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Phytophthora species in tanoak trees, canopy-drip, soil, and streams in the sudden oak death epidemic area of south-western Oregon, USA †

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Abstract

Various *Phytophthora* species were recovered from tanoak trees, tanoak canopy drip, soils, and streams, which were sampled as part of a larger survey and management effort aimed at limiting the spread of *Phytophthora ramorum* Werres, De Cock & Man in't Veld (the causal agent of sudden oak death) in an epidemic area encompassing native forest and urbanised forest areas in south-western Oregon. Environmental samples were analysed by baiting with either green pear fruits or rhododendron and tanoak leaves. Tanoak bark samples and baits from environmental samples were plated on media semi-selective for the isolation of *Phytophthora* spp. After incidence of *P. ramorum* growing on isolation plates was recorded, other *Phytophthora* species growing on the isolation plates were sub-cultured for identification. DNA sequencing was used to identify the unknown *Phytophthora* species. A total of seventeen *Phytophthora* species and one *Halophytophthora* species were identified across all substrates. Over an 8-year period, *P. ramorum* was detected in cultures from 41% of samples from over 1600 diseased tanoak trees, while other *Phytophthora* species were detected in 14% of these samples. Of 5189 tanoak canopy drip samples collected over a 4-year period, *Phytophthora* species other than *P. ramorum* were detected in 106 samples (2.0%). Of 5967 soil samples baited over an 8-year period, *Phytophthora* species other than *P. ramorum* were detected in 71 samples (1.2%). *Phytophthora ramorum* was detected in ca. 10% of 642 stream samples over a 3-year period, and other *Phytophthora* species were detected in ca. 86% of these stream samples.

Keywords: Forest *Phytophthora; P. cactorum; P. cambivora; P. cinnamomi; P. gonapodyides; P. nemorosa; P. pseudosyringae; P. siskiyouensis; P.* taxon Pgchlamydo; *P.* taxon Salixsoil.

† *Based on a paper presented at the fifth meeting of the IUFRO working party S07-02-09, Phytophthora Diseases in Forests and Natural Ecosystems, 7 – 12 March 2010, Auckland and Rotorua, New Zealand.*

Introduction

Forests in south-western Oregon are undergoing an epidemic of sudden oak death (SOD) disease, which is caused by *Phytophthora ramorum* Werres, De Cock & Man in't Veld. The primary host for *P. ramorum* in this area is tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H.Oh), for which infection is lethal. The epidemic area encompasses native mixed evergreen-hardwood forest, forest plantation, urban fringe, and urbanised areas (see Hansen et al., 2008 for greater detail). This paper focuses on the diversity of other *Phytophthora* species identified in the course of survey and monitoring activities in support of the SOD eradication effort.

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SOD is being managed in Oregon, in part, by an effort to find and eradicate inoculum sources (Hansen et al., 2008). Diseased tanoaks are detected primarily by aerial survey and confirmed through ground survey culminating in isolation of the pathogen. *Phytophthora ramorum* (and other *Phytophthora* species encountered) is confirmed by culture (Hansen et al., 2008). After detection of the disease, infection centres are treated as quickly as possible to eradicate all known host plants within 15 – 30 m of any symptomatic host. Eradication treatments consist of cutting and burning vegetation (Kanaskie et al., 2006). The preferred treatment includes treating tanoak trees with herbicide (imazapyr; [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-nicotinic acid]) to prevent re-sprouting (Kanaskie et al., 2011, this issue).

Additional activities were undertaken to evaluate the effectiveness of eradication treatments, and to monitor possible sporulation, spread, or survival of the pathogen (Hansen et al., 2008). Rain traps were installed in eradication areas to monitor sporulation of *P. ramorum* during and after the eradication treatment. Soil surveys were conducted to monitor survival of *P. ramorum* in the soil after eradication treatment. Streams flowing out of eradication areas, and streams in nearby uninfested watersheds were monitored to detect *P. ramorum* spores moving out of eradication areas, and to provide for early detection of the pathogen in pre-symptomatic areas (Sutton et al., 2009). These survey and detection activities provided an opportunity also to systematically collect and identify other *Phytophthora* species occurring in the samples collected for monitoring *P. ramorum*.

The purpose of this publication is to bring together the results of these various survey efforts, some of which have been partially reported elsewhere, to develop an understanding of overall *Phytophthora* diversity and substrate specialisation in the forest ecosystem and adjacent forest-urban interface areas of south-western Oregon, USA.

