assembly, overall completeness of coverage of the “gene-space” of the three genomes was estimated using BUSCO. This estimation relies on detecting the presence of a number of “core” genes expected to be present within the genomes of the target organism family. In this case, a set of 234 genes expected to be common to all Alveolata (the larger classification of Protista containing Oomycetes). Table 3 summarises the BUSCO output for the three genome assemblies.

Genes can be classified as complete (single copy or duplicated), fragmented, or missing. BUSCO genes are typically expected to occur as single copies within a genome and where multiple complete copies are identified, this can be an indication of a highly heterozygous genome or a duplicated genome (e.g. *P. palmivora*), or an interspecific hybrid (as in *P. alni* and *P. cambivora*). Genes may be classed as fragmented when only a portion of the gene’s sequence is identified in the assembly.

Table 3. Completeness of coverage summary

	<i>P. europaea</i>	<i>P. foliorum</i>	<i>P. obscura</i>
Complete	229 (97.9%)	229 (97.9%)	231 (98.7%)
Complete, single copy	226 (96.6%)	228 (97.4%)	230 (98.3%)
Complete, duplicated	3 (1.3%)	1 (0.4%)	1 (0.4%)
Fragmented	3 (1.3%)	1 (0.4%)	0 (0.0%)
Missing	2 (0.8%)	4 (1.8%)	3 (1.3%)

The completeness levels of 98 – 99% indicate a very good coverage of the gene-space was achieved for all three species, with the low rate of duplication indicating low heterozygosity, possibly due to all three species being homothallic. A number of the published genomes show a similar level of completeness and low duplication, such as *P. agathadida*, *P. sojiae* and *P. ramorum*. However others, most notably *P. alni*, *P. cambivora* and *P. palmivora* show a much lower completeness and higher rate of duplication, possibly as a result of being duplicated or hybrid species and heavily fragmented assemblies.

Identifying repeat content and gene complement

Analysis of the repeat content of the three assemblies using RepeatModeler and RepeatMasker identified 35.5% of the *P. europaea* genome as being repetitive, 29.0% of *P. foliorum* and 28.7% of *P. obscura*. Comparison of these values with the repeat content of published genome assemblies for other species shows a comparable repeat content to similarly sized genomes such as *P. ramorum*, *P. sojiae* and *P. pisi* with larger genomes containing proportionally more repetitive sequence: up to 67.6% for *P. infestans* with the largest genome assembly of 228.5 Mbp.

Gene models were predicted using Augustus, trained with gene structures derived from closely related species: *P. rubi* (clade 7) gene structures were used to aid with *P. europaea* gene predictions and *P. austrocedri* (clade 8) gene structures were used to aid with *P. foliorum* and *P. obscura* gene predictions. Gene model counts for all three assemblies were very similar: *P. europaea* with 19,658 models, *P. foliorum* with 19,484 and *P. obscura* with 19,441.

Counts of predicted gene models vary greatly among previously published assemblies, ranging from 10,051 for *P. kernoviae* to 75,671 for *P. cambivora* (possibly a result of being such a highly fragmented assembly of a hybrid species). The gene model counts obtained for the three assemblies here are comparable to counts for the similarly-sized assemblies of *P. fragariae* (18,876), *P. rubi* (19,255), *P. pisi* (19,363), *P. pinifolia* (19,687) and *P. capsici*

(19,805) but are considerably more than the much larger *P. infestans* assembly (17,785 genes).

Generally, gene counts are likely to be overestimates as gene prediction algorithms tend to be very “greedy” and may misidentify non-gene features as genes, split a single genes into multiple models, or merge two physically close genes into one. However, the *P. infestans* gene number may be the most accurate, since that species has received a much greater degree of annotation effort and curation; the gene numbers for three new genomes reported here are only a little higher than that estimated for *P. infestans*.

