

Report on Phyto-threats project team meeting October 5th 2016

Held at

APHA headquarters, Sand Hutton, York YO41 1LZ

This event provided an opportunity for the project team to meet in its entirety, including new team members Peter Thorpe (JHI), Louise Barwell (CEH) and Debbie Frederickson-Matika (FR), together with an overseas member of the Expert Advisory Panel (EAP) Susan Frankel (US Forest Service), and other UK-based EAP members David Slawson (OPAL), John Morgan (FC), Richard MacIntosh (Defra) and Kelvin Hughes (APHA). The objectives of the meeting were to share research updates for each workpackage (WP) in the morning, and to promote team-wide understanding of the plant trade and nursery sampling challenges through a visit to Johnsons of Whixley nursery in the afternoon.

09.30-10.20: WP1 Presentation - David Cooke (JHI) and Leighton Pritchard (JHI)

David Cooke outlined WP1 objectives as using metabarcoding to analyse *Phytophthora* community structure in nurseries and associated ecosystems with the aim of informing disease management and best practice and to model *Phytophthora* communities. David reviewed WP1 sampling methods and reiterated the need to extend networks for nursery sampling. David is involved in a number of related projects that can feed into this one, including a THAPBI Phase 2 project on Early Detection and a Scottish Government funded project looking at *Phytophthora* diversity in different ecosystems in Scotland. Data on *Phytophthora* diversity in a range of wider ecosystems can be modelled together with data on nursery *Phytophthora* diversity. David then gave an overview of the metabarcoding approach to be used from sampling in nurseries through to analyses of *Phytophthoras* present in each sample.

So far the WP1 team has taken five sets of samples from four nurseries (one in England and three in Scotland). This equates to 316 samples in total, mostly in triplicate. Of these, 228 samples have been PCR tested for *Phytophthora* to date; only 40 were positive. There were 106 samples from water filters and also samples from the buffer solution in which filters are stored immediately after sampling. Also, 96 samples consisted of plant roots collected from 35 different hosts. Host plants were selected for sampling based on either being known *Phytophthora* hosts, showing *Phytophthora*-like symptoms, or because the nursery managers requested that they be tested. There were some practical challenges encountered during this first round of sampling, including the length of time needed to filter water samples, cleaning the pumping equipment between samples, and problems when trying to filter water runoff from plants in pots. An efficient and effective nursery sampling methodology is essential.

In terms of the PCR data, there have been issues with reproducibility of results, ie replicates from the same batch giving different results. The next stage is to run a lot of controls to test the DNA extraction and PCR method and to develop a good positive PCR control.

David summed up with lessons learned to date; sampling is time consuming and they need to allow sufficient time to sample premises, particularly the larger ones. There is also no 'one size fits all' approach to sampling as different operations have different practices and there is great host diversity across sites. In general nurseries are working to a high standard and staff have been very supportive.

Future plans for WP1 will be to sample more nurseries in Scotland in October and England/Wales in November. David aims to complete the first Illumina plate with controls by November and send sampling protocols for the broad scale nursery survey to APHA and SASA for comments and completion.

Leighton Pritchard then spoke on the bioinformatics pipeline, which they have been developing at JHI for the metabarcoding, and in particular the issues with ITS1 classification. Essentially this project will be identifying and profiling *Phytophthora* species in nurseries using sequence analyses of the ITS region. However ITS sequences can be highly variable, even within species. For example *P. infestans* has 133 ITS copies, *P. cinnamomi* has 2 and *P. cambivora* has 12. Basically there are plenty of ITS regions in a *Phytophthora* genome !. It is important to optimise the ITS1 clustering process within the pipeline so that species are identified correctly. There is also the need for a better database of *Phytophthora* ITS sequences – this is something the JHI team will be working on.

