

A survey of *Phytophthora* and *Pythium* spp. in soil from upland prairie restoration sites in western Oregon

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Introduction:

Native upland prairie and oak savanna habitats were once widespread in the Willamette Valley of western Oregon, but now have been diminished by conversion to other land uses. These threatened habitats are considered essential for rare and endangered species such as the Fender's blue butterfly.



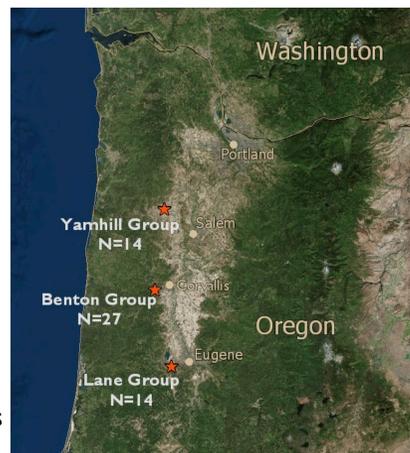
Restoring native upland prairie habitats is a major goal of wildland restoration in Oregon. The inadvertent spread of *Phytophthora* species from nurseries into native ecosystems can have long-term environmental and economic impacts, as has been seen with *Phytophthora ramorum*, *P. lateralis*, *P. cinnamomi*, *P. tentaculata*, and other species. The risk may be particularly great when nursery-grown plants infested with *Phytophthora* spp. are planted in restoration sites, introducing pathogens directly into native habitats¹.

Objectives:

- 1) Survey the distribution of *Phytophthora* and *Pythium* spp. in upland prairie restoration sites in western Oregon.
- 2) Determine if *Phytophthora* and *Pythium* spp. are detected at greater frequency in planted vs. non-planted sites.

Methods:

Soil samples (0-20 cm depth) were collected from 55 upland prairie/oak savanna sites in the Willamette Valley in western Oregon between November 2016 and May 2017. Of these, 27 sites had been planted with seeds, bulbs, or live plants within the last 5 years, and 28 sites had not been planted. Soil was sieved (2-mm) and split into two homogenized subsamples. One set of subsamples was baited with pears. Pear lesions were plated to get pure culture isolates.



DNA was extracted from cultures and species were identified based on Sanger sequencing of the ITS region. From the second set of subsamples, DNA was extracted directly from 10 g of soil. PCR was performed with these extracts using oomycete-specific ITS primers before sequencing on the Illumina MiSeq platform. Samples with low DNA (< 2ng/μl) were excluded. Species abundance was normalized across all samples and adjusted for total amount of DNA recovered from the soil. A species was considered present in soil if its abundance was ≥1% of the sample DNA.

Results:

- We detected 13 *Pythium* spp., 5 *Phytophthora* spp., and 1 *Phytophythium* sp. with the pear baiting and metabarcoding approaches (Figure 1).
- *Pythium attrantheridium* was the most frequently detected species with the metabarcoding approach. It was not recovered from pear baits.
- *Pythium* species were detected in 46 of 55 sites (84%). Only three species were recovered from pear baits.
- *Phytophthora* species (*P. cactorum* cluster, *P. megasperma* complex, *P. cambivora* complex, *P. fragariae* complex, and *P. ramorum*-like) were detected in 7 of 55 prairie sites (13%). Only 2 of the 5 species were recovered from pear baits.
- A quantitative real time PCR assay confirms that the *P. ramorum*-like species has an ATP9-NAD9 region different than *P. ramorum*, despite an identical ITS1 region (data not shown).
- There was no clear association between planting history and the presence of *Phytophthora* or *Pythium* species (Table 1).