Materials and Methods

Tanoak samples

Suspected *Phytophthora ramorum* infection centres were usually located by aerial survey to detect dead or dying tanoak. Ground surveys were also conducted around confirmed infected trees to delineate infection centres and establish boundaries for eradication (Hansen et al., 2008). To confirm infection, samples of bark from stem cankers of diseased tanoak trees were collected and plated on semi-selective agar medium CARP (Corn-meal agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifamycin SV). This work was conducted during 2002 – 2009.

Canopy drip

Rain traps were employed to monitor *Phytophthora* sporulation in the canopy. Rain traps consisted of plastic buckets (23.5 cm dia. by 22.5 cm deep) lined with disposable plastic bags and charged with about 300 mL deionised water. Tanoak and *Rhododendron macrophyllum* D.Don ex G.Don leaf baits (two each) were placed in each rain trap and exchanged at two-week intervals. Rain traps were placed to collect canopy drip under tanoak trees in areas distant from known infection, in known infested stands before eradication treatment began, in infested stands during eradication treatment and around the perimeter of treated areas when operations were complete. Trapping was continued in two phases from November 2006 through June 2010.

Soil samples

During 2002 – 2005 soil sampling plots encompassing ca. 0.3 – 0.4 ha each were established near the stumps of infected tanoak trees at the center of the eradication areas which ranged in size from ca. 1 ha to 6 ha. Soil samples were collected across a square grid with ca. 6.4 metres between samples and between 80 – 100 samples per plot, depending on constraints due to landform and size of eradication area. Each sample consisted of ca. 1 litre of soil collected to a depth of ca. 15 cm after removal of the litter layer. A total of 3067 individual soil samples were collected from 26 sampling plots in this period.

During 2008 – 2009 soil sampling plots were established around stumps of known infected tanoak trees. At each plot a 1-litre composite soil sample was collected from within 2 metres of the known infected stump. An additional 19 individual 1-litre soil samples were collected across a 0.02-hectare circular plot around the same stump. Samples were collected to a depth of ca. 15 cm after removal of the litter layer. A total of 2900 individual soil samples were collected from 145 sampling plots in this period. The spatial distribution of soil sampling locations in the sudden oak death quarantine zone is illustrated in Figures 1A and 1B.

A 0.5-litre portion of each soil sample was assayed for *Phytophthora ramorum*. Samples were placed in plastic bags and flooded with de-ionised water to a depth of 1 – 2 cm above the soil surface. For samples collected in 2002 and 2004 green pears (*Pyrus communis* L.) were used as bait and placed in the water for 7 d. For samples collected in 2003 and 2005 *Chamaecyparis lawsoniana* (A. Murr.) Parl. and *Rhododendron* sp. leaf pieces were used as bait and floated on the water for 3 d. For samples collected in 2008 and 2009 small pieces (ca. 1 mm x 10 mm including midrib) of *Rhododendron catawbiensis* Michx. leaves were placed in a cellulose fibre tea bag with a piece of plastic foam and floated on the water above the soil for 48 h.

