Described as one of the most destructive pathogens of agricultural crops and forest trees, *Phytophthora* is a genus of microorganisms containing over 100 known species. *Phytophthora alni* has caused collar and root disease in alders throughout Europe and a form of the species has recently been isolated in North America. Red alder, *Alnus rubra*, is native to Oregon and has been reported to be suffering from dieback, which prompted a survey of their overall health as well as determining if *P. alni* was present. Over 1200 individual *Phytophthora* isolates were recovered in the survey, which are representative of 22 species and 2 taxa, including *P. alni* subsp. *uniformis*. High levels of mortality for red alder were not observed during the WORE survey, which suggests these *Phytophthora* species are not aggressive pathogens of red alder. In order to test the hypothesis that red alder is not susceptible to the twelve *Phytophthora* species recovered from western Oregon riparian ecosystems, a variety of pathogenicity tests were conducted. Twelve
species of Phytophthora were selected from the Phytophthora species recovered from the western Oregon riparian ecosystem survey for pathogenicity testing. Red alder seedlings were selected for testing because they have adapted to survive in riparian ecosystems, which is where these Phytophthora species have been recovered. Pathogenicity tests conducted for this study demonstrated that the twelve Phytophthora species from the survey of riparian ecosystems were able to cause minor disease symptoms on red alder, but did not cause the same symptoms observed during the WORE survey. Phytophthora species have the potential to have global impacts on forest ecosystems, which can be mitigated by conducting research on indigenous species before they become global issues of forest health.
Pathogenicity of *Phytophthora* Species from Oregon Waterways

by
Sarah M. Navarro

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APPROVED:

Major Professor, representing Botany and Plant Pathology

Head of the Department of Botany and Plant Pathology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sarah M. Navarro, Author
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Pathogenicity of *Phytophthora* Species from Oregon Waterways
CHAPTER 1. Thesis Introduction and Literature Review

General Introduction

Described as one of the most destructive pathogens of agricultural crops and forest trees, *Phytophthora* is a genus of oomycetes containing over 100 known species. They are found worldwide in many different ecological systems, where they can cause severe blight, damping off, or dieback of a broad range of plant species. *Phytophthora* has come to the forefront of forest health in recent decades with the introduction of several non-native species into forests around the world that are causing disease on the landscape level (Holdenrieder, 2004).

At the same time, new species of *Phytophthora* are continually being described, with their ecological roles currently unknown. Through environmental sampling, new *Phytophthora* species are being discovered in natural ecosystems. In western Oregon, riparian ecosystems were systemically sampled for the presence of *Phytophthora* through multiple isolation techniques (Sims and Hansen, 2012a). Red alder (*Alnus rubra*) dominate the riparian ecosystems in western Oregon and have exhibited symptoms characteristic to *Phytophthora* diseases (Sims and Hansen, 2012a). Although *Phytophthora* species were found in association with red alders displaying symptoms of dieback, pathogenicity tests have not been completed. Taking a proactive approach to determine the pathogenicity of these organisms will significantly benefit forest health in western Oregon riparian ecosystems because these newly discovered *Phytophthora* species have the potential to cause disease if
introduced into new natural ecosystems. By conducting more research on these new *Phytophthora* species, forest managers are better able to respond to emerging forest diseases.

**Phytophthora de Bary**

The genus *Phytophthora* is classified under the kingdom Chromista within the class Oomycota in the family Pythiaceae (Cavalier-Smith, 1986). Although *Phytophthora* was originally classified within the kingdom Fungi given its mycelial growth and heterotrophic nutrition; its mycelium is comprised of cellulose and contains no crosswalls, which differentiates the genus from true fungi (Erwin and Ribeiro, 1996).

Additionally, the genus is characterized by the formation of asexual spores, known as zoospores and sexual spores, known as oospores. Zoospores are biflagellate, which allows them to swim through water, giving them the common name of “water molds”. After a period of swimming, zoospores encyst by shedding their flagellae, rounding up, forming a cell wall, and then germinating to form mycelia (Judelson and Blanco, 2005). These asexual spores are produced inside of a sporangium (plural: sporangia), a sac-like structure, which can also act as an inoculum source through the formation of a germination tube depending on the surrounding environmental conditions. Sporangia can differ in the thickness at the apical end of the structure, known as the papilla. Non-papillate describes no apical thickening present on the apical end of the sporangia, while semi-papillate and
papillate refer to varying thickenings of the apical end (Blackwell, 1949). For some species sporangia can be caducoous, which aids in the aerial dispersal of inoculum to new host plants (Erwin and Ribeiro, 1996). In addition to sporangia, Phytophthora are capable of asexually producing thick-walled survival spores called chlamydospores.

