Metabarcoding analysis of *Phytophthora* diversity in spore traps and implications for disease forecasting in the *Phytophthora ramorum* management zone

PHC2019/08 - Policy Summary

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1 Policy Summary

1.1 Background

- *Phytophthora* is a large genus of oomycete plant pathogens. Many are invasive and have caused devastation to native and non-native host species after accidental introduction into new locations outside the pathogen's natural range.
- Since 2009, *Phytophthora ramorum* has been spreading through the UK's commercial larch population, leading to large-scale tree mortality and losses by anticipatory felling to slow the pathogen's spread. One way the disease can spread to new hosts is by aerially dispersed inoculum.
- Two *P. ramorum* lineages, EU1 and EU2, exist in the UK and appear to differ in their pathogenicity on larch. Although sexual recombination between the lineages is unlikely, somatic recombination could occur if the lineages co-infect a tree and come into physical contact. EU2 is found in South West Scotland and the extent of EU1 range overlap in the area is unclear.
- A Scottish Forestry-funded project has previously tested several spore trapping methods to monitor rain and wind-dispersed *P. ramorum* inoculum and improve forecasting and management of severe disease outbreaks. The project used qPCR to assess *P. ramorum* prevalence.
- Metabarcoding is an interesting alternative to qPCR because the method can simultaneously detect a range of Phytophthoras that may threaten our UK tree species. *Monitoring using m*etabarcoding could therefore provide early data on potential shifts in *Phytophthora* species prevalence under climate change or introduction of new *Phytophthora* species.

1.2 Key Research Questions

- To assess the suitability of metabarcoding as an alternative to qPCR, in particular to extend surveying to perform both a monitoring role for *P. ramorum* and provide prevalence data for other *Phytophthora* species.
- To assess the sensitivity of metabarcoding compared to the qPCR assay in detecting low levels of *P. ramorum* inoculum.
- To determine the *P. ramorum* lineage composition in the spore trap samples collected at the study site in South-West Scotland.

1.3 Research Undertaken

- A Scottish Forestry-funded project conducted spore trapping at a site in the Galloway Forest, South West Scotland in 2019 and tested three trap types to optimise capture of wind and rain dispersed *P. ramorum* inoculum. Traps were also set up at three points along a transect from an infected larch stand to test the dispersal distance of inoculum. *P. ramorum* prevalence was estimated on each spore trap by extracting DNA and using a *P. ramorum*-specific qPCR assay.
- Our project took the DNA samples from the Scottish-Forestry project and investigated the suitability of metabarcoding as an alternative to qPCR, to test whether other airborne *Phytophthora* species were present in addition to *P. ramorum*. We compared the frequency of *P. ramorum* detection using metabarcoding with the Scottish Forestry project qPCR data. We also tested the inoculum DNA using a *P. ramorum* lineage-specific qPCR assay to investigate the prevalence of both the EU1 and EU2 lineages in our samples.

1.4 Main Findings

- Our study considered the potential of different methods to detect a broader range of pathogens and lineages while primarily monitoring *P. ramorum* dispersal. The results illustrated these detection methods are appropriate, provide some helpful pointers to the future design of spore networks and suggest scope for further refinement.
- Metabarcoding enabled the detection of *Phytophthora* species other than *P. ramorum* at our forest site, even though the time of year over which the study was conducted may not have been optimal for detection of a broader range of species. The incorporation of metabarcoding-based surveillance for multiple species detection and estimation of their prevalence will provide early data on the potential threats to our trees caused by new arrivals or change of behaviour in a changing environment.
- While the qPCR method did not work optimally for DNA samples from traps, rapid lineage testing of samples of high target DNA content, e.g., infected trees, is possible using the qPCR assays tested here. This will allow for an early assessment of risk due to possible somatic recombination and changes in lineage prevalence given the potential for North American lineages to enter the UK.
- These methods extend the insights which can be gained from the task of sample collection and processing for presence/absence, and with refinement have the potential to enable improved disease forecasting and investigation of the effectiveness of management operations such as felling.

1.5 Recommendations and next steps

- Future spore-trapping studies should be extended into spring and summer as this is the period of most rapid lesion extension by *P. ramorum* (H. Dun *et al.* unpublished) and there is evidence for sporulation on asymptomatic larch needles during summer (Harris, 2015). Other *Phytophthora* species are also likely to be more active at this time.
- Future monitoring should also involve both wind vane and rainfall traps to account for rain-borne and windborne spore dispersal under a variety of weather conditions
- In our study, sample sizes were too small to draw statistically sound conclusions on the dispersal distance of *P. ramorum* inoculum or to make direct comparisons between trap types in terms of capturing *P. ramorum* inoculum. Future projects should increase sampling sizes and sampling distances to investigate these aspects.
- Lineage testing in South West Scotland should be carried out to look at the prevalence of EU1 in the EU2 area and to monitor for presence of North American lineages.
- Development of a new DNA barcode more specific to *Phytophthora* would be beneficial. The current ITS1 barcode primers can detect other windborne oomycetes (e.g., downy mildews) which can dominate the sequencing data at the expense of *P. ramorum* detection.
- Progress in these method refinements would inform the design and implementation of a comprehensive monitoring, surveillance and disease forecasting system for *Phytophthora* in Scotland.

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