Phytophthora diversity in plant nurseries and woodland ecosystems

This summer I was fortunate to be able to work in the Pathology lab at Forest Research's Northern Research Station (NRS) near Edinburgh, where I assisted in multiple projects concerning the fungus-like plant pathogen *Phytophthora*. *Phytophthora* (Greek for 'plant destroyer') species are widespread and have caused significant plant epidemics such as the Irish potato famine (*P. infestans*) and the ongoing disease on larch in the UK and sudden oak death epidemic in the United States (*P. ramorum*). *Phytophthora* species pose a large environmental, ecological and economic threat due to their rapid spread and it being nearly impossible to contain or cure the pathogen. Human activities further risk introducing species into naïve environments as well as potentially extending host ranges by facilitating hybridisations via e.g. the plant trade. Research into *Phytophthora* therefore remains of great ecological and agricultural importance.

Two of the projects I was able to assist with seek to survey the presence, distribution and diversity of *Phytophthora* species in British plant nurseries (LWEC /Phyto-threats), and in sites of recent plantation and undisturbed natural habitats (POnTE) using Illumina metabarcode sequencing as well as traditional isolation techniques. The end goals of these studies are to determine *Phytophthora* diversity in samples from the different habitats, identify which species currently pose the highest threats to the UK, to document alterations in host associations, and ultimately to improve biosecurity measures in the plant trade and beyond. As these projects have only just begun, I was primarily involved in field sampling, extracting DNA, as well as performing PCR. Nursery sampling was done in collaboration with the James Hutton Institute (JHI), and involved water and root sampling. Flow-through water from selected plant species as well as the general water supply and collection tanks was pumped through cellulose filters in triplicate, which were sent to JHI for DNA extraction. At the reforestation sites and woodlands, soil was sampled around 10 trees per site. We used augers to collect 24 soil cores surrounding each tree. The soil samples were dried and ground up at NRS prior to further processing. I extracted DNA in triplicate from each sample using an established protocol and kit, before cleaning the extracts to reduce the likelihood of polyphenols interfering with the PCR. Nested PCR was performed using *Phytophthora*-specific primers. The samples that showed up positive on agarose gels will be then prepared and sent to JHI for sequencing on an Illumina platform. Preliminary results suggest that the undisturbed environmental sites and new planting sites sampled so far have had a relatively low *Phytophthora* prevalence.

Additionally, I was able to assist in projects in their late stages, which gave me an insight into the bioinformatics that will have to be undertaken for the LWEC and POnTE projects. I analysed sequence data from the end of a pipeline of a Scottish *Phytophthora* Metabarcoding project using NCBI Blast on the most frequently occurring operational taxonomic units (OTUs) in blocks of aligned sequences. I was responsible for determining new 'signature sequences' for closely related species as well as newly discovered species from recent publications, so that they can be checked up manually in cases of ambiguous Blast results, and wrote new codes for these.

I gained further insights into the traditional isolation techniques which will be performed on our soil samples, again assisting in an older project. Baiting was performed using apples and leaves of various species. Lesions were subsequently plated onto SMA and V8 agar, from which the leading edges of colonies were submerged in pond water then examined under the microscope for presence of sporangia to determine whether we had in fact isolated *Phytophthora*. DNA was extracted from the mycelium of positive colonies and sent for sequencing. Ultimately, traditional baiting serves as proof of concept: if a species is detected in soil samples via DNA extraction, one should be able to isolate it in its living form (and vice versa).

Overall, I generated data and footwork needed for these recently started Forest Research projects and relieved some of the backlog from older projects. I would like to thank Dr Sarah Green for giving me the opportunity to work on all these varied projects and to Dr Béatrice Henricot for kindly supervising me, as well as everyone at NRS who made it a great work experience (when I wasn't trying to annihilate myself with Lyme's disease or by setting my hands on fire). Many thanks also to the BSPP for their generous funding.

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