Oospores, which can also act as resting spores, are the product of the union of the “female” gametangium, an oogonium (plural: oogonia), and the “male” gametangium, an antheridium (plural: antheridia) (Erwin and Ribeiro, 1996). Antheridia can become attached to the oogonium through two different orientations, amphigynous, encircling the hyphal stalk of the oogonium, or paragynous, along side the stalk of the oogonium (Blackwell, 1949). In order for sexual reproduction to occur, some species of Phytophthora are homothallic (self-fertile), which requires only one mating type; other Phytophthora species require two different mating types for reproduction and are known as heterothallic.

Following traditional taxonomic classification systems, morphological characteristics were first utilized for species designation within the genus Phytophthora. This was completed through the use of characteristics such as sporangial shape and size, presence of apical thickening, antheridial orientation, caducity, presence of hyphal swelling and chlamydospores (Waterhouse, 1963). Based on these morphological characteristics, Waterhouse developed six taxonomic groups for the species of Phytophthora. Through advancements in molecular
techniques, the species of *Phytophthora* are currently organized into ten clades based on gene-wide phylogenetic analysis of two mitochondrial gene regions in addition to the nuclear internal transcribed spacer (ITS) region (Cooke and Duncan, 1997; Cooke et al., 2000; Martin and Tooley, 2003). New *Phytophthora* species are now characterized by this clade system, which has since been validated using seven loci with 8700 nucleotide bases (Blair et al., 2008).

Originally described by Heinrich Anton de Bary in 1875, the genus *Phytophthora* has since grown to over 101 formally described species (Bary, 1876; Kroon et al., 2012). Meaning “plant destroyer” in Greek, *Phytophthora* is described as one of the most destructive plant pathogens of forest and agricultural systems (Erwin and Ribeiro, 1996). *Phytophthora infestans* was the first species to be formally described, as it was the cause of late potato blight in Europe in the 1840s (Erwin and Ribeiro, 1996). Currently, new species are continually being formally described, with an estimated 200 to 600 species yet to be identified (Brasier, 2009).

Through the increased use of molecular diagnostic techniques within the last 20 years, *Phytophthora* species are being revealed by large-scale environmental sampling in addition to the re-examination of culture collections (Brasier, 2009; Jung and Burgess, 2009; Reeser et al., 2011). Additionally, new species have been identified through the increase in the international movement of plants, which has brought about new diseases not previously known in natural ecosystems and the nursery trade (Brasier, 2009).
**Phytophthora species' roles in forest ecosystems**

As reported by Brasier in 2009, 38% of the known species and taxa of *Phytophthora* identified are associated with forests and natural ecosystems. *Phytophthora* surveys worldwide have increased and forest *Phytophthora* populations are being described, however, their ecosystem roles have yet to be determined (Balci and Halmschlager, 2003; Hwang et al., 2009; Milenkovic et al., 2012; Reeser et al., 2011). Additionally, through the implementation of systematic surveys for established forest pathogens, such as with the stream monitoring in California and Oregon for the presence of *P. ramorum*, new species of *Phytophthora* have been discovered and described (Hansen et al., 2003; Reeser et al., 2007).

The ecological roles of these recently discovered *Phytophthora* species have yet to be determined, as they were found through baiting methods and are not associated with disease systems on any forest trees. A majority of *Phytophthora* species belong to the ITS clade 6, and are widely found to inhabit aquatic as well as riparian ecosystems (Brasier et al., 2003). Clade 6 species are routinely isolated from fallen leaf debris in streams and submerged streamside root systems throughout Europe and North America (Brasier et al., 2003; Reeser et al., 2011). Although only a few disease symptoms have been found in association with these clade 6 Phytophthoras thus far, with increasing globalization leading to the introduction of non-native organisms and shifting climates, novel forest diseases could arise (Brasier et al., 2003; Brasier, 2008).
**Phytophthora and Alder**

Of the *Phytophthora* species present in forest ecosystems, *P. alni* has devastated trees in parts of Europe and has recently been discovered in North America (Adams et al., 2008; Brasier et al., 2004; Sims et al., 2012). Originally known as the "alder *Phytophthora*", *P. alni* subsp. *alni* was first isolated in 1993 from declining alders (*Alnus* spp.) from various locations in southern Britain (Brasier et al. 1995). Symptoms observed on the declining alders included crown dieback and bleeding cankers, which are characteristic symptoms of *Phytophthora* diseases (Brasier et al. 1995). Isolates of the pathogen were extracted from bleeding cankers as well as from soil taken near the roots and collars of the dying alders, which were located along streams as well as from the surrounding woodland area (Brasier et al., 1995). Since its discovery in 1993, *P. alni* subsp. *alni* has been recovered from alders in other parts of Europe, including France, Netherlands, Belgium, Sweden, Germany, Spain, Austria, and Hungary (Brasier et al., 2004; Pintos Varela et al., 2012). The spread of the pathogen throughout Europe has been attributed to the nursery trade and through outplantings of infected nursery stock along riverbanks for riparian restoration (Jung and Blaschke, 2004).

