Phytophthora Species Associated With Tanoak Stem Cankers in Southwestern Oregon[1]

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Abstract

From 2001 through 2006 stem cankers on tanoak (*Lithocarpus densiflorus*) were sampled during surveys to detect and eradicate *Phytophthora ramorum* from forests in southwestern Oregon. Pieces of bark from stem canker margins were plated on cornmeal agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifampicin. *P. ramorum* was usually identified on the isolation plates by the presence of characteristic hyphae, chlamydospores, and sporangia. Other colonies resembling *Phytophthora* were isolated into pure culture and identified by examination of morphological features and by DNA sequence analysis. *Phytophthora* species other than *P. ramorum* isolated from tanoak stem cankers include *P. cambivora*, *P. cinnamomi*, *P.* species "Pgchlamydo," *P. gonapodyides*, *P. nemorosa*, *P. pseudosyringae*, and *P. siskiyouensis*. Additional isolates may represent new species of *Phytophthora* and have not yet been identified. Selected isolates were tested for pathogenicity by inoculating healthy tanoak stems. Lesion margins were plated for re-isolation of the pathogen.

Introduction

Since 2001 the Oregon Department of Forestry (ODF) has conducted an intensive quarantine and eradication effort in southwestern Oregon to limit the spread of *Phytophthora ramorum*, cause of sudden oak death (SOD). Diseased tanoak (*Lithocarpus densiflorus*) trees were sampled to verify infection by *P. ramorum*. In some cases sampled infections yielded a species of *Phytophthor*a other than *P. ramorum*. We identified these species and tested some of them for their pathogenicity on tanoak.

Materials and Methods

Aerial surveys were conducted by the Oregon Department of Forestry twice yearly (2001 through 2006) to locate dead or dying tanoak trees. Cause of death or disease was determined by follow-up ground surveys. Cankers or lesions in tanoak stems were sampled for the presence of *P. ramorum* by plating bark from the leading edge in corn meal agar amended with 10 ppm Na-natamycin, 200 ppm Na-ampicillin, and 10 ppm rifampicin (CARP). *Phytophthora* colonies were purified by re-plating on fresh CARP and then sub-cultured on corn meal agar amended with 20 ppm β-

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sitosterol (CMAβ) for DNA extraction and long-term storage. Isolates were stored at room temperature as agar plugs in sterile de-ionized water.

For morphological identification isolates from 2006 were grown on 15 percent clarified V8 agar amended with 20 ppm β-sitosterol (V8S). Plugs from colony margins were transferred into stream water to generate sporangia. Isolates which did not form oogonia in single culture on V8S were paired with standard A1 and A2 tester strains on V8S to generate oospores and determine mating type. Structures for microscopic observation were fixed and preserved in 3.7 percent formaldehyde. Isolates were identified by comparison of features with published descriptions (Brasier and others 2003, Hansen and others 2003, Jung and others 2003, Reeser and others 2006, Waterhouse 1963). Representative isolates from each species identified were selected for DNA sequencing. DNA extracted from mycelium grown on CMAβ was used as template to amplify nuclear rDNA internal transcribed spacer (ITS) for sequencing. ITS sequences were compared with Genbank database using BLAST Search to aid identification.

Isolates from 2001 through 2005 were identified by sequencing the mDNA COX spacer and comparing with sequences of known reference isolates (Frank Martin, personal communication). Representatives from each group identified were confirmed by morphological examination as described above.

Five isolates of *P. cambivora,* four isolates of *P. gonapodyides,* one isolate of *P. nemorosa,* and one isolate of *P. siskiyouensis* were inoculated into green twigs of tanoak seedlings by placing a CMAβ culture plug over a pinprick wound and sealing with parafilm. Each inoculated twig also received an agar control. Lesion size was measured after 10 to 15 days, and lesion margins were plated on CARP for re-isolation of the pathogen.

One isolate each from *P. cambivora, P. cinnamomi, P. gonapodyides, P. nemorosa, P. siskiyouensis,* and *P.* species 'Pgchlamydo' was tested for pathogenicity on tanoak stems in the field. A 5 mm diameter plug from the margin of a V8S culture was placed in a hole in the bark, covered with wet cheesecloth and sealed with aluminum foil and duct tape. Each isolate was inoculated into five different stems. Each stem received three different isolates and an agar control. After four weeks bark was removed to reveal lesion development. Lesions were measured (length by width), and pieces from four points on the lesion margin were plated in CARP to re-isolate.

Results

Results of tanoak stem canker isolations are listed in table 1. More than one half of the tanoak cankers sampled were culture negative for *Phytophthora*. *P. ramorum* and *P. nemorosa* were the species most frequently isolated. Eight additional species were identified, three of them being un-named ('Pgchlamydo') or undefined ('ilicis clade' sp.1 and sp.2). Each of these eight species occurred only rarely.

Infection in green twigs of tanoak seedlings was variable. All four isolates of *P. gonapodyides* tested produced relatively large lesions in green tanoak twigs. Only one of the five isolates of *P. cambivora* tested produced lesions in green tanoak twigs. The one isolate of *P. nemorosa* tested produced moderately sized lesions in

green tanoak twigs, and the one isolate of *P. siskiyouensis* tested did not form any lesion.

Table 1—*Phytophthora* species isolated from tanoak stem cankers sampled during sudden oak death surveys in southwest Oregon from 2001 through 2006.

Species isolated	**Number of stem cankers**
Negative culture for *Phytophthora*	575
P. cambivora A1	4
P. cinnamomi A2	1
P. species 'Pgchlamydo'	1
P. gonapodyides	6
P. nemorosa	102
P. pseudosyringae	3
P. 'ilicis clade' sp.1	1
P. 'ilicis clade' sp.2	1
P. ramorum	359
P. siskiyouensis	4
Total stem cankers sampled	1057

In contrast, when inoculated in stems of field-grown tanoak, *P. cambivora, P. cinnamomi, P. gonapodyides, P. nemorosa, P. siskiyouensis,* and *P*. species 'Pgchlamydo' were all pathogenic after 4 weeks, causing substantial bark lesions relative to agar control (table 2). In all cases, the species used for inoculation was recovered on re-isolation from lesion margins.

Table 2—Pathogenicity of selected isolates of *Phytophthora* from tanoak stems. Isolates were inoculated into tanoak stems in the field.

Isolate	**Species**	**n**	**mean lesion area (cm^2) [a]**	**s.d.**
control		12	0.2	0.10
9641.1	*P. cinnamomi*	5	11.5	10.48
9569	*P. siskiyouensis*	5	13.4	84.63
9675-BK	*P. cambivora*	5	16.5	5.55
9364	*P*. sp. 'Pgchlamydo'	5	18.1	6.13
9684.2	*P. nemorosa*	5	18.4	14.75
9616	*P. gonapodyides*	5	18.6	7.59

[a] lesion area computed as approximate rhomboid (l x w)/2 - inoculum area

Discussion

Prior to the recent SOD epidemic, no species of *Phytophthora* were known to cause cankers in forest grown tanoak. The discovery of *P. ramorum* as a pathogen of tanoak in California was quickly followed by the discovery of *P. nemorosa* and *P.*

pseudosyringae associated with tanoak cankers. Six years of diagnostic support of survey and detection for *P. ramorum* in tanoak forests of southwest Oregon has revealed the occurrence, at very low frequency, of at least five additional species of *Phytophthora* causing stem cankers in tanoak.

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