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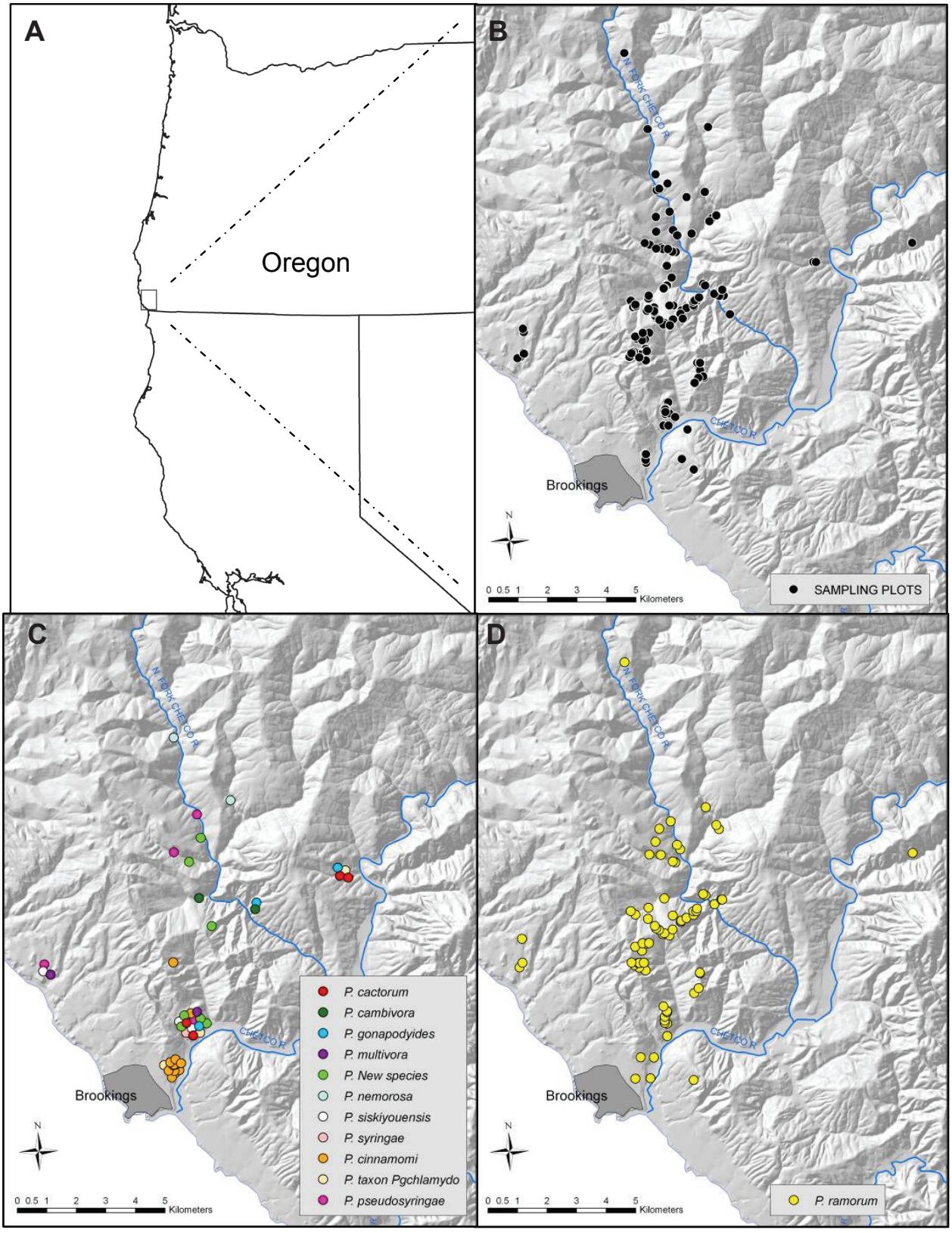

FIGURE 1: Distribution of soil sampling locations and *Phytophthora* species recovered from those locations in the 2008 – 2009 period. (A) Area locator; (B) Distribution of soil sampling locations in the Oregon sudden oak death epidemic area; (C) Distribution of *Phytophthora* species other than *P. ramorum*; and (D) Distribution of *P. ramorum*.

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Streams

Phytophthora species in water samples were detected using a baiting technique (Sutton et al., 2009; Reeser et al., 2011). In brief, streams in the sudden oak death quarantine zone in south-western Oregon were selected in areas considered to be at high risk for future infestation, including watersheds with concentrations of tanoak near the perimeter of infested areas. Bait stations (typically one per stream) were usually placed upstream from road crossings in the upper reaches of perennial streams draining watersheds ranging in area from 8 to 3634 ha. Tanoak and *R. macrophyllum* leaf baits (two each) were placed in open-weave nylon mesh bags and floated in relatively slow-moving water. Leaf baits were exchanged at two-week intervals. A total of 60 streams were monitored between 2002 and 2004, although not every stream was monitored over the entire period.

Isolation from baits

Leaf baits used in rain traps and streams and pear baits used in soils were rinsed in tap water to remove surface contamination. Petioles and symptomatic areas of leaf baits and firm lesion areas of green pear baits were excised. Leaf bait pieces used in soils were blotted dry without rinsing.

Prepared bait material was plated in CARP+ (CARP amended with 25 ppm hymexazol (99%) and 30 ppm benomyl (50WP). Isolation plates were incubated at 20 °C in the dark and examined at approximately 3 and 7 days. When *Phytophthora* species other than *P. ramorum* grew on isolation plates, hyphal tips were transferred to fresh CARP for confirmation of purity, then to CMAβ (Corn meal agar amended with 20 ppm β-sitosterol) for characterisation, DNA extraction, and storage. Colonised agar plugs were stored at room temperature in sterile de-ionised water with or without hemp seed pieces.

Identification of isolates

Phytophthora ramorum was routinely identified on the isolation plate based on characteristic hyphae, sporangia and chlamydospores (Werres et al., 2001). In many cases *P. nemorosa* E.M. Hansen & Reeser could also be identified on isolation plates when characteristic oogonia, antheridia, and blistered hyphal swellings were present (Hansen et al., 2003). For all other putative *Phytophthora* isolates, DNA was extracted from colonised agar plugs using a hexadecyl trimethyl-ammonium bromide (CTAB) buffer with ethanol precipitation protocol (Winton & Hansen, 2001) and analysed by either single-strand conformational polymorphism (SSCP) or one-way sequence of the mitochondrial cytochrome c oxidase (COX) spacer region.

