North American Fungi

Volume 8, Number 7, Pages 1-8
Published May 11, 2013

***Phytophthora pluvialis*, a new species from mixed tanoak-Douglas-fir forests of western Oregon, U.S.A.**

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Reeser, P., W. Sutton, and E. Hansen. 2013. *Phytophthora pluvialis*, a new species from mixed tanoak-Douglas-fir forests of western Oregon, U.S.A. *North American Fungi* 8(7): 1-8. <http://dx.doi.org/10.2509/naf2013.008.007>

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Abstract: A new species, *Phytophthora pluvialis* is described. *P. pluvialis* has been recovered from streams, soil and canopy drip in the mixed tanoak-Douglas-fir forest in Curry County, Oregon, and in two additional streams in other areas of western Oregon. It has been found only rarely in association with twig and stem cankers on tanoak but not with any other plant host. The earliest isolate of *P. pluvialis* was from soil in 2002. *P. pluvialis* is classified in ITS Clade 3.

Key words: Oomycetes, canopy drip, rainwater, streams, soil, environmental *Phytophthora*

Introduction: In 2011 we reported the occurrence of *Phytophthora* “New species 3” (Reeser et al. 2011b) in a stream from southwestern Oregon. Subsequently we

reported the recovery of additional isolates of this species from a variety of environmental substrates and one plant species, and proposed to describe it as a new species (Reeser et al. 2011a).

This new species, *Phytophthora pluvialis*, is described below.

Materials and Methods: Methods for recovering and analyzing *Phytophthora* isolates from various substrates were reported in Reeser et al. (2011a) and are summarized briefly here. Streams were sampled by immersing tanoak (*Notholithocarpus densiflorus*) and *Rhododendron macrophyllum* whole leaf baits for 2 wk. Soils were sampled by flooding with de-ionized water and floating leaf bait pieces (*Rhododendron* sp. and *Chamaecyparis lawsoniana*) for 2–4 d or semi-immersing whole green pears for up to 1 wk. Isolates of *Phytophthora* were recovered from leaf bait or pear bait pieces plated in cornmeal agar (CMA) amended with 200 ppm Na-ampicillin, 10 ppm rifamycin-SV, 10 ppm natamycin, 30 ppm Benlate 50WP and 25 ppm Hymexazol. Additional isolates were recovered from the margins of tanoak twig and bark lesions plated in CMA amended with 200 ppm Na-ampicillin, 10 ppm rifamycin-SV, and 10 ppm natamycin. Purified isolates were grown on CMA amended with 20 ppm β -sitosterol to obtain mycelium for DNA extraction and for storage as agar plugs in sterile de-ionized water with and without hemp seed pieces. Cultures for morphological study were grown on V8S (15% clarified V8 neutralized with 14.3 mg/mL CaCO_3 , 1.5% Bacto™ agar and 20 ppm β -sitosterol). Oogonia and oospores were produced in 20–30 d cultures on V8S and fixed and preserved in 3.7% formaldehyde. Sporangia were produced by transferring disks from margins of mycelium grown on V8S into natural stream water. Sporangia were fixed and preserved in 3.7% formaldehyde. Specimens were mounted in the fixative and viewed with brightfield illumination on a Zeiss standard microscope (sporangia at 40X, oogonia at 63X) and measured with an eyepiece micrometer. DNA extracts from *P. pluvialis* isolates were amplified with primers DC6 and ITS4 (Cooke et al 2000). Amplified products were sequenced with DC6, ITS2, ITS3 and ITS4 as sequencing

primers (White et al 1990). Published sequences of other *Phytophthora* species were downloaded from GenBank and aligned with Clustal X (1.81) (Thompson et al. 1997). The phylogenetic tree was generated with Clustal X (1.81) and displayed with TreeView (Win32) (Page 1996). The mitochondrial *cox* I and *cox* II 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>). Mitochondrial *cox* spacer sequences were aligned with Clustal X (1.81) and compared with in-house references.

