

Phytophthora communities in a western Oregon (USA) river

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Introduction

Horticultural nurseries typically harbor multiple *Phytophthora* species. One potential source of contamination is untreated water from ponds and rivers used for irrigation.

Objectives

- To detect pathogenic oomycetes species in a western Oregon river using Illumina MiSeq amplicon sequencing.
- To compare the communities detected using water filtration and leaf-baiting techniques.
- To determine seasonal effects on the oomycetes diversity.

Sampling to sequencing

We sampled river water approximately every 2 weeks from April 2015 to May 2016 and filtered 1L through 5µm Millipore nylon membranes. A subset of unfiltered water samples was used to bait rhododendron leaves. DNA was extracted directly from filters and baited leaves, and oomycete-specific primers^[1] ITS6 and ITS7 were used to amplify the ITS1 region for Illumina MiSeq 250bp paired-end sequencing.

Data analyses

The paired sequences were first cleaned, quality filtered and queried against a custom oomycete reference ITS database^[2,3] using a nucleotide megablast search. Operational taxonomic units (OTUs) were identified based on percent sequence similarity to the reference sequence of known oomycetes species. OTU abundance and diversity analyses were performed using QIIME^[4]. Mock communities composed of known oomycetes were used in this study to estimate cutoffs.

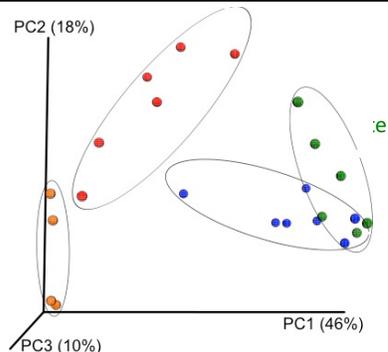
References

- Vannini et al. 2010. *FEMS microbiology ecology* 85:433-442.
- Robideau et al. 2011. *Mol Ecol Resour* 11:1002-1011.
- Grünwald et al. 2011. *Plant Disease* 95:337-342.
- Caporaso et al. 2010. *Nat Methods* 7:335-336.
- Dixon P. 2003. *Journal of Vegetation Science* 14:927-930.

Acknowledgments

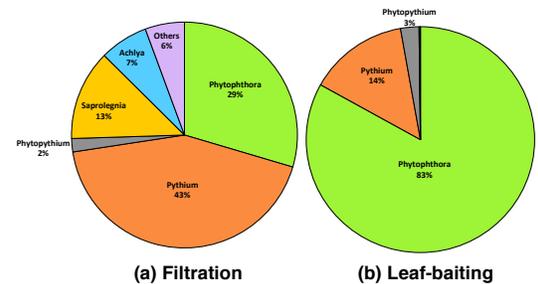
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A. Species diversity in river water



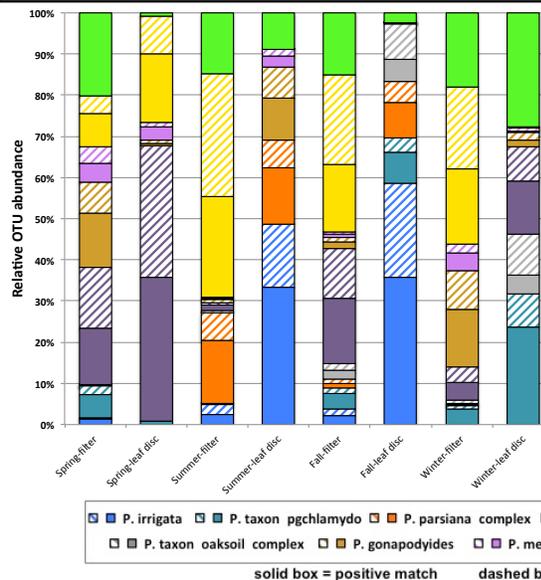
Oomycetes species diversity differed across seasons, as depicted in the Principal Component Analysis plot (above) for filter samples. Species richness (Chao index^[4]) increased from spring to winter (peak), while fall samples showed more diversity (Shannon-Weiner^[4] and Simpson's index^[4]) (data not shown). Bray-Curtis^[5] between sample diversity was estimated using 1,900 sequences per sample (depth).

B. Oomycetes detected in river water



With filtration (a), *Pythium* was the most abundant oomycete genus found in river water, followed by *Phytophthora*, *Saprolegnia*, *Achlya*, and *Phytophythium*. Primarily *Phytophthora* species grew on leaf-baits (b), followed by *Pythium* and *Phytophythium*, but species richness was quite low for leaf-baits. Although a greater number of species were detected on filters, the leaf-baiting technique was useful to detect active plant pathogenic species.

C. Seasonal variation in *Phytophthora* species



- P. irrigata* dominated on leaf-baits in summer and fall; while the *P. riparia* complex and *P. taxon pgchlamydo* dominated in spring and winter respectively.
- P. citricola* complex was observed on filters in every season, but only in spring did it amplify on leaf-baits.
- P. parsiana* complex was only identified in summer and spring; while *P. taxon oaksoil* complex was only identified in fall and winter.
- P. gonapodyides* did not grow on leaf-baits in fall.

Limitations: Illumina MiSeq amplicon sequencing technology is very effective and sensitive towards detecting community structure. However, shorter (250 bp) MiSeq sequences are unable to differentiate between two or more species in some cases, and often result to unresolvable species complexes.

Conclusions: All the *Phytophthora* species identified in this study were found in the filtered water samples, while only a subset of these species were present on leaf-baits. The ability of *Phytophthora* species to grow on leaf-baits greatly varied across seasons.

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