Investigation into the spread of *Phytophthora austrocedri* in soil and water at an infected Juniper site in Perthshire

This summer I worked in Sarah Green's laboratory at the Northern Research Station of Forest Research, located just outside Edinburgh. Our study area in Perthshire was Glen Artney, a Site of Special Scientific Interest. This area has a population of Junipers (*Juniperus communis*) that has been badly affected by the pathogen *Phytophthora austrocedri* causing high levels of mortality and die back in infected trees. Symptoms of infection include discoloured lesions under the bark and bronzing and dieback of foliage. *P. austrocedri* has motile zoospores and can spread through soil via ground water. I investigated the extent of *P. austrocedri* spread across the site through DNA analyses of samples taken from soil transects, streams and rainwater traps using a quantitative real-time PCR assay specific to the pathogen.

Soil samples were collected with an auger from three transects spread across the site, running approximately north to south down a slope, and DNA was extracted from the soil. Water samples from three streams were initially filtered onsite using a back pack sprayer and DNA extracted from the filters. However, control samples found that the back-back sprayer still contained pathogen DNA even after cleaning, therefore the method was amended to collecting 5 litre stream samples separately in bottles and using in-lab vacuum filtration to minimise likelihood of DNA carry-over between samples. I also investigated the possibility of aerial spread of *P. austrocedri* by collecting and analysing rain water. Rain water traps were set up at ground level (4 traps), and at 1m (2 traps) and 2m (2 traps) height, across the site. The results from weekly collection, filtration and DNA analyses of rainwater samples suggested some pathogen DNA cross-contamination was occurring during the filtering process so cleaning methods were adjusted to reduce this, including immersing all equipment in 1:10 bleach solution for 25 minutes between samples.

qPCR results gave the quantity of *P. austrocedri* DNA present in total DNA extracted from each soil and filtered water sample. All the soil samples gave consistently positive qPCR results, suggesting that *P. austrocedri* is widespread in soil across the site.

After improving the between-sample cleaning process during water filtration, the majority of rain and stream water samples were negative for *P. austrocedri* DNA, although some samples were positive, mainly in the ground level rain water traps. This could have been caused by splash back from the surrounding soil. The 2m high traps were not positive for pathogen DNA suggesting that *P. austrocedri* is not aerially spread.

Nested PCR with *Phytophthora*-specific primers was also used on a subset of samples to amplify DNA for sequencing, although *Phytophthora* DNA from most soil samples did not amplify well in standard PCR and time constraints prevented DNA clean up and repetition. Subsequent BLAST analyses of the samples which gave a band after nested PCR showed the presence of *P. austrocedri* in some of the soil, rain water and river samples and also showed other species: *Peronospora spp.*, *Hyaloperonospora parastica* and *Paraperonospora leptosperma*. BLAST returned one sample as having high similarity to *Phytophthora iranica*, a species not found in the UK, and further analysis of this DNA sequence showed some differences between it and other known sequences for this species. This sample was amplified again and an electrophoresis gel was used to separate out *Phytophthora* species from other contaminants. The DNA was eluted from the excised gel bands and sent off for more sequencing. Further study is required establish if this is a new species.

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Heather sampling in the field

The British Society for Plant Pathology

Bursary Report