Conclusions:

- *Pythium* appears to be nearly ubiquitous in upland prairie soils of western Oregon. The ecological role(s) of *Pythium* in this habitat are not known but *P. attrantheridium* has been shown to affect the distribution of native plants elsewhere^{2,3}.
- *Phytophthora* was detected relatively infrequently. Species complexes include plant pathogenic species of potential concern to wildlands.
- Pear baiting resulted in detection of only 6 species. Illumina MiSeq expanded the number of species or species complexes detected to 15.
- Results of this study provide a snapshot of the current distribution of *Phytophthora* and *Pythium* species in prairie restoration sites in western Oregon and can serve as a baseline for recognizing future introductions.

Figure 1: Oomycete species detected in prairie soils in western Oregon using pear baiting and Illumina MiSeq sequencing (55 sites). Species detected on pear baits are represented as striped bars. A complex consists of closely related species that are indistinguishable based on the ITS1 region. A cluster consists of closely related species that are identical between the priming sites.

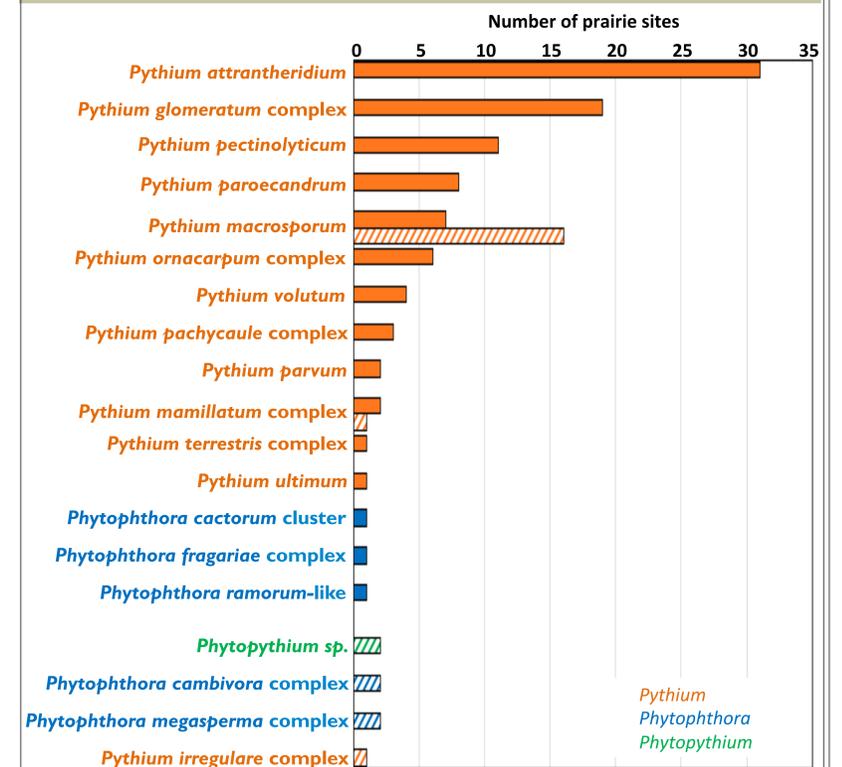


Table 1. Contingency tables using a Pearson's test for independence showed that there was no statistical relationship between detection of *Pythium* or *Phytophthora* and planting history.

	planted		nonplanted		
	obs	exp	obs	exp	
Pythium					p = 0.503
present	24	22.58	22	23.42	
absent	3	4.42	6	4.58	

	planted		nonplanted		
	obs	exp	obs	exp	
Phytophthora					p = 0.389
present	5	3.44	2	3.56	
absent	22	23.56	26	24.44	

¹Garbelotto, M., Frankel, S. J., and Scanu, B. 2018. Soil- and waterborne *Phytophthora* species linked to recent outbreaks in Northern California restoration sites. *Calif. Agric.* 72:208-216.

²Allain-Boulé, N., Tweddell, R., Mazzola, M., Bélanger, R., and Lévesque, A. C. 2004. *Pythium attrantheridium* sp. nov.: taxonomy and comparison with related species. *Mycol. Res.* 108:795-805.

³Packer, A., and Clay, K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature.* 404:278-281.