Characterizing the Community of *Phytophthora* Species in an Oregon Forest Stream[1]

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Introduction

Phytophthora species are best known as pathogens of agricultural crops, or invasive pathogens destroying forests. Little is known about indigenous species, especially in wild ecosystems. Previous work showed that *Phytophthora* species are relatively abundant in natural streams in forests, but the species present are poorly characterized, and their ecology is essentially unknown. The aim of this work was to compare methods for the collection, identification, and enumeration of *Phytophthora* spp. in streams. Three methods of isolate collection were compared (leaf baits, pear baits, and filtration), and species identification was carried out using morphological and growth characters, and by DNA sequencing two regions of the genome (ITS rDNA, mitochondrial COX spacer region). Stream sampling was conducted at five locations in the Oak Creek drainage near Corvallis Oregon.

Oak Creek

Oak Creek starts in forest, then runs through an agricultural valley and an urban area, where it joins Marys River and then the Willamette River. Sampling Site 1 was at the beginning of the stream, in forest. Site 2 was downstream at the edge of the forest, before the first habitations. Site 3 was located in the plain with scattered housing, a few orchards, meadows and crop fields. Site 4 was in Marys River, just after Oak Creek flows into it. Site 5 was in the Willamette River, which flows through a fertile valley with many orchards, fields and forests. Tree species present at all sites included Douglas-fir, grand fir, big leaf maple, and red alder.

Methods

- Isolations were made on CARP + (CMA with 25ppm hymexazol (99 percent), 20ppm delvocid (50 percent natamycin salt), 200ppm ampicillin sodium salt, 10ppm rifampicin SV sodium salt and 30ppm benlate (benomyl 50WP)) then transferred to carrot agar.
- Isolates were collected every other week for 12 weeks, by filtration, leaf baiting, and pear baiting. 700 ml of stream water, in 50, 100, and 200ml

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portions, was passed through 5 μm nitrocellulose filters using a portable vacuum filtration unit. Filters were plated on CARP +.

- Two *Rhododendron* leaves, one tanoak leaf and three small pieces of Port-Orford-cedar foliage were placed in a mesh bag and immersed in the stream. After two weeks, leaves were removed, washed, and plated on CARP +. One d'Anjou Pear was also immersed in the stream. After three days, pears were removed, washed and tissues with lesions were plated on CARP +. All isolates were grown up on CMA and DNA was extracted from colonized agar plugs using a CTAB buffer protocol. The COX gene spacer region was PCR amplified using primers FMPH8 and 10, and sequenced in one direction. These single strands were aligned with other known (reference) sequences for initial species identification. Isolates not matching a reference isolate were then amplified and sequenced using ITS4 and 5, and blasted against the GenBank database.

Results

A total of 514 isolates were recovered from baits or filters. About 57 of these proved to be *Pythium* or zygomycetes. Another 20 isolates were not characterized because of sequencing problems or other difficulties. 437 *Phytophthora* isolates were analyzed. An average of 13 *Phytophthora* colonies were grown from each liter of stream water filtered.

Phytophthora Diversity

ITS and COX spacer DNA analysis distinguished at least 18 groups of isolates. These 18 groups formed nine clusters.

- Four of the clusters matched known species (*P. citricola, P. gonapodyides*, *P. pseudosyringae* and *P. syringae*).
- Three clusters contain groups of isolates that match and/or whose sequence is very similar to a known species (*P. cambivora*, *P. megasperma and P. sp. "Pg chlamydo"*).
- Two clusters contain groups of isolates that are similar but not identical to a known or unnamed species (*P. europaea* and Clade 6 "Kalamazoo").
- More than 90 percent of all isolates were in ITS clade 6: *P. gonapodyides, P. megasperma*, *P.sp. "Pg chlamydo"*, and unnamed related groups ("Kalamazoo" types).

Capture Method

Water filtration yielded more different species groups than either leaf baits or pears baits. *P. megasperma* and related isolates were only recovered from filters. On the other hand, *P. cambivora* and variant group and *P. syringae* were only recovered from baits (Table 1).

Table 1ð Recovery of *Phytophthora* species groups/clusters on filters, leaf baits, and pears.

	Filter	Leaf	Pear	Total
P. cambivora cluster	0	3	6	9
P. citricola	7	11	5	23
Clade 6 "Kalamazoo" cluster	41	15	26	82
P. europaea cluster	2	0	1	3
P. gonapodyides	92	74	20	186
P. megasperma cluster	7	0	0	7
P. sp. "*Pg chlamydo"* cluster	48	66	8	122
P. pseudosyringae	0	0	1	1
P. syringae	0	1	3	4
All Phytophthoras	197	170	70	437

Sample Time and Location

Phytophthora species varied by sample time and location within the watershed (Tables 2 and 3). *Phytophthora syringae* was recovered from 3 different locations but only at one June sample time. *P. cambivora* and cluster isolates and *P. europaea* cluster isolates came only from sample sites 1 and 2. In contrast, *P. citricola* was recovered only from downstream sites, late in the season, while *P. megasperma* and cluster isolates came from downstream sites early in the sampling period. The clade 6 groups (*P. gonapodyides*, *P.sp. "Pg chlamydo"*, and the cluster of variants similar to P. "Kalamazoo") tended to dominate at all locations and sample times.

Table 2ð Recovery of *Phytophthora* species group/clusters by sampling time, Juneï August 2006.

	Site1	Site2	Site3	Site4	Site5	Total
P. cambivora cluster	7	2	0	0	0	9
P. citricola	0	0	4	17	2	23
Clade 6 "Kalamazoo" cluster	1	9	30	26	16	82
P. europaea cluster	3	0	0	0	0	3
P. gonapodyides	37	55	52	32	10	186
P. megasperma cluster	0	2	2	2	1	7
P. sp. "*Pg chlamydo*" cluster	4	58	25	30	5	12
P. pseudosyringae	0	1	0	0	0	1
P. syringae	0	1	1	2	0	4
All Phytophthoras	52	128	114	109	34	437

Table 3ð Recovery of *Phytophthora* species group/clusters by sampling location in Oak Creek and downstream sites.

	Time1	Time2	Time3	Time4	Time5	Time6	Total
P. cambivora cluster	0	0	0	1	6	2	9
P. citricola	0	0	1	1	7	14	23
Clade 6 “Kalamazoo” cluster	6	2	7	22	14	31	82
P. europaea cluster	0	0	0	2	0	1	3
P. gonapodyides	49	33	29	21	16	38	186
P. megasperma cluster	4	2	1	0	0	0	7
P. sp. “*Pg chlamydo*” cluster	10	16	22	21	23	30	122
P. pseudosyringae	0	0	1	0	0	0	1
P. syringae	0	4	0	0	0	0	4
All Phytophthoras	69	57	61	68	66	116	437

Conclusions:

- Water filtration provided more isolates and more species than baiting and also enabled quantification of propagules.
- 12 to 20 *Phytophthora* propagules/liter of water were usually detected.
- At least 18 different groups of isolates were distinguished.
- 12 groups did not match named species.
- *P. gonapodyides* was most numerous, followed by *P.sp. “Pg chlamydo”* and its variants (cluster), and another unidentified group of taxa from ITS clade 6.
- The *Phytophthora* community changed through the season, and differed at different locations in the watershed.

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