We modified the SSCP fingerprinting protocol of Kong et al. (2003) by incorporating an additional marker locus and using fluorescent-labelling chemistry to allow quantitative matching of unknown isolates. The internal transcribed spacer (ITS) 1 region of nuclear ribosomal DNA (rDNA) was amplified with primers ITS6 and ITS7 (Cooke et al., 2000) labelled with hexachlorofluorescein (HEX) and 6-carboxyfluorescein (FAM), respectively, yielding an approximately 300 base pair (bp) product. The mitochondrial COX spacer region was amplified according to the method of Martin et al. (2004) with the modified primers FMPh8 and FMPh10 (http://www.ars.usda.gov/Research/docs.htm?docid=8737) labelled with HEX and FAM, respectively, yielding an approximately 400 bp product. Amplified products were mixed with formamide and GeneScan-500 (ROX) marker, heated to 95 °C for 3 min then cooled in ice for 5 min. Samples were run at 25 °C on an ABI 3100 Capillary Sequencer (Applied Biosystems, Carlsbad, CA, USA) with a 36 cm array and 4% GeneScan polymer with 10% glycerol and 1X Tris-borate-EDTA (TBE) buffer. Fluorescence was analysed with GeneScan software v. 3.7 (Applied Biosystems, Carlsbad, CA, USA) and electrophoretic mobility was reported as scan number. Maximum fluorescence values for each isolate were aligned manually with values generated from a set of reference isolates included in each sequencer run.

For COX spacer grouping, DNA was amplified using primers FMPh8 and FMPh10 as described above and sequenced in one direction on an ABI 3100 capillary sequencer at 25 °C. This yielded an approximately 400 bp product that was trimmed to about 300 bp for alignment. These single-strand sequences were aligned with in-house reference sequences for initial species identification. For isolates that did not match a reference isolate the ITS region was amplified using primers ITS4 (White et al., 1990) and DC6 (Cooke et al., 2000) for sequencing. The ITS sequences were aligned with in-house reference sequences then compared with the GenBank database using BLAST. Finally, isolates were grouped into species units if they were morphologically similar and belonged to the same well-supported terminal ITS and COX spacer clades. Names were assigned to isolates based on sequence similarity to validated reference isolates.

Results

Species of *Phytophthora* (or *Halophytophthora*) were recovered from nearly 20% of all samples collected. Species other than *P. ramorum* were identified in about 7% of the samples. Eighteen distinct taxa were identified, including seven species that were recovered from all four different substrates sampled (Table 1).

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TABLE 1: Incidence of *Phytophthora* species recovered from various substrates sampled during the course of survey, detection, and monitoring efforts related to managing the sudden oak death epidemic in south-western Oregon.

***Phytophthora* Species**	**Substrate**			
	Tanoak Cankers n[a]=1637	**Canopy Drip** n=5189	**Soil** n=5967	**Streams** n=642
P. ramorum	667	821	151	65
P. nemorosa	181	49	2	56
P. gonapodyides	15	1	6	133
P. cambivora	6	0	5	12
P. siskiyouensis	5	5	5	18
P. pseudosyringae	6	22	7	11
P. taxon Pgchlamydo [c]	4	9	7	214
P. New species 3[b]	3	17	10	1
P. cinnamomi	1	0	13	0
P. cactorum	0	1	8	0
P. multivora	0	0	6	0
P. plurivora	0	0	0	3
P. psychrophila	0	2	0	0
P. syringae	0	0	2	0
P. taxon Oaksoil [c]	0	0	0	6
P. taxon Salixsoil [c]	0	0	0	67
P. megasperma	0	0	0	2
Halophytophthora sp.	0	0	0	6

[a] *n refers to the number of individual samples for tanoak cankers, canopy drip and soils (171 eradication sites). For streams n refers to the number of two-week sample periods for 60 streams.*
[b] Phytophthora *New species 3 is discussed in Reeser et al. (2010).*
[c] *Species not formally described, discussed in Brasier et al. (2003).*

Tanoak trees

In the period 2002 – 2009, bark lesion samples from 1637 diseased tanoak trees were plated in semi-selective media for recovery of *Phytophthora* species. *Phytophthora ramorum* was detected in 667 of these samples. Other *Phytophthora* species were also detected at much lower frequency (see Table 1), amounting to 221 isolations of 8 different species. The pathogenicity of these *Phytophthora* species to tanoak was reported previously (Reeser et al., 2008).