Based on initial morphological observations, *P. alni* was determined to be a novel species of *Phytophthora*. *P. alni* reassembles the described species *Phytophthora cambivora* with similar oogonial and sporangial structures (Brasier et al. 1995). However, the two species differ in sexual reproduction; *P. alni* is
homothallic, while *P. cambivora* is heterothallic (Brasier et al. 1999). In addition to the morphological differences, *P. cambivora* is not known to cause disease in alders. Not until 2004 was the species formally described by Brasier, who recognized three intraspecific variants. This species was described through the combined use of morphological characteristics and DNA sequencing. The three variants include *Phytophthora alni* subsp. *alni* (*Paa*), *Phytophthora alni* subsp. *multiformis* (*Pam*), and *Phytophthora alni* subsp. *uniformis* (*Pau*) (Brasier et al., 2004). With its close resemblance to *P. cambivora*, it was believed to be a newly evolved hybrid species, with the hybridization event occurring within a nursery setting (Brasier et al. 1999). However, further genetic analysis of each of the subspecies and *P. cambivora* demonstrated that *Paa* probably arose through the hybridization of *Pau* and *Pam* (Ioos et al., 2006). While *Pau* could have evolved from *P. cambivora* and *Pam* probably self-generated (Ioos et al., 2006).

Additionally, differences in pathogenicity to alders exist between the subspecies, with *Paa* being the most virulent and responsible for the decline in alder stands throughout Europe (Brasier et al., 2004). *Pau* and *Pam* are only rarely isolated from dying alders and are less aggressive pathogens when compared to *Paa* (Brasier et al., 2004; Brasier and Kirk, 2001). Currently known European alder species that are susceptible to *P. alni* include: Italian alder (*Alnus cordata*), common alder (*Alnus glutinosa*), grey alder (*Alnus incana*), and green alder (*Alnus viridis*) (Hansen, 2012). Heavy cone production, thin crowns with only small yellowing
leaves, and collar rot are all symptoms exhibited by these alder species following infection by the alder *Phytophthora* (Hansen, 2012).

In addition to *P. alni*, alder trees in Europe are susceptible to other *Phytophthora* species found in natural ecosystems. However, pathogenicity of these other *Phytophthora* species has only been observed through in-vitro testing (Haque and Casero, 2012; Jung and Nechwatal, 2008; Santini et al., 2006). Through in-vitro inoculation methods, Haque was able to demonstrate the susceptibility of common alder seeds and seedlings to *P. cinnamomi, P. citrophthora, P. nicotianae*, and *P. palmivora* (Haque and Casero, 2012). Thus far, these *Phytophthora* species have not been discovered to cause disease symptoms on common alder in natural ecosystems.

*Phytophthora alni subsp. uniformis* in Alaska

Following reports of severe dieback and mortality of thinleaf alder (*Alnus incana subsp. tenuifolia*) in the interior of Alaska, a systematic survey for the alder *Phytophthora* was conducted collaboratively by Dr. Gerald Adams and the United States Forest Service (USFS) in 2007 (Adams et al., 2008). Baiting of soils, roots, and stream water was performed during July 2007 from thinleaf alder stands throughout Alaska. Two separate sites, on the Kenai Peninsula and near Denali National Park produced *Pau* isolates baited from the soil rhizosphere during the initial survey (Adams et al., 2010). Thus far, all reported isolates have been collected through baiting methods, mostly soils, and not directly from cankers present on alder stems (Adams et al., 2010). Although *Pau* has been recovered in association
with thinleaf alders, the reported decline of thinleaf alder is believed to be caused by Cytospora canker (Trummer et al., 2007). As of 2009, the US Forest Service has conducted additional surveys of declining alder, which has resulted in new Pau isolates extending for 1,000 miles of roads between Fairbanks the Kenai Peninsula (Trummer and Wittwer, 2009). With such a wide distribution over south central Alaska, it was suggested that Pau is a native pathogen in the riparian ecosystems of thinleaf alder. Recently, the genetic diversity between the European and North American populations of Pau was analyzed in order to determine the origins this oomycete in each location (Aguayo et al., 2013). When comparing the genetic diversity of each population, the European population had markedly lower levels of genetic diversity than the North American population (Aguayo et al., 2013). Because of diversity levels, it is suggested that the North American population is probably native, while the European population probably arose from the introduction of the organism (Aguayo et al., 2013).

