

Assessing spread of *Phytophthoras* in Scottish forests by recreational and harvesting activities using comparative qPCR and metabarcoding techniques

Policy Summary



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Please cite this report as follows: C. Riddell, A. Armstrong, P. Cock & T. Clark (2023). Assessing spread of *Phytophthoras* in Scottish forests by recreational and harvesting activities using comparative qPCR and metabarcoding techniques: Policy Summary. PHC2018/17. Scotland's Centre of Expertise for Plant Health (PHC). DOI: 0.5281/zenodo.10671319

Available online at: planthealthcentre.scot/publications

Dissemination status: Unrestricted

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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.
- Disease outbreaks are frequently associated with human activity and soil movement, as well as climate change.
- The costs of managing a disease outbreak are generally high and there is an urgent need to provide forest practitioners with tools to forecast and manage new diseases.
- Since 2009, *Phytophthora ramorum* has been spreading through the UK's commercial larch population, leading to large-scale tree mortality and loss of forest stands through controlled felling to slow the pathogen's spread. Whilst some spread has been by airborne spores, there remains concerns over the potential for inadvertent spread via soil attached to footwear, tyres and machinery.
- A previous University of Stirling undergraduate project used a qPCR detection method to investigate the potential for spread of *P. ramorum* via movement of soil along recreational trails in public forests.
- Metabarcoding has been shown to be a more informative detection method than qPCR as it has a broader range of detection and can, in theory, detect a broader range of *Phytophthora* species, in addition to *P. ramorum*. The use of this technology has the potential to provide new insights into the diversity of *Phytophthora* species in an environment.

1.2 Key Research Questions

- Can metabarcoding be used to detect *Phytophthora* species present in sites in which both recreational and harvesting activities are being carried out?
- How sensitive and reliable is metabarcoding in detecting *P. ramorum* in relation to the targeted species-specific *P. ramorum* qPCR assay?
- What risk does recreational and harvesting activities pose in introducing and spreading pathogens within forests and potentially to new sites?

1.3 Research Undertaken

- A previous undergraduate project at Forest Research assessed the prevalence of *Phytophthora ramorum* in three public Scottish forests (Glentress, Glentool and Kirroughtree) in 2016 using a species-specific qPCR assay and baiting for live propagules. The study determined the level of *P. ramorum* DNA that could be detected from soil removed from boot and bike treads of recreational users and compared detection at forests that were experiencing *P. ramorum* outbreaks in larch to those from a forest in which the larch showed no symptoms of *P. ramorum*.
- This project took the soil DNA samples from the undergraduate project and used this to do both metabarcoding and qPCR in order to compare the sensitivity and reliability of both methods for the detection of *P. ramorum*.
- In addition, the project aimed to determine whether the metabarcoding method could detect the presence of other *Phytophthora* species in addition to *P. ramorum* in the soil samples.

- The sampling was expanded to include metabarcoding, qPCR and baiting analysis of soils collected from treads of timber harvester and forwarder machines in the Galloway Forest where extensive felling was underway to manage the outbreak of *P. ramorum* on larch.

1.4 Main Findings

- Our study confirmed that metabarcoding wasn't as sensitive as qPCR for detecting *P. ramorum* in soil DNA, however, it proved useful in detecting a broader range of *Phytophthora* species in some samples. Quantitative PCR remains a sensitive and reliable method for being able to quickly detect a single-targeted species. Improving bioinformatic analysis methods would be an important step in increasing the reliability of this metabarcoding technology in the future.
- Metabarcoding detected several other species of *Phytophthora* in all forests. This provides additional insights on samples that had previously only been screened for *P. ramorum* using qPCR. Across the recreational sites, ten known *Phytophthora* species and 3 other *Phytophthora* sequences reported as complexes were detected. Several of these species are known to be pathogenic and known to infect UK tree species. This included species associated with decline of European oak forests (*P. cinnamomi*) and native forest in Australia (*P. gibbosa/gregata* complex) and pathogens of many tree species including larch (*P. pseudosyringae*), Northofagus (*P. pseudosyringae*), fruit trees (*P. chlamydospore*), juniper (*P. austrocedri*) and horse chestnut (*P. obscura*).
- There were substantial differences in *Phytophthora* species diversity between the forest sites tested. Glentrool had the greatest diversity of *Phytophthora* species (10), compared to 8 for Kirroughtree and 5 for Glentress. Only two species were found at Galloway.
- Two sequences were obtained from one of the recreational sites via metabarcoding that showed high similarity in each case to *P. pinifolia*, which has had severe impacts on *Pinus radiata* in Chile but is not known to be present in the GB. This is now being investigated as priority with further monitoring at the forest in collaboration with Scottish Forestry. This highlights the value of methods such as metabarcoding with broad pathogen detection, that can alert to the potential presence of new pathogens in our forests.
- On the harvester sites, *P. gonapodydies* was the only other *Phytophthora* species detected. A key finding was that harvesting machines arrived on site with soil (and plant debris) already attached to treads from which *P. ramorum* was detected. This indicated that *P. ramorum* has been moved from one forestry site to another via the machinery treads.
- Collectively, the project findings highlight the contribution of both recreational and harvesting activities in moving *Phytophthora* diseases within our forests with the potential that infectious propagules could be moved to healthy sites with the possibility that new infections are initiated. Further work to determine pathogen viability after being moved to a new location and the potential to start new infections would be valuable.

1.5 Recommendations

- Our confirmation of the risk of pathogen spread via soil (or other plant debris) on footwear, bike tyres and treads of harvesting machinery in Scottish forests underlines the need to continue good biosecurity practice as advocated by campaigns such as 'Keep it clean'. Recommendations for forestry practitioners would be to ensure boots and machinery are thoroughly cleaned before moving to a different location. Forests used by the public for recreational activities would also benefit from biosecurity messaging around cleaning boots and bike tyres before and after entering the forest.

- Although we have demonstrated the utility of DNA based metabarcoding in detecting presence of a range of *Phytophthora* species the method cannot indicate whether the pathogen is viable and able to cause disease. Future steps could include the development of an RNA-based metabarcoding analysis of soils, as this would indicate the viability of detected organisms.
- Future studies could work to optimise the baiting protocol to improve recovery of viable pathogen species. Except for *P. gonapodyides*, we were unable to bait *Phytophthora* from our soil samples even when qPCR or metabarcoding indicated they were present. Digital PCR could be trialled as an alternative to qPCR in species-specific assays as it is able to detect and quantify low levels of pathogen DNA against background noise more reliably compared to qPCR.
- Several *Phytophthora* species were detected via metabarcoding including sequences which closely matched *Phytophthora pinifolia* and sequences that could not be fully resolved via metabarcoding. If these are true detections, the risk of these species is unknown and would warrant further investigation. In the first instance, additional bioinformatic analysis would be useful to try and resolve if these are true positives and, if so, to obtain more genome information to aid identification. It would also be beneficial to also try rebaiting to obtain live isolates of the unknowns and to clarify whether viable propagules are present in the forest. Once additional information is obtained, along with live isolates, the risks of these organisms can be better determined.
- We have no understanding of whether understorey plants act as a reservoir for pathogens. Therefore, further work is required to investigate the presence of *Phytophthora* in understorey plants and to examine the risks associated with understory vegetation communities.

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