Plant nursery networks

Suitability of existing pathogen data and opportunities for new data collection to parameterise a plant nursery network model

Beccy Ganley

REPORT INFORMATION SHEET

REPORT TITLE	SUITABILITY OF EXISTING PATHOGEN DATA AND OPPORTUNITIES FOR NEW DATA COLLECTION TO PARAMETERISE A PLANT NURSERY NETWORK MODEL
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EXECUTIVE SUMMARY

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Authors: Beccy Ganley

This project

The movement of live plants through professional nurseries is known to be an effective means to disseminate plant pathogens. The aim of the Biosecurity Network Interventions project is to develop a network model linked with the potential for pathogen spread through nursery pathways. Ultimately the model will allow stakeholders to identify opportunities for interventions to either contain or slow the rate of spread of unwanted pathogens, thereby providing methods to safeguard New Zealand's plant-based economy.

The objective of this report is to review the suitability of existing pathogen data for plant nursery networks, highlight the gaps that exist with the current data and identify opportunities or methods to generate new data to parametrise or validate models.

Recommendations and conclusions

- The pathogen datasets for New Zealand are limited and the ability to generate new datasets for model parametrisation in a timely fashion are low. Although New Zealand specific data is limited there is considerable information from overseas studies that could be used.

- It is recommended that *Phytophthora* spp. are used as a model pathogen system as these include pathogens that are soil and aerially dispersed, and in some cases both. *Phytophthora* spp. are well documented for their spread through nurseries internationally either in the soil or roots, or via infected stem or leaf material.

- Validation is critical to this project. The exact project design of any validation is dependent on pathogen(s) selected for parametrisation and the subsequent networks generated.

- It is recommended that an eDNA, or a similar approach is used for the validation section of this project as it is more likely to detect small, cryptic infections than the other techniques available.

- Specific stakeholder engagement with MPI's Incursion Response team and Plant Health and Environment Laboratory to mitigate risks associated with new to New Zealand identifications is recommended. It is suggested this involvement is formalised at the MPI stakeholder engagement meeting on 2 August 2016.

- It is critical that nursery information used in this project is kept confidential to protect nursery growers. Nursery names or locations must not be released, and where possible linkages to host species should be avoided.

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June 2016

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Introduction

The movement of live plants through professional nurseries is known to be an effective means to disseminate plant pathogens. The aim of the Biological Heritage National Science Challenge Biosecurity Network Interventions project is to develop a network model linked with the potential for pathogen spread through nursery pathways. Ultimately the models will allow stakeholders to identify opportunities for interventions to either contain or slow the rate of spread of unwanted pathogens, thereby providing methods to safeguard New Zealand's plant-based economy and native flora.

The objective of this report is to review the suitability of existing pathogen data for plant nursery networks, highlight the gaps that exist with the current data and identify opportunities or methods to generate new data to parametrise or validate models.

Data collections

There are limited databases available that specifically document pathogen, pest or disease records in nurseries. Informal discussions with industry representatives indicate that most sectors do not have nursery surveillance programmes in place. While some industries have regulated nursery standards, these are new or do not include assessments of pathogen, pest or disease incidence. The forest industry had a nursery surveillance system in place, but this has been discontinued for some time; any pathogen, pest or disease reports from those surveys are recorded in the Forest Health Database (FHDB).

Forest Health Database (FHDB)

The FHDB is a collection of diagnostic pest, pathogen and abiotic records that have been taken from tree species across New Zealand since 1960. The database contains records from native, urban and commercial tree species. There are over 200,000 records in the FHDB, approximately 50,000 are results of formal identifications made in the diagnostic laboratory, and the remainder are identifications based on field observations. Details include agent identification, host species, location (GPS coordinates) as well as other data. Depending on individual entries, most records include data or information on disease symptoms and severity.

Location: Administered by Scion, Rotorua
Contact: Beccy Ganley
Ownership: Scion and the Forest Owners' Association
Data access: Permission to use data for the duration of the project
Confidentiality: All locations and business names must be kept confidential.
Data storage/termination: Not for archiving

Landcare Research NZFungi2 Database

Landcare Research's NZFungi2 database contains information on fungal species that have been identified on host plants in New Zealand, the year in which the association was recorded and the source of the record, since 1847. The majority of records come from assessments of plants showing disease symptoms, although the database does not contain information on disease symptoms or severity.

An extracted version of this dataset of records from between 1847 and 2012 is available for use. This extracted version contains approximately 76, 000 entries and has had duplicates removed, it also includes New Zealand host-fungal records from the HerbIMI database

There will be duplication between the FHDB and the NZFungi2 database as new to New Zealand pathogens identified in Scion's diagnostic laboratory are recorded in the FHDB and in NZFungi2.