The talks were followed by a discussion session, summarised as follows;

- Kelvin Hughes (APHA) asked about the issues with false positives that sometimes crop up in the bioinformatics pipeline. This was answered by Peter Thorpe (JHI) who said that this problem was due to ITS similarities between certain species. The project needs a reference database that really works. Some other research groups are looking at the *cox* region for *Phytophthora* ID. David Cooke countered that there are a sufficient number of species (ie most!) that can be resolved with ITS1. Kelvin Hughes agreed that it was better to have false positives than negatives. Peter Thorpe made the point that read number is important, ie very low reads for a species (1 or 2) might be due to sequence error and could be discounted, although Beatrice Henricot (FR) commented that in one of her other metabarcoding projects looking at *Phytophthora* diversity in public garden sites, she was able to bait *P. ramorum* into culture from a sampling location yielding only 2 sequence reads of the species.
- There then followed a general discussion of complementary metabarcoding projects that team members are working on. All agreed that there is a need for a really good and comprehensive manually curated ITS1 database for *Phytophthora* which should include all ITS1 variation within a species.

10.20-11.10: WP2 Presentation – Mariella Marzano (FR) and Gregory Valatin (FR)

Mariella started her presentation with a review of work planned for the social and economic research. This package will cover the feasibility analyses and development of 'best practice' criteria for a nursery accreditation scheme. There are three key parts: social-the applicability of best practice criteria; cost-benefit analysis; the development of best practice criteria to underpin guidelines for an accreditation scheme.

Stakeholder mapping: the WP2 team now have a database of over 100 organisations and individuals but need to fill in more detail. The team needs to seek out the networks and communications among stakeholder groups. The social scientists will also join the nursery sampling teams whenever possible.

Context building: this will involve nursery visits with the sampling team, interviews with members of the EAP and interviews with participating nurseries from January 2017.

Mariella posed the question to the group 'which questions to ask when interviewing nurseries?' Also, which stakeholder events should they target for attendance and are there opportunities for focus groups?.

- Tim Pettitt (University of Worcester) went to the National Plant Show and the Four Oaks show earlier this year and distributed leaflets about the project.
- David Slawson (OPAL) suggested that Mariella should use user groups and industry groups as points of contact.
- Mariella's group needs to interview the public about what they'd be willing to pay for – ie the public needs to be convinced about the benefits of nursery accreditation.

Consumer survey: The WP2 team will outsource a contractor to undertake a consumer survey mainly targeting the plant-buying public later this FY – this will be an online survey of 3-4000 people. Mariella outlined some of the proposed survey questions in her presentation and stated the potential benefits of using networks and memberships, for example Dobbies Members, the RHS and National Trust as well.

- David Slawson pointed out that landscapers are the most important consumers in terms of impact and should be included in the survey. Mariella said that landscapers were the hardest to reach and she requested help with names of useful contacts in the landscaping, retail and wholesale sectors.
- It was also mentioned that the Horticultural Trades Association are looking at accreditation, and the Defra Tree Health Policy Group have an interest in this. The Woodland trust has accreditation scheme plans and is in the process of setting this up for their members.
- Susan Frankel (US Forest Service) commented on the *Phytophthora* outbreak problems encountered in California from sectors they hadn't considered before, for example flood defence schemes and major construction projects involving large-scale plantings. Susan suggested that utility companies should be included in the stakeholder surveys. Sarah green (FR) offered to provide contacts for Cumbrian Water.
- Gregory Valatin gave an overview of his plans for cost-benefit analyses of nursery accreditation options and defined the objectives of the economics analyses as cost-benefit options from the nursery perspective as well as from the perspective of society as a whole. The analyses would take into account the implications of 'doing nothing' with the main expected impacts on different stakeholders (best scenario, worse scenario) as a result of disease outbreaks. They also need to take into account costs imposed on other parts of society due to *Phytophthora* outbreaks. Some baseline information is needed such as size (£Millions/year) of markets for tree seedlings and how this is likely to change with the introduction of new pathogen strains, which plants would be most susceptible without the introduction of a scheme