Canopy drip

In the period 2006 – 2009, a total of 5189 tanoak canopy drip samples were collected and baited for the detection of *Phytophthora* species. *Phytophthora ramorum* was detected in 821 samples and other *Phytophthora* species were detected in 106 samples (Table 1).

Soils

In the period 2002 – 2005 there were 3067 soil samples taken from 26 different sudden oak death eradication sites. Some of these sites were sampled repeatedly in this period and some were sampled just once. *Phytophthora ramorum* was detected at 9 of these eradication sites, and *P. ramorum* and other *Phytophthora* species were detected together at three of these eradication sites. There were no *Phytophthora* species detected at the remaining 14 sites. In the period 2008 – 2009 there were 2900 soil samples taken from 145 eradication sites. *Phytophthora ramorum* was detected at 53 of these eradication sites, other *Phytophthora* species were detected at 18 of these sites, and *P. ramorum* and other *Phytophthora* species were detected together at nine of these sites. There were no *Phytophthora* species detected at the remaining 65 sites. Table 1 lists the number of soil samples in which each species of *Phytophthora* was detected for the cumulative 5967 individual soil samples. The spatial distribution of *Phytophthora* species recovered from soil is shown in Figures 1C and 1D.

Streams

Data for streams were previously reported as part of a larger study of *Phytophthora* in streams of Oregon and

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Alaska, USA (Reeser et al., 2011). Species recovered in south-western Oregon, USA are shown in Table 1 for comparison with occurrence in the other substrates shown.

Discussion

Hansen et al. (2006) reported at least fifteen species of *Phytophthora* from trees, soil or streams in oak forests in Oregon and California, USA. Here we report on 17 species of *Phytophthora*, and one species of *Halophytophthora* from habitats in south-western Oregon alone. Seven of these species (*P. ramorum*, *P. nemorosa*, *P. gonapodyides* (Petersen) Buisman, *P. siskiyouensis* Reeser & E.M. Hansen, *P. pseudosyringae* T. Jung & Delatour, *P.* taxon Pgchlamydo, and *P*. New species 3 were present in at least one sample in all substrates examined.

Very little is known about the occurrence of *Phytophthora* species in native forest ecosystems. Activities associated with survey, detection and monitoring of *P. ramorum* in south-western Oregon provided a rare opportunity to collect data on the occurrence of other *Phytophthora* species in this limited forest area and in the adjacent forest-urban interface. Although the sampling procedures were biased towards *P. ramorum*, and methods were very different for the different substrates, the *Phytophthora* assemblage discovered is surprising for its diversity and ubiquity. While nine species were recovered from cankers on tanoak, each of these, and the remaining nine taxa were also identified from substrates not associated with specific disease symptoms.

It is interesting to contrast the distribution and relative abundance of the invasive *P. ramorum* with the other species. While *P. cinnamomi* Rands, and possibly other species, have been introduced to this area, they are not behaving as invasives. They are encountered infrequently and usually in the absence of overt disease symptoms. Even when killing trees, they are found on scattered single trees, in contrast to the expanding mortality centres associated with *P. ramorum*.

Phytophthora ramorum was the species most frequently recovered from tanoak bark lesions, infected tanoak canopy drip, and from soils in infested areas. This is consistent with presence of the species in the environment as an invasive pathogen, and the targeting of infested areas for the sampling. *Phytophthora ramorum* was less frequently encountered in streams since many of the streams sampled were not in infested areas.

Phytophthora nemorosa was the species second most commonly associated with tanoak bark cankers. It is interesting to note that *P. nemorosa* was relatively common in streams and in canopy drip of trees which showed bark infection by *P. ramorum*, but was relatively rare in soils around these trees. This previously unknown species was described only recently (Hansen et al., 2003) when it was found in association with bark cankers in oaks and tanoaks being sampled for *P. ramorum*. It is still not certain whether *P. nemorosa* is exotic or native to California and Oregon where it was first discovered.

It was something of a surprise to find *P. gonapodyides* as the third most common species of *Phytophthora* associated with tanoak cankers in Oregon. The association is not incidental, as pathogenicity has been demonstrated (Reeser et al., 2008). Historically *P. gonapodyides* has not been considered a serious plant pathogen, although it is relatively common in soils and water in both Europe and North America (Brasier et al., 2003), and its occurrence here and in beech forests in Europe (Jung 2008) suggests a re-appraisal of this species as a pathogen.