Comparison of gene/protein content between *Phytophthora* species

Protein sequences translated from the gene models predicted for the three species were compared with the protein sequences from the 23 species shown by BUSCO to be over 85% complete (*P. alni*, *P. cambivora*, *P. lateralis* and *P. palmivora* were excluded as they were less than 85% complete). The approach taken here was to perform MCL clustering using Orthofinder to identify orthologous sequences between the protein complements of the 26 species, or subsets of more closely related species to identify core sets of proteins common to all species and presence/absence of proteins from the three species sequenced here.

Comparison of the protein complement of all 26 species identified a total of 54,963 protein groups. However, 33,254 of these are single-protein groups containing only a single sequence from a single species – it is likely that the majority of these sequences will not correspond to genuine gene products, rather reflecting miscalled genes arising from gene prediction tools. The remaining 21,709 clusters contain sequences from more than one species or multiple sequences from a single species. Of these 8,666 clusters contain sequences from at least 20 of the 26 species (>75%) and 5,097 of these can be classed as single-copy orthologues with the clusters containing a single sequence from at least 20 species.

In order to refine this search the Orthofinder analysis was repeated with subsets of *Phytophthora* species:

- **Clade 7:** *P. europaea* was compared with *P. cinnamomi*, *P. rubi*, *P. fragariae*, *P. pisi* and *P. sojae*
- **Clade 8:** *P. foliorum* and *P. obscura* were compared with each other, and with *P. cryptogeta*, *P. austrocedri* and *P. ramorum*

Clade 7 comparisons

A total of 10,584 clusters were found to be common to all five species, with 101 of these being present in the four pathogens but absent from *P. europaea*. Searching the *P. europaea* genome for these 101 sequences using Exonerate showed that sequences from these clusters were indeed present but had not been identified by the gene prediction tool and that 36 of these contained internal stop codons or insertions/deletions that could lead to a truncated or non-functional gene product.

Clade 8 comparisons

A total of 9,798 clusters were common to all five species, with 101 present in the pathogens but not *P. foliorum* and 133 present in the pathogens but not *P. obscura*. A total of 40 clusters were present in the pathogens but absent from both *P. foliorum* and *P. obscura*. Searching for sequences from these 40 clusters in the assemblies for both species showed that sequences from 32 were present in the genomic sequence for both species but had been missed by the gene prediction tool, four were present in the genomic sequence for *P. foliorum* but were disrupted by internal stops or frameshifts arising from indels in *P. obscura*, three were disrupted by internal stops or frameshifts in both *P. foliorum* and *P. obscura* and one was absent from both species

The 36 sequences from *P. europaea* that appear to be disrupted by internal stop codons or frameshifts (when compared with orthologous sequences from the pathogens), and the 8 that are either missing from or disrupted in *P. foliorum* and *P. obscura* may be of interest for further study to determine if they are 1 – genuinely absent or disrupted (and not an artefact of sequencing and assembly) and 2 – of functional importance in the pathogenicity of *Phytophthora* species. Gene content can then be used to predict which newly discovered *Phytophthora* species have the potential to be most damaging.

Work package 5: Development of a collaborative learning platform

The objectives of this work package were to develop a collaborative Learning Platform to facilitate day-to-day management of the project and communication between science, policy, nursery and other practitioners as well as promoting interdisciplinary working and facilitating stakeholder participation and knowledge exchange. It aimed to include important sectors such as plant-buyers and commercial enterprises.

The collaborative Learning Platform was developed through the establishment of both a project Board comprised of work package leaders, project co-ordinator and an industry stakeholder, and an Expert Advisory Panel comprised of external scientists from the UK and overseas, UK policymakers, Plant Health practitioners and industry representatives, whose role was to provide guidance and expert opinion, and to ensure the project maximised its relevance to the different sectors. Monitoring of project progress against the timetable and milestones was achieved through quarterly Board meetings and annual all-project team meetings which rotated around collaborating institutes. Effective communication between science, policy and industry was achieved by promoting interdisciplinary working and facilitating stakeholder participation and knowledge exchange to help build the evidence base to support the development of an accreditation scheme. This was done through use of project management software which enabled sharing of data and reports among the project team as well as the establishment of a Phyto-threats project website which hosted information and reports on team meetings, stakeholder workshops and other engagement activities.