and which 'best practice' scenarios to focus on. For each best practice scenario they need to determine in discussion with nursery owners and plant buyers which risks are reduced and by how much, the relationship between uptake and reduction of risk, and how to frame estimated risks with reduction impacts. Gregory is also considering the potential of an MSc student for an in-depth analysis on consumer willingness to pay for accreditation. Beth Purse (CEH) asked whether anyone had looked at how public opinion on willingness to pay changes following publicised outbreaks. Gregory explained that this is known as 'behavioural economics' and in the context of this project would be about understanding how cognitive aspects affect willingness to pay scenarios.

- David Slawson (OPAL) commented that a British Standard for plant production from nursery to landscape has been published, but costs £234 for a copy [BS 8545:2014 Trees: from nursery to independence in the landscape – Recommendations].
- In general the project team need to be aware of all the other initiatives in terms of accreditation schemes, for example Richard MacIntosh (Defra) mentioned the Woodland Trust Accreditation Scheme.
- Gregory Valatin asked what the expected uptake of an accreditation scheme might be, pointing out that if low the overall impact would be small.
- There was also a short discussion on a 2016 paper by Whittet et al in Land Use Policy journal "Supplying trees in an era of environmental uncertainty: Identifying challenges faced by the forest nursery sector in Great Britain"
<http://nora.nerc.ac.uk/514159/1/N514159JA.pdf>
This study involved a survey of forest nurseries in Britain in relation to supply of locally sourced seed and domestically produced planting stock for native woodland and hedging markets. It will be important to follow up on which nurseries were surveyed to avoid overlap.
- The discussion ended with the comment that consumers' willingness to pay will be affected by the availability of cheap plant imports.

11.30-12.20: WP3 Presentation – Beth Purse (CEH), Dan Chapman (CEH) and Louise Barwell (CEH)

Beth outlined the approaches for WP3 objectives 1) assessing risk of introduction of *Phytophthoras* to the UK via trade and recreational spread, 2) risk of establishment and spread following arrival, and 3) scoping knowledge gaps to try to predict likely future introductions. The WP team will identify and rank *Phytophthora* threats to the UK and link invasiveness to different traits.

Dan will focus his work on objective 1, estimating risks of *Phytophthora* introduction to the UK through identifying important trade and recreational pathways and analyses using quantitative models. He will model invasion by transport networks using volume of imports and origin of imports as two key parameters, and cited a paper [Seebens *et al.* 2013. *The risk of marine bioinvasion caused by global shipping. Ecol. Lett*] describing analyses of important shipping routes and risk of marine bioinvasions at different ports. The authors identified that distant sources have more non-native species, short journeys are more survivable and similar local environments promote establishment. Therefore, one of the outcomes of the paper was the hypothesis that ports at intermediate distance from source were most likely to be invaded. There also tends to be a 20 year time lag between

globalisation of a country and invasion of new locations by organisms from that country. Dan used recent work on ragweed invasion as an example of approaches he will use in this project, and there have been other projects looking at trade networks and plant pest invasions that he can draw on. For *Phytophthora* the analysis will focus on ~40 species for which there are details on global distribution, looking at different movement networks and characteristics of importing countries. Arrival risks for different species will be modelled.

- Susan Frankel (US Forest Service) commented that when looking at risk you need to ask what is the main damage concern?. Is it natural vegetation?. A short discussion followed on disease impact risks.
- David Cooke (JHI) was interested in the 20 year lag between globalisation of countries and *Phytophthora* introductions, and suggested that it would be good to look at historical data on *Phytophthora* introductions.
- David Slawson (OPAL) also stressed the importance of understanding substrate in terms of introduction risk, ie soil vs plant and suggested all databases should be consulted for data on arrival in each country.
- Louise Barwell (CEH) has been compiling information of which pathogens are quarantine regulated in each country.
- Beth Purse (CEH) said that they can try to model changes in trade patterns as well, for example changes due to Brexit. They will also look at spread of *Phytophthoras* across Europe and link to traits.