Results: All *Phytophthora pluvialis* isolates examined yielded identical ITS sequences. *P. pluvialis* resides in ITS Clade 3 (Cooke et al. 2000) along with *P. ilicis* Buddenhagen & Young, *P. nemorosa* E.M. Hansen & Reeser, *P. pseudosyringae* T. Jung & Delatour, and *P. psychrophila* T. Jung & E.M. Hansen. It is readily distinguished by ITS DNA sequence from related species in Clade 3 (Figure 1). Phylogenetic analysis of *cox* spacer sequences supported this placement (data not shown). Based on *cox* spacer sequence analysis we have identified seven unique haplotypes (Table 2) among the 38 isolates of *P. pluvialis* analyzed.

Taxonomy

Phytophthora pluvialis, Reeser, Sutton and E Hansen., *sp. nov.* Figs. 2–3.
MycoBank MB803172

Phytophthora pluvialis is homothallic, forming oogonia in single-strain culture. Oogonia are terminal, smooth, globose, $32 \pm 2.8 \mu\text{m}$ in diameter with amphigynous antheridia (ca $17 \times 13 \mu\text{m}$). Oospores are globose, aplerotic, $28 \pm 1.8 \mu\text{m}$ in diameter. Sporangia formed in water are ovoid or slightly irregular (occasionally bifurcate), semi-papillate, terminal or sub-terminal, and partially caducous with medium length ($<5\text{--}22 \mu\text{m}$) pedicels. Sporangia averaged

61 x 42 μm (isolate means 53–67 x 39–45 μm). Length to breadth ratio averaged 1.47 (isolate means 1.35–1.58). Sporangiphores are simple or sympodial. Hyphal swellings formed in water are globose and intercalary with radiating hyphae to form loose networks. Chlamydospores are not formed in agar. The colony pattern on carrot agar was angular and petaloid, with hyphae appressed. Radial growth on carrot agar was about 1.2–1.4 mm/d at 15 C, with a growth optimum 15–20 C. Slow growth was evident at 5 C, and no growth was observed above 25 C.

Holotype. OSC 147940, dried culture from OSU LC-9.2-020508, isolated in February, 2008 from baited rainwater near Brookings, OR (N42.11185, W-124.25657).

Additional specimens examined.

Subcultures of LC-9.2-020508 are deposited at American Type Culture Collection MYA-4930. DNA sequences derived from the holotype are deposited in GenBank (Table I). Isolates examined for this study are listed in Table 1.

Etymology. “*pluvialis*” refers to the rain-associated canopy drip from which many isolates were recovered.

Commentary. *Phytophthora pluvialis* can be distinguished from all other *Phytophthora* species not in ITS Clade 3 by the combination of mostly ovoid, semi-papillate, partially caducous sporangia with variable length pedicels between 5 and 20 μm borne on sporangiphores in monochasial sympodia, and terminal, globose, non-ornamented oogonia with amphigynous antheridia produced in single culture.

While *Phytophthora pluvialis* shares some common features with other species in ITS Clade 3, there are some distinctive features. Average diameters of oogonia and oospores of *P. pluvialis* are larger than those of *P. pseudosyringae* and *P. ilicis*, but smaller than those of *P. nemorosa* and *P. psychrophila*. *P. pseudosyringae* has both

amphigynous and paragynous antheridia while *P. pluvialis* has exclusively amphigynous antheridia. Sporangia are on average larger than in these four species. Hyphal swellings produced by *P. pluvialis* both in water and in agar are distinctive compared with *P. nemorosa* which produces small blistered hyphal swellings in agar only, and *P. pseudosyringae* which produces catenulate hyphal swellings in branched chains. Hyphal swellings are not known in *P. ilicis* and may occur only rarely in *P. psychrophila*. Colony pattern on carrot agar will separate the species in ITS Clade 3, but absent reference cultures, the comparison would be difficult to make. As *P. pluvialis* is differentiated from *P. ilicis*, *P. nemorosa* and *P. psychrophila* by size of oogonia and sporangia which are otherwise similar in appearance, accurate identification of unknown isolates may need to rely on ITS or *cox* spacer DNA sequencing.