**Alder Dieback in Oregon**

Spurred by a concern about the possible presence of *P. alni* ssp. *alni* in Oregon, a survey of western Oregon riparian ecosystems was conducted through 2010-2012 as a collaborative effort of the USFS, Oregon Department of Forestry, and Oregon State University. Forest inventory surveys conducted by the United States Forest Service (USFS) underrepresent riparian ecosystem health, given their linear nature on the landscape, Thus, a Forest Health Monitoring project was developed to
determine the species of *Phytophthora* present in western Oregon riparian ecosystems (WORE), including the presence of *P. alni* subsp. *alni*. Additionally, this project was created to describe damage associated with *Phytophthora* species as well as other insects and pathogens present in WORE.

The survey included eighty-eight 100 by 10 meter transects adjacent to waterways spread out along three sub regions of western Oregon, the coast region, the Willamette Valley, and the southern region (Figure 1). Three major river systems throughout western Oregon were selected based on the risk map developed for *P. alni* ssp. *alni* by the Forest Health Technology Enterprise Team of the USFS (Figure 1). In addition to the risk map analysis for selection of the streams sampled, varying degrees of human impact on the riparian ecosystem influenced the installation of the transects. Alder trees, red alder (*Alnus rubra*) and white alder (*Alnus rhombifolia*), were observed and dieback levels were determined. Root, soil, bark and water samples were collected at each streamside transect. Soil and washed root samples were baited with *Rhododendron* leaves and plated onto *Phytophthora* selective media, while water samples were filtered and the filter paper was then directly plated onto the same selective media. Bark samples were taken from red alder trees, with necrotic margins and then plated onto the selective media.

From this sampling, over 1200 individual *Phytophthora* isolates were recovered, which are representative of 22 species and 2 taxa over 7 ITS clades, including *P. alni uniformis* (Sims and Hansen, 2012a; Sims et al., 2012). Of the isolates, about 82%
were from clade 6 and many known deciduous tree pathogens were found. Although many *Phytophthora* isolates were recovered, only *Phytophthora siskiyouensis* was isolated from above ground bark samples of red alder (Sims et al., 2012).
Figure 1. Map of the *P. alni* subsp. *alni* susceptibility potential in Oregon with an overlay of the 88 WORE survey transect locations. Susceptibility potential map *P. alni* subsp. *alni* produced by FHTET Fort Collins, CO, 2007. Map overlaid with 2010 TIGER/Line Shapefiles.
Comparatively more *Phytophthora* isolates were recovered from water samples than from the red alder root samples (Sims et al., 2012). In terms of alder tree health, of the 2310 alder trees observed 42% had reported dieback (Sims and Hansen, 2012a).

**Red Alder- *Alnus rubra* Bong.**

Classified in the family Betulaceae, red alder occurs along many streams and rivers throughout the Pacific Northwest (Hibbs and Bower, 2001). Common traits of this family include the presence of male catkins, simple leaves, and a nut fruit (Hitchcock and Cronquist, 1973). Red alder is a deciduous tree species and is characterized by it’s serrate green leaves, staminate catkins, and thin, gray bark. Under optimal growing conditions, trees can reach 75 centimeters in diameter and up to 40 meters in height (Deal and Harrington, 2006).

As the most common hardwood in the Pacific Northwest, red alder is found from southeastern Alaska (lat. 60° N) to southern California (lat. 34° N) and within 200 km of the coastline (Burns and Honkala, 1990). Red alder is typically found at elevations below 750 m; it rarely grows east of the Cascade Range and Sierra Nevada Mountains in Oregon, Washington, and California respectively (Deal and Harrington, 2006). Preferring humid to super humid climates, which leads to high soil moisture, red alder forms pure stands along riparian zones and low slopes (Deal and Harrington, 2006).
Although pure stands do occur throughout its range, red alder typically forms mixed stands with coniferous trees. Commonly associated conifers include: Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), grand fir (*Abies grandis*), Sitka spruce (*Picea sitchensis*) (Goldman, 1961).