Beth, on 3.2., described the approaches to be used for fine-scale mapping pathogen niches and selecting focal species for modelling. The aim is to determine which species have greatest capacity for establishment and spread, leading to a map of UK areas most at risk. Over 1-2 yrs they will evaluate drivers and models for establishment patterns to see if UK distribution of *Phytophthoras* can be reproduced from global occurrences and if there is any evidence of niche shifts. Focal species will include those already here e.g. *P. ramorum*, *P. rubi*, *P. austrocedri* and species yet to arrive such as *P. acerina*, *P. pinifolia*, *P. pluvialis*. They have already acquired data on *Phytophthora* occurrence in the UK from a number of plant health advisory bodies and surveys, for example from the RHS. Beth also gave a breakdown of *Phytophthora* occurrence literature including the global niche model already available for *P. cinnamomi*.

Louise Barwell (CEH) spoke about the *Phytophthora* global traits database that the project team pathologists have been compiling for objective 2 (ie Ana Perez-Sierra (FR), Beatrice Henricot (FR) and Anna Harris (FR)). There are 169 species currently in the database (with new species added as they are described) and data on biological traits are complete for 90 species. The team aim to have the traits data completed for all species by December this year.

Louise presented on the ecological traits by which species should be categorized in any models, including traits which affect long term persistence (ie do they produce oospores, chlamydospores or both), or propensity for aerial or aquatic dispersal (ie are sporangia caducous-spore breaks off and can be dispersed- or non-caducous). Mating system of a species is also an important characteristic to consider. Louise also asked which value is most important when considering optimal temperatures for growth, ie min, max, or mean? (in terms of matching to the UK climate). Louise also asked how best to categorise host specificity.

For the traits hypothesis, those traits linked to invasiveness will be i) a long distance dispersal mechanism, ii) thick walled survival structures, iii) broad temperature tolerance, iv) wide host spectrum. Other hypotheses will be formed around substrates and disease symptoms, mating system and tolerance to soil pH (broad vs narrow).

- A skype meeting will be held between modellers and pathologists on the team once the traits database is complete later this year to discuss further which traits to focus on, and how traits can be best categorized for the models.

Beth went on to speak about objective 3 – horizon scanning for knowledge gaps. This will involve understanding how plant products move around the globe as people move around and requires input from the social scientists. In general WP3 will have potential policy impact, for example in updating the UK Plant Health Risk Register. Beth outlined the plan for the next few months, which will be to finalise the traits database, collate global occurrence data with help from colleagues and collaborators overseas and to test trade models.

12.20-13.00: WP4 Presentation – Sarah Green (FR) and Leighton Pritchard (JHI)

Sarah (standing in for Paul Sharp, University of Edinburgh, who was unable to attend the meeting) gave a brief overview of the objectives of WP4 which aims to predict risk via analysis of *Phytophthora* genome evolution. This will enable a better understanding of the genetic mechanisms by which Phytophthoras can infect woody hosts, adapt to new hosts, and the extent to which they can acquire new genes through hybridizations or horizontal gene transfer. WP4 does not start until April 2017, however the project has funding to target sequence three *Phytophthora* species (to be completed this year) and the focus of Sarah's presentation was how to select these species.

The WP team will take the approach suggested by Paul Sharp which is to look at the *Phytophthora* phylogeny, classify species according to important phenotypes ie whether they infect woody or non-woody hosts, highly pathogenic vs weakly pathogenic etc and mark on the phylogeny those species for which genome data is already available. Three species will be selected which contrast best with existing genomes. This approach is not easy for *Phytophthoras*, however, because so little is known about the true phenotypes of so many.