A science-policy-practitioner network (SPPN) was established which included those involved in the commercial sourcing, supply and growing of trees and amenity plants. Other key stakeholders included policy-makers, conservation groups, landscapers and the public and other indirect interest groups. The SPPN aimed to develop and implement a collaborative engagement and knowledge exchange protocol facilitating active interaction with nurseries and other key stakeholders through three annual workshops which are detailed below (year one, scene setting and building relationships; year two, identifying effective management options; year three, securing resilient outcomes – scoping the potential of an accreditation scheme and building a framework for its continued development), and project participation in organised events to enhance understanding and build trust. An example of the latter included annual attendance over three years at the National Plant Show. This is one of the largest plant trade shows in the UK, featuring over 160 exhibitors and receiving around 1400 visitors, representing garden centres and retail nurseries, as well as wholesale nurseries, online and mail order retailers, landscapers, garden designers, consultants and local authorities. The purpose of the Phyto-threats stand and seminar was to raise awareness of the link between the plant trade and *Phytophthora* outbreaks in the wider environment, and to inform growers of the role they can play in helping to reduce the spread of *Phytophthora* through best management practice. It was also an opportunity for different members of the project team to gain a greater understanding of what drives the plant trade, and how trade networks work across Europe and beyond.

Stakeholder workshops enabled tracking of wider attitudes towards accreditation

Three multi-stakeholder workshops were held over the course of the project, each with c45 attendees representing nursery managers, landscape architects, garden designers, Plant

Health inspectors, foresters, policy makers, academics and others. The first workshop on 'Improving nursery resilience against threats from *Phytophthora*' was held in 2016. The objectives of this workshop were to introduce the scientific aims of the Phyto-threats project, develop collaborative networks across individuals and groups with an interest in working towards collective best practice in nurseries and to share lessons and experiences around the challenges and opportunities of managing disease threats. The workshop included perspectives on Plant Health from a Defra policymaker, two forest nursery managers and a trader followed by a panel discussion to elaborate on some of the challenges faced by different sectors. The use of correct tools for disease detection was said to be important, as was the closure of high-risk pathways in terms of transmission of pests and diseases. The value that plants add to the environment was believed to be highly under-valued by society, which facilitates the desire for cheaper products and imports. Reducing bureaucracy was considered key to making changes in the sector as well as seeking opportunities to increase the quality and quantity of UK plant production. Feedback from an afternoon workshop session suggested that accreditation might need to be tailored for different stakeholders but possibly under a single umbrella. A scheme could include different levels of standards to encourage businesses to improve their practices, but it should have minimal bureaucracy and there would need to be consumer support for any scheme to provide an incentive for nurseries to be involved. Decisions over what the scheme should include would best be made by representatives from a mix of sectors. Brexit might provide an opportunity for the UK to promote its own best management practices and to have more control over quality of imports. There are practices (e.g. mail orders, TV garden shows, illegal trade in plants) that could undermine an accreditation scheme, so the scheme would require consumers to be informed and supportive.

The second workshop in 2017, entitled 'Reducing *Phytophthora* in trade and designing effective accreditation', aimed to share science findings to date from the project which might help underpin accreditation, understand existing UK assurance schemes and how they might be supported, and generate ideas for how an accreditation scheme should work in order to be effective. Although attendance was similar to the previous year's workshop it was notable how the overall appetite for accreditation had increased across the range of stakeholders. The key outcomes of the workshop are summarised as follows;

- A single, all-encompassing UK accreditation scheme is preferred.
- Accreditation needs to cover the entire supply chain if it is to have impact.
- Demand for accredited products should be ensured to incentivise uptake in the scheme.
- Accreditation could also be incentivised by allowing access to grant funding, and through financial reimbursement when contracts to supply accredited plants are cancelled/altered.
- It is necessary to raise public awareness about the need and benefits of accreditation through highly visible campaigns and signage, including a recognisable logo.
- Training for growers and others in the sector should be integral to accreditation so that practices continue to improve.
- For a scheme to be respected and to generate improvement it must be effectively policed.