Phytophthora siskiyouensis is another recently described species (Reeser et al., 2007) which was discovered as a consequence of *P. ramorum* survey and detection activities in south-western Oregon. It was originally found in soil and stream water samples. Later it was found once in association with blighted myrtlewood shoots growing near ground level, and rarely in tanoak bark cankers. *Phytophthora siskiyouensis* is of great concern as a pathogen of Italian alder (*Alnus cordata* (Loisel.) Duby) in California, and European alder (*Alnus glutinosa* (L.) Gaertn in Australia (Rooney-Latham et al., 2009). Although found in soil and streams in an area where red alder (*Alnus rubra* Bong.) is abundant in the landscape, and demonstrated experimentally capable of infecting red alder stems, *P. siskiyouensis* has not to date been observed causing disease on red alder in the area where these two species are known to overlap.

Phytophthora pseudosyringae T. Jung & Delatour was not known outside Europe (Jung et al., 2003) before its discovery in California and Oregon during surveys for *P. ramorum* (Wickland et al., 2008). More recently it has been found widespread in forest streams in western North Carolina (Hwang et al., 2007). In our experience, isolates from bait pieces in CARP and CARP+ are slow-growing and emerge days later than other *Phytophthora* species. Because of this, we may be underestimating the occurrence of this species in the baited substrates (canopy drip, soils and streams).

Phytophthora taxon Pgchlamydo was recovered quite frequently from stream baits. While this abundance in streams suggests an 'aquatic' habit, this species was also found in some soil and canopy drip samples, and was recovered from tanoak bark cankers in four different trees. Pathogenicity in tanoak was demonstrated by Reeser et al., (2008). Recovery of *P.* taxon Pgchlamydo from canopy drip was unexpected.

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This could be due to an unrecognised aerial phase, or to occasional soil splash into the rain traps despite low rates of recovery directly from soil samples.

Phytophthora New Species 3 (Reeser et al., 2011) is the third new species from phylogenetic ITS clade 3 (Cooke et al., 2000) to be discovered in western Oregon. The first of these, *P. ilicis* Buddenhagen and Young, was described by Buddenhagen and Young (1957) infecting the exotic English holly (*Ilex aquifolium* L.) in plantations in western Oregon and Washington. The species is presumed to be endemic to this area, but has not been found on any native host, nor in any of the extensive stream sampling that has occurred in recent years. The second of these, *P. nemorosa,* was described in 2003 (Hansen et al., 2003).

Phytophthora cinnamomi is a serious forest pathogen in temperate forests in Western Australia and around the world (Hansen, 2007). In the forest area of south-western Oregon that we studied, *P. cinnamomi* was recovered from a tanoak stem canker on only one occasion. It was not found either in canopy drip or in streams. While this was the *Phytophthora* species third most frequently encountered in soil samples, these samples were all from forest-urban interface lands in Brookings, OR (data not shown). One forested city park in particular is characterised by ornamental plantings of *Rhododendron* species, and most of the soil samples yielding *P. cinnamomi* were collected along a high-traffic foot path in this park.

Phytophthora multivora P.M. Scott & T. Jung is a recently described species, first recognised in Western Australia from isolates previously identified as *P. citricola* Sawada, and also comprising isolates from Europe, Canada, South Africa, and Asia (Scott et al., 2009). According to Jung and Burgess (2009) the isolates designated as CIT3 in work by Oudemans and others (1994) should be re-classified as *P. multivora*. This group includes isolates from nine different host genera in California. Our finding of *P. multivora* in soil samples constitutes a first report of this species from Oregon.

Phytophthora plurivora T. Jung & T.I. Burgess is another recently described species derived from isolates previously identified as *P. citricola*. *Phytophthora plurivora* is found on a wide range of hosts throughout Europe and North America in association with root and collar rots, bark cankers and with dying shoots and necrotic leaves in a large number of woody hosts (Jung & Burgess 2009). *Phytophthora plurivora* is found regularly in Oregon ornamental plant nursery settings (Grunwald, N. United States Department of Agriculture, Agricultural Research Service, Corvallis OR, personal communication, August 17, 2010). We found *P. plurivora* only rarely in streams, and not at all in the other substrates sampled. The occurrence of this species was not associated with any observable forest decline or disease situation.

Phytophthora psychrophila T. Jung & E.M. Hansen was described from baited soil beneath declining oak trees in Bavaria, Germany and southern France (Jung et al., 2002). However, the species was only weakly pathogenic to roots of *Quercus robur* L. in soil infestation tests. In contrast to the apparent soil-borne nature of the discovery in Europe, *P. psychrophila* encountered in this study was found only in baits from rain traps under tanoak trees. Prior to this discovery, the species was not known to occur in North America.

