Sword Fern Research Project

Progress Report

April 3, 2020

Haven
Ecology & Research

Accomplishments

Project Management

In February and March, I supported research collaborations with Ylva Lekberg and Susan Fawcett. I assisted Susan Fawcett in applying for a grant to fund a genomics study to compare symptomatic, healthy and recovering sword ferns, along with other *Polystichum* species. I discussed methodology with both collaborators and also consulted with Brett Younginger. We agreed that June is likely a better time to do metagenomic work with sword ferns and that physical pooling of samples will enhance the accuracy of our results given the limited sample size of the pilot study and the lack of within-individual replication. Lastly, I completed most of the permit application for Seattle Parks.

Research

In January I wrote a 2-page summary report titled *Summary of the State of Knowledge on the Decline of Sword Fern (Polystichum munitum)* to assist our collaborator Ylva Lekberg quickly familiarize herself with the die-off phenomenon. This report summarized what we know about the symptoms, spatial patterns, regional extent, pertinent null results and recent breakthroughs regarding transmission and ecophysiological aspects of the disease.

In my review of literature, I found several promising primer pairs for identifying oomycetes. The cox2, ITS and RAS regions are most typically used as barcodes in metagenomic studies on oomycete communities. Unfortunately, all primer pairs have trade-offs between the scope and specificity of taxa that can be identified. For example, the 18Ph2F/5.8S-1R primers excel at detecting *Phytophthora* species but can omit other oomycete taxa such as *Peronospora* (Legeay et al., 2019). After further discussion with Ylva Lekberg, I will likely recommend one of the following procedures: ITS3oo/ITS4ngs primer set for targeting the ITS2 region (Riit et al., 2016; Tedersoo et al., 2014); nested PCR using Oom18S/ITS7 followed by 18ph2F/5.8-S1R targeting the ITS1 region for *Phytophthora* only (Legeay et al., 2019); or the ITS6/ITS7-a.e. primers for targeting the ITS1 region (Esmaeili Taheri et al., 2017). All three primer sets are compatible with MiSeq workflows using 300bp paired-end reads.

Next Steps

I will continue to prepare for field work under the assumption that we will be able to sample in June. However, that timeline may need to be revised depending on how the covid-19 epidemic progresses. In early April, I will submit a permit application for sampling in Seward Park and also reach out reach to nearby land managers for potential backup sites. Between April and the end of May, I will narrow down

the sampling protocols through conversations with Ylva Lekberg and Susan Fawcett. I will also be procuring necessary supplies and materials. As soon as the Washington stay-at-home order is lifted, I will inspect samples from the greenhouse experiment under a microscope.

References

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