Understanding the drivers of emergence of *Curreya pithyophila* and associated impacts on Caledonian pine

Policy Summary

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1 Policy Summary

1.1 Background

This project followed on from a previous project 'PHC2022/07: Understanding a new health threat to Caledonian Scots pine (*Pinus sylvestris*)' caused by a new and unusually widespread infestation of two genetically and morphologically distinct forms (phragmospore and dictyospore) of a previously rare stroma-forming fungus *Curreya pithyophila* (syn. *Cucurbidothis pithyophila*). Both forms are infesting shoots and branches of Scots pine across Scotland in a symbiotic association with the native Scots pine adelgid, *Pineus pini*, which causes feeding wounds. Affected Scots pine also exhibits abundant blackened branch cankers resulting from invasion of feeding wounds by the native pine pathogen *Crumenulopsis sororia*. This project focused on investigating potential drivers of emergence of *C. pithyophila* and its impact on Caledonian Scots pine.

1.2 Key Research Questions

- How can studies of the genomes and morphological relationships among isolates assist in our understanding of differences between the phragmospore and dictyospore forms of *C. pithyophila* and how each form interacts and spreads?
- How do British specimens of *C. pithyophila* from Scots pine compare with global specimens from various Pinaceae hosts, and can these comparisons shed light on the origin of British specimens?
- What are the impacts of *C. pithyophila* and cankers on Caledonian Scots pine, and are these affected by location (east vs west) and tree age?
- Do genetic factors such as source population and family influence the response of Caledonian Scots pine to *C. pithyophila* and cankers?

1.3 Research Undertaken

Whole genome sequences were assembled for a phragmospore isolate and a dictyospore isolate of *C. pithyophila* from Scotland and gene content analysed. Further genomic analyses were conducted for 20 phragmospore and 20 dictyospore isolates from Britain. Analysis of fine scale genetic variation was undertaken to investigate how populations of *C. pithyophila* differ. Laboratory compatibility tests on agar plates were also carried out to determine whether isolates of *C. pithyophila* remain separate or merge. This helps us understand relatedness of isolates and the gene flow within fungal populations. A total of 48 herbarium specimens comprising both forms of *C. pithyophila* were collected from *Abies, Larix, Picea* and *Pinus* hosts across continental Europe, Asia and North America. The morphology of these specimens was compared with British specimens.

Field surveys were carried out at two native Caledonian Scots pine populations, Glen Affric in the west and Rothiemurchus in the east, to determine the incidence and impact of *C. pithyophila* and cankers. Within each population, 50 young trees and 50 mature trees were surveyed for crown dieback, presence of *C. pithyophila,* presence of cankers and percentage visible disease on the six lowest live branches. Cankers were dated using dendrochronological methods. To investigate the influence of host genetic factors, the same variables were surveyed at three Caledonian Scots pine common garden trials located in the east, west and south of Scotland. Inoculation experiments were carried out to determine whether both forms of *C. pithyophila* can infect Scots pine in the absence of *P. pini.*

1.4Main Findings

Summary of key findings:

- The two forms of *Curreya pithyophila* (dictyospore and phragmospore) are genetically distinct and represent separate species.
- The phragmospore form may have been introduced into Britain more recently than the dictyospore form and may have been spread through planting.
- Both forms most closely resemble global herbaria specimens from Scots pine in Europe.
- Infestations in native pine populations may have started in the mid-1990s with wetter site conditions favouring *C. pithyophila*.
- *Curreya pithyophila* appears to have a unique genetic adaptation to growth within the bark layers of Scots pine.
- Susceptibility to infestations of *C. pithyophila* may be a genetically inherited characteristic of Scots pine, meaning that future adaptation is possible.

Significant genetic differences were revealed between dictyospore and phragmospore forms of *C. pithyophila*. Both forms of *C. pithyophila* contain lignin-degrading genes not yet found in an ascomycete species which may help it to infest Scots pine bark. Both forms have two different mating type strains, allowing sexual reproduction. Almost all isolates of *C. pithyophila* were unable to merge with each other regardless of whether they were the same mating type or not. This suggests that sexual reproduction occurs at a high frequency within each population, helping to maintain diversity and adaptability. The fungus was unable to grow in the tissues of Scots pine without the presence of *P. pini*.

Dictyospore and phragmospore isolates were genetically very different from each other, providing further evidence for their separation into distinct species. Dictyospore isolates from Britain showed more genetic diversity than phragmospore isolates, with dictyospore isolates from south-west Scotland separating from other Scottish isolates. This suggests that the dictyospore form has been present in Britain for longer than the phragmospore form. The presence of genetically similar strains in widely differing geographical locations suggests that introductions have occurred through non-natural pathways, such as planting. Ascospore sizes in British specimens of *C. pithyophila* most closely matched specimens from Scots pine in continental Europe, which may be their origin, although there was no consistent variation in spore size based on either host or region.

Incidence of *C. pithyophila* was higher in Caledonian pine at Glen Affric than at Rothiemurchus which may reflect the wetter site conditions at Glen Affric. The low overall incidence of this fungus and the high incidence of blackened cankers typical of secondary *Crumenulopsis sororia* infections in both pine populations suggests higher levels of past infections. This is because native Scots pine growing on good sites are not typically susceptible to *Crumenulopsis sororia*. Most cankers sampled within these two populations dated back to the mid-1990s onwards indicating that disease may have started earlier in native pine populations than at planted sites in Scotland. Surveys of Caledonian pine common garden trials revealed that susceptibility to *C. pithyophila* infestations may have a degree of genetic control, potentially allowing Scots pine to adapt resilience to the fungus if climate change favours future prevalence of *C. pithyophila*. Incidence of secondary cankers caused by *C. sororia* may be more affected by site conditions than genetic factors.

1.5 Recommendations

- Plant only healthy stock which is visibly free of adelgids or fungal infection and pay particular scrutiny to any Pinaceae hosts imported from overseas.
- Plant Scots pine only on sites deemed suitable for this species.
- Monitor Scots pine plantings for signs of *C. pithyophila* infestations and report any findings to the Tree Health Diagnostic and Advisory Service of Forest Research via Tree Alert (forestresearch.gov.uk).
- Scots pine on wet sites are particularly vulnerable and should be the focus of more targeted monitoring.
- Report any *C. pithyophila* stromata observed on other Pinaceae hosts in Scotland.
- Surveys for *C. pithyophila* on Scots pine should be conducted in England and Wales.

1.6 Next Steps

Curreya pithyophila does not kill Scots pine but infested trees are likely to continue to experience annual re-infestations resulting in accelerated lower crown dieback. New plantings are also susceptible. There is much still to be explored in the *C. pithyophila* genome data which can allow us to further understand the differences between the two forms, whether they are likely to have been recently introduced to the UK, and where in the world they have originated. Data from the common garden trials can be further analysed to test whether Scots pine provenances are locally adapted to the environmentally contrasting trial sites, providing insights into the resilience of this species under future biotic and biotic stresses. These genomic and provenance analyses would support national efforts to enhance Scots pine resilience under changing climate and pest pressures.

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