As an early successional species within its range in the Pacific Northwest, red alder forms dense stands in recently disturbed, open, moist sites when abundant seed is available (Deal and Harrington, 2006). Disturbances such as clearcuts, road construction, and forest fires lead to bare mineral soil and canopy gaps, which are both required for red alder regeneration (Burns and Honkala, 1990). A highly shade intolerant species, densely grown red alder stands typically lead to rapid mortality of lower leaves and branches as well as small stems of the species (Deal and Harrington, 2006). Red alder has a life span of 60 to 70 years, with 100 years being reported as the maximum age (Deal and Harrington, 2006).

Throughout the Pacific Northwest, riparian zones provide many important ecological functions of the forest, such as fish habitat and maintaining stream water quality (Hibbs and Bower, 2001). In riparian zones, alders act as bank enforcement, with their fibrous root system, which is typically exposed along the watercourse (Goldman, 1961). Additionally, alders act as a high nitrogen source for other riparian plant species. A symbiotic relationship exists between *Frankia alni*, a nitrogen fixing actinomycete, and alder trees (Compton et al., 2003). *F. alni* forms
nodules on the alder roots, red in color, which can act as a distinguishing factor for the root system (Compton et al., 2003).

With recent increases in wood value and rapid growth rates, red alder is managed in the Pacific Northwest in pure stands as well as part of a mixed regime of Douglas-fir (Deal and Harrington, 2006). Although once viewed as an undesirable species by industrial forest managers, today, red alder is utilized for its ability to fix nitrogen onto low productivity sites and shorter harvest rotation time (Deal and Harrington, 2006). Additionally, red alder can be planted onto sites throughout the Pacific Northwest that historically contain conifer specific fungal pathogens such as laminated root rot (Phellinus weirii) or Swiss needle cast (Phaeocryptopus gaeumannii) (Deal and Harrington, 2006). Through the development of the Hardwood Silviculture Cooperative at Oregon State University, perception and management of red alder has improved over the course of the last 25 years. Today, red alder sawlogs can fetch the same price or more compared to Douglas-fir and are utilized in furniture making, cabinetry, and fuelwood (Deal and Harrington, 2006).

Insect and disease damage agents of red alder have been reported throughout its range in the Pacific Northwest, however, extensive damage has not been observed (Burns and Honkala, 1990). The insects of great importance for alder health include the alder flea beetle (Macrohaltica ambiens), green alder sawfly (Monsoma pulveratum), and western tent caterpillar (Malacosoma californicum). The alder flea beetle has a been reported in the Pacific Northwest and has the
potential to cause complete defoliation of alders, however, the damage is not permanent as the tree produces leaves the following spring (Woods, 1917). Native to Europe and just recently reported in Washington and Oregon, the green alder sawfly has the potential to contribute to the defoliation of red alder (Flowers, 2012). Although defoliation has increased on thinleaf alder in Alaska since the initial observations in 2007, green alder sawfly does not currently appear to cause excessive defoliation in Oregon on red alder (Kruse et al., 2010). As a reported host of western tent caterpillar red alder experiences a range of defoliation, from a single branch to a whole stand (Ragenovich and Ciesla, 2008).

Disease agents of red alder to note include root rot and stem canker fungi. Red alder are susceptible to the root rot pathogens *Heterobasidion annosum* and *Armillaria* species; when planted in a mixed conifer stand, root rot has been noted in red alder (Deal and Harrington, 2006). *Neonectria* species and *Valsa melanodiscus* are known to cause stem cankers on red alder, but thus far large-scale mortality has not been reported (Omdal and Ramsey, 2009, Stanosz, et al, 2008). A field study by Omdal and Ramsey (2009) found *Neonectria major* to be the causal agent of stem cankers on red alder in western Washington, but was determined to be a weak pathogen as no mortality was reported. Commonly known as the disease Cytospora canker of alder, *V. melanodiscus*, has been confirmed as a pathogen of alder on multiple occasions, including in the interior of Alaska on thinleaf alder (Stanosz, et al, 2008).
Forest diseases caused by *Phytophthora* in Oregon

The threat of introduced pathogens is not a new concern in Oregon, as two of the most important forest diseases, *P. lateralis* and *P. ramorum*, were both introduced into the natural ecosystems. Although the origin of *P. lateralis* is unknown, it was introduced into the ornamental trade in Seattle, Washington in 1923 on Lawson’s cypress (Zobel et al., 1985). In 1952, *P. lateralis* was first reported in the native range of Port-Orford-cedar (POC), its main host, in Coos County from an infested out planting of ornamental rhododendrons (Betlejewski, 2003). Since the initial introduction, *P. lateralis* has continued to spread throughout the range of POC via waterways and infected soils. Once infection occurs through the roots, the pathogen grows up into the inner bark of the tree killing living plant tissue along the way (Hansen et al., 2000). Eventually, the hyphae of *P. lateralis* will continue into the phloem of the tree creating a necrotic lesion extending above the root collar. Mortality occurs within one year for larger forest tress and within a few weeks for seedlings (Hansen et al., 2000).

