

***Phytophthora* Species Associated With Stem Cankers on Tanoak in Southwestern Oregon¹**

Paul Reeser,² Wendy Sutton,² and Everett Hansen²

Abstract

In effort to eradicate *Phytophthora ramorum* from Oregon forests, tanoak over its entire range in southwestern Oregon is surveyed intensively for stem disease. Pieces of bark from the leading edge of tanoak stem cankers were plated on cornmeal agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifamycin SV (CARP) to favor the isolation of *Phytophthora*. *Phytophthora ramorum* was usually identified on the isolation plates based on the presence of characteristic hyphae, chlamydospores, and sporangia. Hyphae from the leading edge of other colonies resembling *Phytophthora* were isolated into pure culture. Isolates were grown out to obtain morphological features and DNA extracts for identification. Selected isolates were tested for pathogenicity by inoculating stems of tanoak seedlings. *Phytophthora* species isolated from tanoak stem cankers include the following: *P. cambivora*, *P. cinnamomi*, *P. species* "Pg chlamydo," *P. gonapodyides*, *P. nemorosa*, and the proposed new species *P. siskiyouensis*. An additional isolate may represent a new species of *Phytophthora* that has not yet been described.

Key words: *Lithocarpus densiflorus*.

Introduction

Prior to activities associated with sudden oak death eradication efforts in southwestern Oregon little or nothing was known about *Phytophthora* species occurring on tanoak in this area. During the course of *Phytophthora ramorum* surveys other species of *Phytophthora* were obtained from diseased tanoak stems. This presented an opportunity to learn about the *Phytophthora* species associated with tanoak stem cankers and mortality.

Materials and Methods

Diseased tanoak trees were located by aerial or ground survey during *P. ramorum* detection efforts. Observations on crown condition (leafless, brown, fading, green) and canker characteristics (position on the stem, percent girdling, presence of bleed spots) of each diseased tanoak were recorded.

All suspect trees were sampled to confirm *P. ramorum* infection. Samples from the leading edge of stem cankers were plated in corn meal agar amended with 20 ppm Delovcid® (50 percent Na-natamycin), 200 ppm Na-ampicillin, and 10 ppm rifamycin

¹ A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

² Oregon State University, Botany and Plant Pathology, 2082 Cordley Hall, Corvallis, OR, USA, 97331; reeserp@science.oregonstate.edu.

SV (CARP). Isolates resembling *Phytophthora* species, but not characteristic of *P. ramorum*, were purified on fresh CARP, then transferred to corn meal agar amended with 20 ppm β -sitosterol (CMA β). Isolates were stored as mycelial plugs on CMA β in sterile water with and without chopped hemp seed.

Sporangia were produced in natural stream water (filtered to 5 μ m) from margins of colonies grown on 15 percent clarified V8 agar amended with 20 ppm β -sitosterol (V8S). Oogonia from homothallic isolates were produced on V8S in single culture. Heterothallic isolates were induced to form oogonia by pairing with standard A1 and A2 testers of *P. cinnamomi* on V8S. 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, Reeser and others 2006, Waterhouse 1963).

Representative isolates from each species identified were selected for DNA sequencing. DNA extracted from mycelium grown on CMA β 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.

Pathogenicity of selected isolates from each species was tested in green twigs of tanoak seedlings by placing mycelium grown on CMA β over a pinprick wound and covering with Parafilm[®]. After seven days lesions were measured and photographed. Leading edges of lesions were plated in CARP for re-isolation.

Results and Discussion

During 2006 stem cankers from 263 tanoak trees were sampled. Of these, 97 were culture negative for *Phytophthora* species and 123 were culture positive for *P. ramorum*. Other *Phytophthora* species isolated from tanoak stem cankers were *P. cambivora* (four isolates), *P. cinnamomi* (one isolate), *P. gonapodyides* (four isolates), *P. nemorosa* (31 isolates), an undescribed *Phytophthora* species (one isolate), and the proposed new species, *P. siskiyouensis* (three isolates). *Phytophthora* taxon “Pg chlamydo” was isolated from a tanoak stem canker in 2005.

Examination of tree and canker attributes suggested that there was no relationship between crown condition, or canker appearance, and *Phytophthora* species isolated. Based on the field observation data, cankers yielding other *Phytophthora* species were indistinguishable from cankers yielding *P. ramorum*. The geographic distribution of the isolates showed no apparent pattern for occurrence, except that all isolates of the proposed new species, *P. siskiyouensis*, were recovered from trees growing in coastal stream drainages.

Selected isolates of *P. cambivora*, *P. cinnamomi*, *P. gonapodyides*, *P. nemorosa*, an undescribed *Phytophthora* species, the proposed new species, *P. siskiyouensis*, and *P. taxon* “Pg chlamydo” were tested for pathogenicity in green tanoak stems. Four isolates of *P. gonapodyides* produced the largest lesions, followed by one isolate of *P. nemorosa* and one isolate of *P. cambivora*. Each pathogen was re-isolated from its respective lesion. The other species tested did not produce noticeable lesions when inoculated into green tanoak stems.

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