In 2019 a final stakeholder workshop was held to share the latest science findings from the project, to provide an interactive demonstration of science outcomes and tools and to explore how the project's science outcomes can best be used to support the continued development of accreditation and Plant Health policy. An update was provided on the 'Plant Healthy Assurance Scheme' being developed by the Horticultural Trades Association together with industry and Defra. It was clear that the project's work on analysing *Phytophthora* diversity in nurseries has identified hosts and practices of high biosecurity risk that can be targeted in the Plant Health Management Standard which forms the basis of the 'Plant Healthy' scheme. The work on assessing the feasibility of accreditation from consumer and nursery perspectives will

assist in securing uptake and consumer support for accreditation. Continued liaison with those developing the Plant Healthy Assurance Scheme as well as with policymakers and practitioners over predictive models will ensure that project outcomes are used to support pest risk analyses and the risk register.

6. Knowledge exchange

(a) Scientific research outputs, including presentations at conferences, meetings attended etc.

Publications

Barwell, L. 2018. UK project mapping global *Phytophthora* and a short questionnaire on the role of tourism, International Society of Plant Pathology newsletter <http://www.isppweb.org/newsletters/mar18.html>

Dunn, M., Marzano, M., Forster, J. 2019. Buying better biosecurity: Plant buying behaviour and the implications for an accreditation scheme in the horticultural sector. *Plants, People, Planet* 00:1–10. <https://doi.org/10.1002/ppp3.10076>

Dunn, M., Marzano, M., Forster. Summary Report: Attitudes and behaviours of the UK's plant-buying public. Forest Research.

Dunn, M., Marzano, M. Summary report: Nurseries and garden centres survey on attitudes towards biosecurity and accreditation. Forest Research.

Green, S. 2016. Fighting *Phytophthoras*. *The Arb Magazine*. Issue 175; 36.

Green, S., Dunn, M., Marzano, M., Forster, J. 2018. Fighting *Phytophthora* - The Phyto-threats project: The UK gets serious in the battle against *Phytophthora*. New Zealand: Commercial Horticulture.

Green, S., Riddell, C.E., Frederickson-Matika, D., Armstrong, A.C., Elliott, M., Forster, J., Hedley, P.E., Morris, J., Thorpe, P., Cooke, D.E.L., Sharp, P.M., Pritchard, L. 2020. Diversity of woody host-infecting *Phytophthora* species in public parks and botanic gardens as revealed by metabarcoding, and opportunities for mitigation through best practice. *Sibbaldia* 18, 67-82.

Riddell, C.E., Frederickson-Matika, D., Armstrong, A.C., Elliot, M., Forster, J., Hedley, P.E., Morris, J., Thorpe, P., Cooke, D.E.L., Pritchard, P., Sharp, P.M., & Green, S. (2019). Metabarcoding reveals a high diversity of woody host-associated *Phytophthora* spp. in soils at public gardens and amenity woodlands in Britain. *PeerJ*, DOI 10.7717/peerj.6931

Conferences attended (in chronological order)

28th USDA Interagency Research Forum on Invasive Species held in Annapolis, Maryland, USA, January 10-13, 2017. Presentation by Sarah Green (FR) entitled 'Tackling emerging forest *Phytophthoras* in the UK: Mitigating risk of new introductions and managing diseased landscapes for the future'.

8th Meeting of the International Union of Forest Research Organisations Working Party (IUFRO) 7.02.09, *Phytophthora* in forests and natural ecosystems. Sapa, Vietnam 18-25th March 2017. Presentations by Sarah Green (FR) and David Cooke (JHI) covering aspects of the Phyto-threats project.