Location: Administered by Landcare Research, extracted dataset available from BioProtection
Contact: Peter Johnston (Landcare Research), Jennifer Bufford, BioProtection
Ownership: Landcare Research and BioProtection
Data access: Permission to use data for the duration of the project
Data storage/termination: Not for archiving

Ministry for Primary Industries (MPI) Databases

The Plant Health and Environment Laboratory (PHEL) in Auckland is responsible for diagnostics of biosecurity risks in pre- and post-border plants, excluding woody plants which are processed almost exclusively at Scion's Forest Health Laboratory. Pre-border detections are not directly applicable to this project as they involve diagnosis of plant material that has not entered the country. Post-border detections are directly applicable and they are recorded in MPI's Plant Pest Information Network (PPIN) (http://archive.mpi.govt.nz/applications/ppin) and Laboratory Information Management System (LIMS) databases. The PPIN database mainly contains new to New Zealand, new host detections or new bioregion detections of pathogens. Some of the entries in MPI's PPIN database will be duplicated in Scion's FHDB and in Landcare Research's NZFungi2 databases. The LIMS database captures information on all of PHEL's identifications and this database could contain valuable information on pathogens in nurseries.

Location: Ministry for Primary Industries
Contact: http://archive.mpi.govt.nz/applications/ppin
Ownership: Ministry for Primary Industries
Data access: Need to obtain permission to use data for the duration of the project. Data may be filtered before provided. Suggest using the MPI stakeholder meeting to progress obtaining this data.
Data storage/termination: TBC

Relevant Scion reports

1. Gardner, J F; Dick, M A; Bulman, L S, 2006. Detection of disease in forest nurseries. Scion output 40490.

Objectives of report:

- To determine the optimal method of pest detection survey and sampling in forest nurseries and to recommend best practice for nursery survey methods in the future.

- To review the world literature relating to incursions to forest nurseries, to demonstrate the quarantine risk associated with forest nurseries and to determine the extent of the risk that New Zealand forest nurseries may act as a pathway for incursions.

Includes pest detection results from limited nursery surveys – these records will be present in the FHDB.

Model pathogen systems

In general, nurseries do not sell plants that show symptoms of disease. Although plants may appear healthy, they can host cryptic or latent infections of undesirable pathogens (Baskarathevan et al. 2016; Crone et al. 2013a; Crone et al. 2013b; Evira-Recuenco et al. 2015). Chemicals are used to control disease but these measures do not necessarily destroy the pathogen completely (Crane and Shearer 2014) and may mask symptoms, which can result in nurseries unknowingly transferring infected material.

To model the biosecurity risks associated with nursery plant movement across New Zealand, data on pathogens that represent those that could be transported unknowingly in soil or cryptically in plant material are needed. The pathogens can be broadly characterised into two groups: soil-borne pathogens that are transported in the roots and associated soil, and aerial pathogens that have cryptic infections in plants.

Examples of soil-borne pathogens are numerous, and in some cases pathogens can be both soil-borne and aerially dispersed. For this project, it is recommended that *Phytophthora* spp. are used as a model system for soil-borne pathogens. *Phytophthora* spp. are amongst the most destructive plant pathogens worldwide. These species present some of the most difficult pest management challenges. The introduction of these species into new systems has led to severe declines such as potato blight caused by *Phytophthora infestans* (Akino et al. 2014), sudden oak death caused by *Phytophthora ramorum* (Grünwald et al. 2012), and phytophthora dieback in the native ecosystems of southern Australia and Cork oak decline in Spain and Portugal, both of which are caused by *Phytophthora cinnamomi* (Duque-Lazo et al. 2016; Vila et al. 2003). Some *Phytophthora* spp. have very board host ranges, for example *Phytophthora cinnamomi* has the broadest host range of any Phytophthora spp. and is known to infect herbaceous plants through to large trees (Robin et al. 2012).

Within New Zealand there are 31 *Phytophthora* species that cause disease on a range of agricultural, horticultural and forestry species (Scott and Williams 2014). Either the *Phytophthora* genus itself could be used to model and validate the biosecurity risk associated with plant movement in nurseries, or a specific *Phytophthora* species already present in New Zealand, such as *Phytophthora cinnamomi* could be used. The broad host range of plants that can be infected by *Phytophthora*, and the cross-industry issues with this genus makes it a suitable option as a model pathogen for this project.