Sudden oak death (SOD) is caused by *P. ramorum* and has led to the decline of tanoaks (*Notholithocarpus densiflorus*), throughout 14 California counties and Curry County, Oregon. *P. ramorum* was introduced into the forest of California in the mid-1990s and detected in southwestern Oregon in 2001, where it has caused tanoak mortality (Rizzo et al., 2005). Although the initial introduction event of *P. ramorum* in the tanoak forests of southwestern Oregon is not known, it is speculated
that the infestation was the result of the pathogen moving from infected nursery stock (Grünwald et al., 2012). Through the introduction of SOD, large overstory tanoaks are being removed from these ecosystems, with the consequences of this mass removal not presently known. However, it has been speculated that tanoak will eventually be removed completely from the landscape. With these two forest pathogens already established in Oregon forests, it is of great importance to prevent future introductions of new *Phytophthora* species and to determine the species are already present in natural ecosystems.

**Species to be used**

*P. alni uniformis, P. cambivora, P. gonapodyides, P. lacustris, P. lateralis, P. pini, P. plurivora, P. pluvialis, P. pseudosyringae, P. riparia, P. siskiyouensis, P. taxon Oaksoil, P. taxon Pgchlamydo* were selected for pathogenicity testing for this research. With the exception of *P. lateralis*, these species were recovered from the WORE survey. *P. lateralis* was selected for inclusion in this study based on its presence throughout southwestern Oregon and it's known pathogenicity to another native tree in Oregon, Port-Orford-cedar. These species are representative of five of the ten ITS clades known in the genus *Phytophthora*, which in turn exhibit different morphological traits (Table 1). All of the species used for this study have been previously described in the literature.
Table 1. Morphological characteristics of *Phytophthora* species tested in thesis

<table>
<thead>
<tr>
<th>Species</th>
<th>clade</th>
<th>sporanigiohpore</th>
<th>sporangia shape</th>
<th>papillate</th>
<th>caducity</th>
<th>homothallic</th>
<th>heterothallic</th>
<th>amphigynous</th>
<th>paragynous</th>
<th>hyphal swellings</th>
<th>chlamydomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. alni</em> subsp. <em>uniformis</em>&lt;sup&gt;2&lt;/sup&gt;</td>
<td>7</td>
<td>simple</td>
<td>ellipsoid, obpyriform, ovoid</td>
<td>np</td>
<td></td>
<td>*</td>
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<tr>
<td><em>P. cambivora</em>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>7</td>
<td>simple sympodial</td>
<td>ellipsoid, ovoid</td>
<td>np</td>
<td></td>
<td>*</td>
<td>*</td>
<td>-</td>
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<tr>
<td><em>P. gonapodyides</em>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6</td>
<td>sympodial</td>
<td>ellipsoid, obpyriform, ovoid</td>
<td>np</td>
<td></td>
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<td>*</td>
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<tr>
<td><em>P. lacustris</em>&lt;sup&gt;4&lt;/sup&gt;</td>
<td>6</td>
<td>simple sympodial</td>
<td>obpyriform, ovoid</td>
<td>np</td>
<td></td>
<td>-</td>
<td></td>
<td>-</td>
<td>*</td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>P. lateralis</em>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>8</td>
<td>simple sympodial</td>
<td>obpyriform, ovoid</td>
<td>np</td>
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<td>*</td>
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<tr>
<td><em>P. pini</em>&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2</td>
<td>simple sympodial</td>
<td>ovoid</td>
<td>sp</td>
<td>*</td>
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<tr>
<td><em>P. plurivora</em>&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2</td>
<td>simple sympodial</td>
<td>obpyriform</td>
<td>sp</td>
<td>*</td>
<td>-</td>
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<tr>
<td><em>P. pluvialis</em>&lt;sup&gt;7&lt;/sup&gt;</td>
<td>3</td>
<td>simple sympodial</td>
<td>obpyriform</td>
<td>sp</td>
<td>*</td>
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<tr>
<td><em>P. pseudosyringae</em>&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3</td>
<td>simple sympodial</td>
<td>ellipsoid, limoniform, ovoid</td>
<td>sp</td>
<td>*</td>
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<td>-</td>
<td>*</td>
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<tr>
<td><em>P. riparia</em>&lt;sup&gt;9&lt;/sup&gt;</td>
<td>4</td>
<td>simple</td>
<td>obpyriform, ovoid</td>
<td>np</td>
<td></td>
<td>-</td>
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<tr>
<td><em>P. siskiyouensis</em>&lt;sup&gt;10&lt;/sup&gt;</td>
<td>2</td>
<td>simple</td>
<td>ellipsoid, ovoid</td>
<td>sp</td>
<td>*</td>
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<tr>
<td><em>P. taxon Oaksoil</em>&lt;sup&gt;1, 11&lt;/sup&gt;</td>
<td>6</td>
<td>na</td>
<td>na</td>
<td>np</td>
<td></td>
<td>NA</td>
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<tr>
<td><em>P. taxon Pgchlamydo</em>&lt;sup&gt;11, 12&lt;/sup&gt;</td>
<td>6</td>
<td>na</td>
<td>limoniform, obpyriform, ovoid</td>
<td>np</td>
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<sup>1</sup>(Kroon et al., 2012), <sup>2</sup>(Brasier et al., 2004), <sup>3</sup>(Erwin and Ribeiro, 1996), <sup>4</sup>(Nechwatal et al., 2012), <sup>5</sup>(Hong et al., 2011), <sup>6</sup>(Jung and Burgess, 2009), <sup>7</sup>(Reeser et al., 2013), <sup>8</sup>(Jung et al., 2003), <sup>9</sup>(Hansen et al., 2012), <sup>10</sup>(Reeser et al., 2007), <sup>11</sup>(Brasier et al., 2003), <sup>12</sup>(Jung and Nechwatal, 2008)
*P. alni* subsp. *uniformis* Brasier and Kirk (2004) is a subspecies of *P. alni*, known as the alder *Phytophthora* when it was first recovered from necrotic bark tissue in Britain in 1993 (Brasier et al., 2004). Originally isolated from streams in Sweden, *Pau* has since been discovered in streams in Alaska and Oregon in 2007 and 2011, respectively (Adams et al., 2010; Sims et al. unpubl). *Pau* is considered to be a less aggressive pathogen than *P. alni*, however, it has been associated with root and collar rot of various alder species throughout Europe and necrotic lesions on the roots of red alder in Oregon (Brasier et al., 2004; Hansen, 2012).