Cooke, D. 'eDNA and Plant Pathogen Metabarcoding' presentation at SNH 'Sharing Good Practice' event on "The Use of Technology to gather data about the natural environment", June 2017.

Louise J Barwell, Ana Perez-Sierra, Beatrice Henricot, Daniel S Chapman, Sarah Green, David Cooke and Bethan V Purse. Do traits or trait syndromes predict the global impact of plant pathogens in the genus *Phytophthora*? BES symposium on macroecology of alien species (24th – 26th July 2017) (<https://www.britishecologicalsociety.org/events/bes-symposium-2017-macroecology-alien-species-patterns-drivers-consequences-global-biotic-exchange/>).

Mariella Marzano: presentation on social research at IUFRO conference, Freiberg, September 2017

Sarah Green: poster presentation on the Phyto-threats project at IUFRO conference, Freiberg, September 2017

Mike Dunn: presentation on social research at Forest Research coffee break seminar in November 2017

Green, S. Hunting for *Phytophthora* in British plant nurseries; what do we see?. Presentation at a research update seminar held at Forest Research, Alice Holt, January 2018.

Mariella Marzano: presentation on social research at Lincoln University, New Zealand, March 2018

Mariella Marzano: presentation on social research at ISSRM conference, Utah, June 2018.

Green, S. *Phytophthora* in plant nursery settings, engaging with stakeholders and early initiatives on accreditation in the UK. *Phytophthora*, *Pythium*, downy mildews and related genera 2018; Sixth International Workshop; Oomycetes in the era of Plant Health in a global economy, Boston, Massachusetts, USA, July 28th 2018.

White, R.M., Young, J., Marzano, M., and Leahy, S. (2018) Prioritising stakeholder engagement for forest conservation during austerity. Pathways conference presentation, Goslar, Germany 16-19 September 2018

UK DNA Working group Conference 26th-27th November 2018, London. Sarah Green, David Cooke and Leighton Pritchard all presented talks focused on the Phyto-threats project.

PacBio Day Exeter (2019): Presentation by Ewan Mollison titled "Highly contiguous genome assemblies for three *Phytophthora* species generated from PacBio sequencing" at Exeter University PacBio Symposium 6th June 2019

Cooke D. 2019. *Phytophthora*: a European view of the threat. Presentation at National *Phytophthora* preparedness workshop. 5-6 June 2019 St. Kilda, Melbourne, Australia.

Mollison, E.; Sharp, P.; Riddell, C.; Cooke, D.; Pritchard, L.; Thorpe, P.; Jeffries, A.; Green, S. (2019): Poster presented by Ewan Mollison titled "Highly contiguous genome assemblies for three *Phytophthora* species generated from PacBio sequencing" at 20th Oomycete Molecular Genetics Network, 10th – 12th July 2019, Scottish Association for Marine Science, Oban

Cooke DEL, Randall E, Clark B, Thorpe P, Cock PJ, Pritchard L, Pettitt T, Frederickson-Matika D, Green S. 2019. *Phytophthora* eDNA barcoding for natural ecosystem surveillance.

Proceedings of the Oomycete Molecular Genetics Network, 10-12 July 2019. SAMS, Oban, Scotland.

Pritchard P, Cock PJA, Thorpe P, Randall R, Green S, Cooke DEL, 2019. Metabarcoding diagnostics of *Phytophthora* species in environmental samples. Poster at XVIII International Congress on Molecular Plant-Microbe Interactions, July 14-18, 2019 Glasgow, UK

Sarah Green and Mariella Marzano were both invited speakers on the Phyto-threats project at the New Zealand Plant Protection Society *Phytophthora* symposium August 2019.

Mike Dunn presented on the Phyto-threats project at the IUFRO World Congress, Curitiba, Brazil, 29th Sept-5th Oct 2019.

9th Meeting of the International Union of Forest Research Organisations Working Party (IUFRO) 7.02.09, *Phytophthora* in forests and natural ecosystems. 17-25 October 2019, La Maddalena, Sardinia, Italy. Presentations by Sarah Green, David Cooke and Louise Barwell covering work done as part of the Phyto-threats project.