Discussion. In 2011 we reported the occurrence of *Phytophthora* “New species 3” (Reeser et al. 2011b) in a stream from southwestern Oregon. At the IUFRO Forest Phytophthora meeting in Rotorua, NZ in 2010 we reported the recovery of additional isolates from various substrates and proposed to describe them as a new species (Reeser et al. 2011a). The earliest isolates of *P. pluvialis* that we recognized were from baited soil in 2002 and were identified by an SSCP method described in Reeser et al. (2011b).

P. pluvialis was recovered from baited streams, soil and canopy drip in the mixed tanoak (*Notholithocarpus densiflorus*)-Douglas-fir (*Pseudotsuga menziesii*) forest in Curry County, Oregon, USA. We have found it in two additional streams in western Oregon (Clear Creek, lower west slope of north Oregon Cascades and Yachats River, lower west slope of central Oregon Coast Range). It has been found only rarely associated with twig and stem cankers on tanoak. We have not found *P. pluvialis* associated with any other plant host.

Like the Clade 3 species *P. pseudosyringae* and *P. psychrophila*, *P. pluvialis* is not strongly host-associated, and may be endemic. This is in contrast with Clade 3 species *P. ilicis*, which has been found only causing disease on *Ilex* spp. and *P. nemorosa*, which is pathogenic primarily on tanoak and myrtlewood (*Umbellularia californica*).

Acknowledgements: We wish to thank the Oregon Department of Forestry and the USDA Forest Service, Pacific Southwest Experiment Station and Southwest Oregon Forest Insect and Disease Center for participation in, and support of, this work.

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Table I. List of *Phytophthora pluvialis* isolates used in the study. All isolates were found in Oregon, USA.

Isolate	Source	Year	GenBank		ATCC
			ITS	cox spacer	
66-2-022008	raintrap	2008	--	KC853446	--
ELK-4-102908	raintrap	2008	--	--	--
JH4-BAIT-032204	raintrap	2004	--	KC853443	--
JR-2-040908	raintrap	2008	--	--	--
LC-8-010808	raintrap	2008	--	--	--
LC-9.2-020508	raintrap	2008	KC529657	KC529656	MYA-4930
MDR-5-010307	raintrap	2007	--	KC853442	--
TL-5-020508	raintrap	2008	--	--	--
EE-4245-S4-09	soil	2009	--	KC853444	--
WA28-022404	stream	2004	HM004217	--	--
121-WA-2.4	stream	2011	--	KC853445	--
19-W-2.3	stream	2011	--	KC853447	--
9451.1	<i>Notholithocarpus densiflorus</i>	2011	--	--	--

Table II. Based on *cox* spacer sequence analysis we have identified seven unique haplotypes among the isolates of *Phytophthora pluvialis* analyzed to date. Base substitutions which define the *P. pluvialis* haplotypes are shown.

Isolate Number	Haplo-type	Number of Isolates	COX SPACER BASE POSITION									
			59	109	181	182	188	190	192	239	270	292
66-2-022008	1	9	T	A	A	T	A	C	A	T	T	T
EE-4245-S4-09	2	3	T	A	A	T	A	C	T	A	T	T
MDR-5-010307	3	14	T	A	A	T	A	C	T	T	T	T
LC-9.2-020508	4	6	T	A	A	T	A	T	T	T	T	T
JH4-BAIT-032204	5	4	T	A	A	T	T	T	T	T	T	T
121-WA-2.4	6	1	T	T	A	T	A	C	A	T	T	T
19-W-2.3	7	1	A	A	T	C	A	T	A	T	A	G

Fig 1. Neighbor-joining tree based on ITS sequences showing the relationship of *Phytophthora pluvialis* with other *Phytophthora* species in ITS Clades 1 through 8. Sequences were aligned and the tree constructed with Clustal X (1.81). Bootstrap values were based on 1000 iterations. Values below 700 are not shown.

Fig 2. Microscopic structures of *Phytophthora pluvialis*. 2A. Oogonia, formed in agar, with amphigynous antheridia and aplerotic oospores. Scale bar = 20 μm . 2B. Semi-papillate sporangia formed in water. Scale bar = 20 μm . 2C. Hyphal swellings formed in agar (left and center), and in water (right). Scale bar = 20 μm .

Fig 3. Morphology of *Phytophthora pluvialis* on carrot agar after 24 d at 15 C.