*P. cambivora* (Petri) Buisman (1927) is the causal agent of ink disease of sweet chestnut (*Castanea sativa*) as well as a canker disease of golden chinquapin (*Chrysolepis chryophylyla*) (Erwin and Ribeiro, 1996; Saavedra et al., 2007). With a worldwide distribution, *P. cambivora* was originally believed to be one of the hybrid parent species for *P. alni* due to morphological similarities, however recent research has disproven this theory (Brasier et al., 2004; Érsek and Nagy, 2008). Additionally, *P. cambivora* has been found in association with other *Phytophthora* species throughout Europe causing oak decline (Balci and Halmschlager, 2003).

*P. gonapodyides* (Peterson) Buisman (1927) first isolated and subsequently described from submerged apples in a pond in Denmark (Erwin and Ribeiro, 1996). Although it was originally described as a weak root pathogen, it has been found to
cause root rot of seedlings in Douglas-fir plantations in the Pacific Northwest (Brasier et al., 1993). In Europe, *P. gonapodyides* has been found to cause root rot and stem lesions on pedunculate oak (*Quercus robur*) in addition to new findings in association with the decline of holm oak (*Quercus ilex*) in Spain (Corcobado et al., 2010; Jung et al., 1996).

*P. lacustris* Brasier et al. (2012) is a newly described species of *Phytophthora* previously designated as *P.* taxon Salixsoil (Nechwatal et al., 2012). Although morphologically identical to *P. gonapodyides*, *P. lacustris* was separated as a new taxon through ITS sequence analysis (Brasier et al., 2003). From its initial isolation in 1972 from *Salix* roots in southern England, it has since been reported in Australia, New Zealand, Alaska, and western Oregon (Nechwatal et al., 2012). This species has been found in association with declining common ash (*Fraxinus excelsior*) in Poland and was determined to be a pathogen to roots, stems, and leaves of this tree (Orlikowski et al., 2011).

*P. lateralis* Tucker and Milbrath (1942) is a known pathogen of Port-Orford-cedar and threatens native populations in Oregon as well as Northern California, causing the disease known as Port-Orford-cedar root disease (Tucker and Milbrath, 1942). This pathogen was first identified in ornamental plants in Seattle, Washington in 1923 on Lawson’s cypress (Zobel et al., 1985). Additionally, *P.*
*lateralis* has been observed as a pathogen of Pacific yew, *Taxus brevifolia* in riparian ecosystems of southwest Oregon and northwest California (Murray and Hansen, 1997).