UK DNA Working group Conference 27th-28th January 2020, London. Sarah Green presented a talk entitled 'A metabarcoding analysis of *Phytophthora* communities in UK plant nurseries and links to management practice'.

Sarah Green and Mariella Marzano hosted a workshop for the Scottish Plant Health Centre scientific response team involving a presentation on Phyto-threats project outcomes and discussion on 'Is our current plant health biosecurity framework fit for purpose?'. February 2020.

- (b) Interactions with stakeholders and/or potential users of the research outputs. This can include inputs to policy development, advice to stakeholders or information leading to changes in practices.

Dissemination of nursery results to date to all project partner nurseries at intervals throughout the project.

Phyto-threats project stand and seminar slot at the National Plant Show in June 2017, 2018 and 2019.

Three multi-stakeholder workshops held in October 2016 and 2017 and in November 2019 (detailed as part of WP5 in the scientific report).

Project team attended the THAPBI dissemination event 7th Feb 2018.

Mariella Marzano presented on the Phyto-threats consumer survey (biosecurity practices and appetite for accreditation) at APHA Multiples day, 2018.

Cooke DEL, 2018. *Phytophthora* diseases of potatoes, fruit and trees and other crops. Presentation at British Crop Protection Council Diseases Review 2018 – 'Problems in high value crops', NIAB, Cambridge 12 Oct 2018.

Green, S. Presentation to the Society of Garden Designers, Scotland. Edinburgh, December 2018.

Sarah Green and Mariella Marzano met with the Horticultural Manager of the Horticultural Trades Association to discuss how the Phyto-threats project outcomes can support the Plant Health Assurance Scheme, Feb 2019.

David Cooke manned the James Hutton Institute stand at the Royal Highland Show in Edinburgh 20-21 July 2019 discussing the Phyto-threats project's role in forest and natural ecosystem health.

Green, S. Presentation on Phyto-threats nursery sampling and outcomes to the Landscape Institute's North East Branch. February 2020.

(c) Public engagement activities or activities aimed at a general audience.

Publicly available reports on all Phyto-threats project team meetings and stakeholder events including links to presentations; <https://www.forestresearch.gov.uk/research/global-threats-from-phytophthora-spp/phytothreats-meetings-and-events/>

(d) Actions or planned actions taken to promote the flow of knowledge, with other researchers, stakeholders or the public.

A new, two-year EUPHRESKO project (Early detection of *Phytophthora* in nurseries and traded plants of EU and third countries) involving twelve partner countries will ring test the nursery sampling and metabarcoding method developed as part of Phyto-threats. This project takes core biological and social science elements developed as part of Phyto-threats out into the international sphere to develop a co-ordinated strategy for the early detection of *Phytophthora* pathogens in plant nurseries and traded plants for planting across EU and third countries. This new project with its much wider geographical focus will inform international best practice, complement phytosanitary regulation and enhance engagement on Plant Health with traders operating in different countries.

Phyto-threats results and knowledge will feed into three new Plant Health projects funded through the Scottish Plant Health Centre:

- Assessment of large-scale plant biosecurity risks to Scotland from non-specialist and online horticultural sales
- Assessment of large-scale plant biosecurity risks to Scotland from large scale plantings for landscaping and infra-structure projects
- Assessment of large-scale plant biosecurity risks to Scotland from large scale tree plantings for environmental benefits

These projects bring together the Phyto-threats team with new partners from RBGE, SRUC and St Andrews Botanic Gardens and also a broad range of advisors from new sectors such as the construction industry.

7. Declaration

I declare that the information I have given is correct to the best of my knowledge and belief.

Name Sarah Green

Date 31/03/20

Position held Senior Forest Pathologist

8. Consent for the final report to be published

In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible. Therefore it is Defra's intention to publish this form.

Please confirm your agreement to do so. **YES** **NO**

- **ACCESS TO INFORMATION**

The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

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