For aerially dispersed pathogens, it is recommended that a pathogen that is known to have a cryptic or latent infection period in its host plant is used. If an aerial pathogen were to be used as a model system, a decision on the extent of aerial dispersal to inform the model would need to be considered. In the nursery situation where the application of chemicals reduces disease expression, aerial dispersal may be effectively eliminated and under this scenario, the true risk of spread of these aerially dispersed pathogens is from the spread of infected but asymptomatic material. This is true for several high risk pathogens including *Fusarium circinatum*/ pitch canker and *Phytophthora* spp. (Evira-Recuenco et al. 2015; Jung et al. 2016). Even *Puccinia psidii* (myrtle rust) a devastating pathogen of Myrtaceae species, which is notorious for aerial spread, could be contained with chemical control in a nursery (Martins et al. 2011) on less susceptible hosts and could be unwittingly spread by nursery trade via cryptic infections.

There is considerable literature available on aerial and cryptic infections that could be used to inform biosecurity networks. However, the choice of a specific pathogen or genus to model is more difficult as most aerial pathogens tend to be relatively host specific. Any aerially dispersed fungal pathogen that is known to have a cryptic or latent phase could be used to mimic non-aerial spread of pathogens such as *Puccinia psidii* or *Phytophthora ramorum* (an aerial dispersed *Phytophthora* species).

As *Phytophthora* spp. include pathogens that are soil and aerially dispersed, and in some cases both, the *Phytophthora* genus would be a logical choice to model pathogens that could be transported through nursery systems either in the soil or roots, or via infected stem or leaf material.

Although the analysis in this report tends to favour the choice of a soil borne pathogen, this is due to the ease of studying and modelling pathogens that have restricted infection cycles. Whilst an aerially dispersed pathogen is more complicated, a list of the top worst plant pathogens (Dean et al. 2012) is dominated by aerially dispersed pathogens. There are also many examples where spread to locations either within a country or between borders has occurred through the movement of infected plants (Bienapfl and Balci 2014; Migliorini et al. 2015; Santana et al. 2016; Schoebel et al. 2014) rather than aerial spread.

Model parametrisation

The data sets for New Zealand are limited and the ability to generating new datasets in time for model parametrisation are low. Although New Zealand specific data is limited there is considerable information from overseas studies that could be used. There are numerous papers that provide data on the level of *Phytophthora* spp. infection in European nurseries could be applied to the New Zealand situation (Blomquist et al. 2016; Jung et al. 2016; Migliorini et al. 2015; Prigigallo et al. 2015). This would also provide extra support for the use of the *Phytophthora* genus as a model pathogen system. *Phytophthora ramorum*, regarded by many to be one of the mostly globally devastating plant pathogens, was spread internationally via the nursery trade (Grünwald et al. 2012). Whilst numerous other high risk fungal pathogens have been spread via the nursery trade, the information on plant host associations and spread is often limited to a specific host or groups of host plants and is not as applicable to this project, which spans cross-sector nursery trade in New Zealand.

Another factor to be considered for model parametrisation is the establishment potential of a pathogen in a new area. In general, temperature and moisture conditions within nurseries are optimal for most pathogens regardless of their location across the country. For establishment outside of nursery settings there is more variation in disease establishment and severity. Soil borne pathogens tend to have fewer restrictions on disease establishment as microorganisms associated with soil often have structures to withstand adverse conditions. Conversely, these resting structures aid dispersal and establishment of soil borne pathogens. Aerially dispersed pathogens tend to be more dependent on climate conditions and pathogen and disease establishment can vary across the country. A variety of climate maps for different aerial pathogens of *Pinus radiata* (grown across New Zealand) have been produced (Ganley et al. 2011; Ganley et al. 2009; Watt et al. 2011a; Watt et al. 2012; Watt et al. 2011b) and these could be used to predict likely establishment potential of other aerial pathogens across the country. In general the majority of the North Island and the top half of the South Island have higher risks of disease establishment and severity than other parts of New Zealand.

The inclusion of aerial dispersal, if an aerially dispersed pathogen system is chosen, is not a necessity for reasons discussed in the previous section, but could be considered. Aerial dispersal of a pathogen under nursery settings may be limited and the true risk mostly likely lies with the spread of infected material.

Model validation

Validation of the models generated using robust data is a critical part of this project. Although it is achievable within the time frame of this project, it comes with considerable biosecurity risk as there is a high likelihood that new to New Zealand microorganisms could be identified during the validation phase. This risk needs to be managed to mitigate impacts on nursery growers and industries involved in this project.