Phytophthora cactorum (Lebert & Cohn) Schroeter, *P. cambivora* Pethybridge & Lafferty, and *P. syringae* (Klebahn) Klebahn are well-known as pathogens of trees and shrubs in agricultural and ornamental settings worldwide (Erwin & Ribiero, 1996). Recent research is improving our understanding of the role of these species in the forest setting. *Phytophthora cactorum* was found quite frequently in plant and soil surveys in beech (*Fagus sylvatica* L.) forests in Europe (Jung et al., 2008), but not in a survey of oak (*Quercus* spp.) forests in the eastern United States (Balci et al., 2007). In this study we recovered *P. cactorum* from only eight soil samples and only one canopy drip sample. *Phytophthora cambivora* is considered to be relatively common in forest soils in Europe, Argentina and the eastern United States (Balci et al., 2007). It has been known to occur in forests in Oregon and is the cause of a significant root and stem disease in Golden Chinquapin (*Crysolepis chrysophylla* (Douglas ex Hook.) Hjelmqvist) (Saavedra et al., 2007). *Phytophthora cambivora* was also associated with tanoak stem cankers on six occasions and its pathogenicity to tanoak was demonstrated (Reeser et al., 2008). *Phytophthora syringae* has been reported from forests in Europe showing oak decline and from plantation settings in New Zealand. It has been found in forest soils in Argentina (Greslebin et al., 2005) and now, in our study, was found rarely in soil samples. *Phytophthora syringae* has not been detected in surveys in eastern United States forests (Balci et al., 2007).

Three additional *Phytophthora* species and several isolates of *Halophytophthora* (Reeser et al., 2011) were identified only in streams in south-western Oregon. *Phytophthora megasperma* Drechsler*, P*. taxon Oaksoil and *P*. taxon Salixsoil are closely related members of ITS clade 6, along with other primarily aquatic species, including in this survey *P. gonapodyides* and *P.* taxon Pgchlamydo. Their ecological roles in this forest ecosystem remain unknown.

Conclusions

This report constitutes one of a few 'ecosystem' studies of *Phytophthora* species diversity and distribution

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based on extensive sampling from a variety of substrates. It begins to give us a picture of diversity and relative abundance of *Phytophthora* species in a limited area. Although the sampling scheme was driven by the search for *P. ramorum,* and is necessarily biased by that constraint, we took advantage of the opportunity to look behind this invasive species, and get a glimpse at the 'background' *Phytophthora* flora in the forest and adjacent forest-urban interface.

Phytophthora species were relatively rare in the forest landscape, except for the invasive species *P. ramorum*. Certain *Phytophthora* species (e.g. *P. gonapodyides* and *P*. taxon Pgchlamydo) were especially abundant in streams and might be regarded as aquatic residents.

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References

Balci, Y., Balci, S., Eggers, J., MacDonald, W. L., Juzwik, J., Long, R. P., & Gottschalk, K. W. (2007). *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. *Plant Disease*, *91*, 705-710.

Brasier, C. M., Cooke, D. E. L., Duncan, J. M., & Hansen, E. M. (2003). Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides-Phytophthora megasperma* ITS Clade 6 which tend to be high temperature tolerant and either inbreeding or sterile. *Mycological Research, 107*, 277-290.

Buddenhagen, I. W., & Young, R., A. (1957). A leaf and twig disease of English holly caused by *Phytophthora ilicis* n. sp. *Phytopathology*, *47*, 95-101.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., & Brasier, C. M. (2000). A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology, 30*, 17-32.

Erwin, D. C., & Ribeiro, O. K. (1996). *Phytophthora Diseases Worldwide*. St Paul, MN, USA: APS Press.

Greslebin, A., Hansen, E. M., Winton, L. M., & Rachenberg, M. (2005). *Phytophthora* species from soil and streams of declining *Austrocedrus chilensis* forests in Patagonia, Argentina. *Mycologia*, *97*, 218-228.

Hansen, E. (2007). Alien forest pathogens: *Phytophthora* species are changing world forests. *Boreal Environment Research, 13,* 33-41.

Hansen, E., Kanaskie, A., Prospero, S., McWilliams, M., Goheen, E. M., Osterbauer, N., Reeser, P., & Sutton, W. (2008). Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. *Canadian Journal of Forest Research*, *38*, 1133-1143.

Hansen, E., Reeser, P. W., Davidson, J. M., Garbelotto, M., Ivors, K., Douhan, L., & Rizzo, D. M. (2003). *Phytophthora nemorosa*, a new species causing cankers and leaf blight of forest trees in California and Oregon, U.S.A. *Mycotaxon*, *88,* 129-138.

Hansen, E., Rizzo D, & Garbelotto M. (2006). *Phytophthora* species from oak and tanoak forests in California and Oregon. *Proceedings of the Sudden Oak Death Second Science Symposium: State of Our Knowledge, January 18-21 2005 Monterey, CA*. General Technical Report PSW-GTR-196 (pp. 63-65). Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture.