Sarah ran through the *Phytophthora* phylogeny pointing out the 25 species that have already had, or are about to have, their genomes sequenced (with data publicly available). All clades except clade 9 have representative genome sequences to date. There are 11 genomes from pathogens that infect the woody parts of woody hosts, 5 genomes from pathogens that infect foliage of woody hosts and 9 genomes from pathogens infecting mainly herbaceous hosts. Species can also be split into those with broad vs narrow host ranges, aerial vs root infecting, however there are no currently available genomes for Phytophthoras which are regarded (as far as current knowledge stands) as weak pathogens.

Sarah's presentation finished with suggestions of four 'weakly pathogenic' *Phytophthora* species which could be sequenced to contrast with a closely related aggressive pathogen of woody hosts – these were *P. europaea*, *P. foliorum*, *P. multivesiculata* or *P. obscura*. Further discussion will be held among the WP4 team before sending isolates for sequencing by end of this year.

Leighton presented an overview of currently available *Phytophthora* genomes – of which there are 59 assemblies representing 21 species. There are 133 ‘bioprojects’ at NCBI ie statements of intent to sequence Phytophthoras at the species level or higher. There are also 630 short read sets for *Phytophthora* at NCBI. In terms of genome annotation there are 16 assemblies on the Ensembl Protists website. Of ongoing projects that we know about, the Grunwald lab is sequencing *P. syringae* and *P. ramorum* and the Mayr-Stihl-Stiftung lab sequencing *P. quercina* and *P. plurivora*. There are also likely to be others we don’t know about.

Leighton ran through other genomic points of interest, for example that the current assemblies are very fragmented (many contigs) and that the genomes vary greatly in size. For one of the larger genomes (*P. infestans*) previous studies have shown evidence for genome expansion (ie proliferation of repetitive DNA regions) which feeds into the ‘two speed genome’ theory whereby large parts of the genome consist of few ‘effector’ genes (ie genes involved in resistance interactions with plants) dispersed in regions rich in repetitive DNA. Thus the location of effector genes in such regions can facilitate rapid adaptive evolution.

- There was no time for further discussion as Leighton had to give his presentation while eating lunch (or rather watching everyone else eat lunch!). Sarah made a note to allocate 1.5 hr per WP at the next project team meeting to enable longer discussion sessions!.

13.30-17:00: Visit to Johnsons of Whixley plant nursery

After lunch the project team hopped into a minibus driven by David Cooke for the 30 min trip to Johnsons of Whixley, one of the largest wholesale plant nurseries in the country, supplying mainly the amenity sector. The team was hosted for the afternoon by Ian Nelson, production manager, who provided a lively, honest and energetic tour of facilities and account of business practices as well as responding to the many questions asked by the project team. This was a very useful experience for all members of the project team, particularly those not directly working with nurseries, who finished the visit with a much greater understanding of what is driving the trade and associated management practices.

List of Participants

Contact

Jane Barbrook
Louise Barwell
Daniel Chapman
David Cooke
Mike Dunn
Susan Frankel
Debbie Frederickson-Matika
Sarah Green
Béatrice Henricot
Kelvin Hughes
Mariella Marzano
Richard McIntosh
John Morgan
Ana Pérez-Sierra
Tim Pettitt
Leighton Pritchard
Bethan Purse
Alexandra Schlenzig
David Slawson
Peter Thorpe
Gregory Valatin

Organisation

Animal and Plant Health Agency
NERC Centre for Ecology & Hydrology
NERC Centre for Ecology & Hydrology
James Hutton Institute
Forest Research
US Forest Service
Forest Research
Forest Research
Forest Research
Animal and Plant Health Agency
Forest Research
DEFRA
Forestry Commission
Forest Research
University of Worcester
James Hutton Institute
NERC Centre for Ecology & Hydrology
Science and Advice for Scottish Agriculture
Open Air Laboratories (OPAL) Imperial College
James Hutton Institute
Forest Research