The exact project design of any validation is dependent on pathogen(s) selected for parametrisation and the subsequent networks generated. For both soil and aerially dispersed pathogens, validation would involve sampling of soil and/or plant material from selected nurseries. Material from a variety of host plants across multiple sectors, or selected hosts from specific sectors, could be sampled. As many pathogens are able to survive cryptically in plants not considered known hosts (Crone et al. 2013a; Ganley et al. 2015; Swett and Gordon 2015), sampling for pathogens across the nursery sector could provide information on the degree that these associations occur across New Zealand. Validation could also involve determining commonality between nurseries that are networked versus those that are not. Variations in perceived nursery hygiene levels of different sector-specific nurseries could also be tested.

To validate what is present in nurseries or plant material, techniques to identify either specific pathogens or groups of pathogens are needed. This can be culturing and/or DNA-based techniques. For both techniques there is a high risk that new to New Zealand organisms will be identified, the risks associated with this outcome are discussed below in the Project risk section.

Culturing has the advantage of providing data on viable isolates present in the soil or plant material. The disadvantages are that some organisms can be difficult to culture or may be outcompeted by other microorganisms in culture, or suppressed by chemicals. It can also be time consuming and has limited through-put. From a new to New Zealand biosecurity point of view, any cultured microorganisms identified as potentially new to New Zealand will be reported immediately to MPI.

DNA-based techniques could include PCR amplification of specific pathogens or genera. The advantage of this technique is that only the pathogen(s) of interest will be detected, it is relatively quick and has high through-put. The disadvantages are it does not tell you if the organism is viable; in asymptomatic plants the levels of infection may be too low for detection; and as sample sizes are small, there is a high risk a pathogen may be missed in a sample. In contrast, an eDNA technique amplifies DNA from all microorganisms specific to the primers used, including those present in very low levels, which is important when dealing with cryptic infections. Similar to PCR-specific techniques it has high-through put but is more time consuming because of the bioinformatics component. This technique also does not tell you if an organism is viable and there are the same issues with not detecting the pathogen of interest due to small sample sizes. From a new to New Zealand biosecurity point of view, any microorganisms identified as potentially new to New Zealand using eDNA techniques would need to be reported to MPI immediately. As identification of an organism to species level cannot be done using only one gene region, there is scope to work with MPI to develop a process that recognises these difficulties but focuses effort on detection of potential unwanted, high risk pathogens.

Overall there is not one detection technique that is superior over the others. Ultimately the best scenario would be to use both a culture and DNA-based method combined. However, due to the expense and time involved this is not practical. The eDNA approach is recommended over the other techniques simply because it is more likely to detect small, cryptic infections than the other techniques.

Scion is looking into a RNA-based diagnostic method, which indicate the viability of microorganisms in a soil or plant sample. If developed before this section of the project is started, this RNA technique could be used in lieu of, or combined with, an eDNA approach.

Project risks

New to New Zealand identifications

New to New Zealand organisms are known species (or in some cases genera) that have not previously been identified in New Zealand. Organisms that cannot be identified to species would not be reported as new to New Zealand if the genus was already present. For this project, new to New Zealand organisms roughly fall into two categories, those that are considered low risk and those considered high risk. Low risk pathogens are typically those that have been present in New Zealand for a considerable time (either native or introduced) but have not previously been identified as present, or pathogens that are newer introductions but are unlikely to adversely affect New Zealand plant-based economies or native flora.

High risk pathogens are more recently established organisms that pose a serious threat to either specific plant-based industries or to our native estate. Examples of high risk pathogens include *Phytophthora ramorum*, *Puccinia psidii* and *Fusarium circinatum*, all which have been discussed previously within this report.

There is a perception that if a new to New Zealand pathogen is found in a nursery that nursery would be closed by the Ministry for Primary Industries. In general, new to New Zealand identifications do not result in government led closure of businesses or areas. The exception to this are high risk pests or pathogens, which in some cases would result in nursery closure for a specified period of time.

To ensure any new to New Zealand identifications are dealt with effectively, both from the viewpoint of MPI and nursery industries involved, it is recommended that there is specific stakeholder engagement with MPI's Incursion Response team and Plant Health and Environment Laboratory. Individuals from these teams need to be fully aware of the project. It is recommended that this involvement is formalised at the MPI stakeholder engagement meeting on 2 August 2016. This formalised engagement with these groups would also be used to work through any issues with publication of pathogen datasets, if required.

Client confidentially

It is critical that nursery information used in this project is kept confidential to protect nursery growers. Nursery names or locations must not be released, and where possible linkages to host species should be avoided.

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