Hwang, J., Jeffers, S., N., & Oak, S. W. (2007). Occurrence and distribution of *Phytophthora pseudosyringae* in forest streams of North Carolina. *Phytopathology*, *97*, S49.

Jung, T. (2008). Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology*, *39*, 73-94.

Jung, T., & Burgess T. I. (2009). Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia, 22*, 95-110.

Jung, T., Hansen EM, Winton L, Osswald W, & Delatour C. (2002). Three new species of *Phytophthora* from European oak forests. *Mycological Research, 106*, 397-411.

Jung, T., Nechwatal J., Cooke, D. E. L., Hartmann, G., Blaschke, M., Osswald, W. F., Duncan, J. M., & Delatour, C. (2003) *Phytophthora pseudosyringae* sp. nov., a new species

ISSN 0048 - 0134 (print)
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causing root and collar rot of deciduous trees species in Europe. *Mycological Research, 107*, 772-789.

Kanaskie, A., Osterbauer, N., McWilliams, M., Goheen, E., Hansen,E., & Sutton, W. (2006). Eradication of *Phytophthora ramorum* from Oregon Tanoak Forests - Status after Three Years. *Proceedings of the Sudden Oak Death Second Science Symposium: State of Our Knowledge, January 18-21 2005 Monterey, CA.* General Technical Report PSW-GTR-196 (pp. 489-490). Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture.

Kanaskie, A., Hansen, E., Sutton, W., Reeser, P., & Choquette, C. (2011). Application of phosphonate to prevent sudden oak death in southwest Oregon tanoak (*Notholithocarpus densiflorus*) forests. *New Zealand Journal of Forest Science, 41S,* in press.

Kong, P., Hong, C., Richardson, P. A., & Gallegly, M. E. (2003). Single-strand-conformation polymorphism of ribosomal DNA for rapid species differentiation in genus *Phytophthora*. *Fungal Genetics and Biology, 39,* 238-249.

Martin, F. N., Tooley, P. W., & Blomquist, C. (2004). Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. *Phytopathology, 94,* 621-631.

Oudemans, P., Förster, H., & Coffey, M. D. (1994). Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsici* and *P. citrophthora*. *Mycological Research, 98*,189-199.

Reeser, P. W., Hansen, E. M., & Sutton, W. (2007). *Phytophthora siskiyouensis*, a new species from soil, water, myrtlewood (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*) in southwestern Oregon. *Mycologia, 99,* 639-643.

Reeser, P. W., Sutton W, & Hansen, E. M. (2008). *Phytophthora* species causing tanoak stem cankers in southwestern Oregon. *Plant Disease, 92*, 1252.

Reeser, P. W., Hansen, E. M., Sutton, W., Remigi, P., & Adams, G. C. (2011). *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia*, *103*, 22-35.

Rooney-Latham, S., Blomquist, C. L., Pastalka, T., & Costello, L. (2009). Collar rot on Italian alder trees in California caused by *Phytophthora siskiyouensis*. Online. *Plant Health Progress* doi:10.1094/PHP-2009-0413-01-RS.

Saavedra, A., Hansen. E. M., & Goheen, D.J. (2007). *Phytophthora cambivora* in Oregon and its pathogenicity to *Chrysolepis chrysophylla*. *Forest Pathology, 38*, 404-419.

Scott, P. M., Burgess, T. I., Barber, P. A., Shearer, B. L., Stukely, M. J. C., Hardy, G. E. St.J., & Jung, T. (2009). *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia, 22*, 1-13

Sutton, W., Hansen, E. M., Reeser, P. W., & Kanaskie, A. (2009). Stream monitoring for detection of *Phytophthora ramorum* in Oregon tanoak forests. *Plant Disease, 93*, 1182-1186.

Werres, S., Marwitz, R., Man in't Veld, W. A., de Cock, A W. A. M., Bonants, P. J. M., de Weerdt, M., Themann, K., Ilieva, E., & Baayen, R.P. (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum. Mycological Research, 105*, 1155-1165

White, T. J., Bruns, T., Lee, S., & Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.) *PCR Protocols: a guide to methods and applications.* (pp. 315-322). San Diego, CA< USA: Academic Press.

Wickland, A. C., Jensen, C. E., and Rizzo, D. M. (2008). Geographic distribution, disease symptoms and pathogenicity of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in California, USA. *Forest Pathology, 38*, 288-298.

Winton, L. M., & Hansen, E. M. (2001). Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction. *Forest Pathology, 31*, 275-283.

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