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

Background: The oomycete *Phytophthora ramorum* has caused substantial losses of Scottish forests in recent years due to widespread mortality of European larch trees (*Larix decidua*). Infected trees are subject to statutory felling notices in an effort to reduce sporulation potential. Nevertheless, there are concerns about the multiple pathways by which spores might be transferred to new sites. During the first phase of this project there have been assessments of the two main leisure activities with the potential to transport soil/plant material between forest sites - mountain biking and walking/running -resulting in evidence to support the Forestry Commission's Keep-it-Clean campaign. There is now interest in extending the study and developing the methodology to consider impacts of machinery movement between forests.

The new phase of this study aims to extend into the industry sector and assess soil/plant material collected from commercial harvesting equipment (tyres, treads, mud guards etc.). A basic investigation, funded by FC Scotland, will permit simple molecular diagnostics to be performed to examine the presence of *P. ramorum* alone. The PHC support will enable a wider range of diagnostic tests to be carried out – testing the robustness of the simple method and examining the potential for multiple *Phytophthora* species to be transferred.

Impact: The data provided will be valuable in verifying detection methods for future studies of *P. ramorum and* will add to the evidence base underpinning public and sector-facing biosecurity campaigns. It is hoped that this will lead to improved disease management and restricted spread of the disease.

Objectives and research required for this call:
1. Compare qPCR and metabarcoding as techniques to establish the presence of *P. ramorum* in soil samples collected from harvesting machinery and from mountain bikes and walking boot treads.
2. Detect the presence of other *Phytophthora* species in the samples collected.

The detection of *Phytophthoras* in environmental samples is quickly moving towards the world of metabarcoding which has the capacity to detect multiple species from the genus in individual samples. However, there is some evidence to suggest that the more traditional route of qPCR could be more sensitive when detecting *P. ramorum* as a species-specific detection method rather than analysing its occurrence as an element of a cocktail of species (Green et al. unpublished). This project offers an excellent opportunity to explore the above using material from both phases of the project.

All samples will undergo DNA testing and *Phytophthora* baiting to determine the viability of detected *Phytophthora*. Sites with and without the presence of *Rhododendron sp.* will be assessed and compared as evidence suggests that sites with the presence of Rhododendron yield higher returns when baited (Frederickson-Matika unpublished) and therefore may pose a greater risk to biosecurity.

Outputs required: Assess the potential for *Phytophthoras* to spread via soil and plant material moved by human activity providing evidence to support the Keep-it-Clean campaign and provide a comparative study of qPCR and metabarcoding techniques for the detection of *Phytophthoras*, in particular *Phytophthora ramorum.*

Key Milestones:
Confirmation of methodology and plan
Samples collected and processed (DNA extraction and baiting)
qPCR of samples completed
Metabarcoding analysis of samples complete
Final report submitted along with draft of scientific paper

Date all work needs to be completed by: 30ᵗʰ September

Project type: Call-down – collaborative

Maximum funding available (including VAT): £13000