*P. pini* Leonian (1925) emend. Gallegly et al. (2008) was recently redesignated a species through advancements in molecular techniques, which led to its separation from the *P. citricola* clade (Hong et al., 2011). As an established pathogen in Europe and North America, it is of great concern to the nursery industry given its high occurrence in irrigation runoff water (Hong et al., 2011). With the potential for spread through infested irrigation water, recent research has found that encysted zoospores of *P. pini* could serve as inoculum in recirculating water systems (Shay, 2012).

*P. plurivora* Jung and Burgess (2009) was recently separated from the *P. citricola* clade through the use of multiple DNA sequence regions as well as differences in morphology (Jung and Burgess, 2009). Since its species designation, *P. plurivora* has been determined to be a pathogen to European beech (*Fagus sylvatica*) in Europe and in the northeastern United States (Weiland et al., 2010). Additionally, this species was determined to be one of the *Phytophthora* species causing a decline in common ash across Europe (Orlikowski et al., 2011).
P. pluvialis Reeser et al. (2013) has been recovered from southwestern Oregon following disease monitoring for P. ramorum and was originally reported as “New species 3” (Reeser et al., 2011; Reeser et al., 2013). This species was recovered through four different sampling methods, including isolation from tanoak bole cankers, canopy drip, soil baiting, and stream baiting. In addition to southwestern Oregon, this species has been recovered from two other streams in western Oregon, Clear Creek in the north Oregon Cascade Range and the Yachats River in the western Oregon Coast Range (Reeser et al., 2013).

P. pseudosyringae Jung and Delatour (2003) was initially isolated from soil samples in association with oak decline in Europe. Recently P. pseudosyringae was recovered through surveys for other forest pathogens, including the P. alni survey in Alaska and the ongoing survey of P. ramorum in California and Oregon (Adams et al., 2010; Wickland et al., 2008). In addition to causing fine root and stem necrosis of European beech and common alder in Europe, P. pseudosyringae has also been reported to cause bleeding cankers and leaf necrosis on tanoak, coast live oak (Q. agrifolia), and bay laurel (Umbellularia californica) (Jung et al., 2003; Wickland et al., 2008).

P. riparia Reeser et al. (2012) was identified following forest stream surveys in Oregon, California, and Alaska, which was initiated by recent interest in
describing forest Phytophthora populations (Hansen et al., 2012). Although similar in morphology to *P. gonapodyides*, *P. riparia* differs from other species in Clade 6. *P. riparia* has been recovered from both stream water and riparian soil samples, however, its ecological role in riparian ecosystems is currently unknown (Hansen et al., 2012).

*P. siskiyouensis* Reeser et al. (2007) was first discovered in southwestern Oregon through the stream monitoring program developed to detect the presence of *P. ramorum* (Reeser et al., 2007). Since the initial isolation of *P. siskiyouensis* from stream water and soil samples, it has been found to cause bark cankers on streamside tanoaks and blight of bay laurel shoots near ground level (Reeser et al., 2007). Although no disease has been observed on red alder to date, this pathogen has been reported to cause cankers of other species of alder in urban settings in California and Australia (Rooney-Latham et al., 2009; Smith et al., 2006).

*P. taxon* Oaksoil Brasier et al. (2003) has yet to be formally described as a species, but it was separated from *P. gonapodyides* due to its unique ITS lineage (Brasier et al., 2003). Since the discovery of the initial isolate from France, *P. taxon* Oaksoil has been isolated from streams in western and southwestern Oregon (Hansen and Delatour, 1999; Reeser et al., 2011). Additionally, *P. taxon* Oaksoil was
isolated in abundance during a riparian stream survey through it’s survival on red alder leaf debris (Sims and Hansen, 2012b).

*P. taxon Pgchlamydo* Brasier et al. (2003) was informally designated after its unique ITS lineage separated it from other clade 6 *Phytophthora* species (Brasier et al., 2003). Originally isolated from Great Britain in 1971, *P. taxon Pgchlamydo* has since been recovered from both forest and nursery soils in other European countries, Australia, South Africa, Argentina, and North America (Brasier and Jung, 2006; Brasier et al., 1993; Sims and Hansen, 2012a). Initial pathogenicity tests have been completed using bur oak (*Quercus macrocarpa*) and northern red oak (*Quercus rubra*), which resulted in significant lesions being caused by *P. taxon Pgchlamydo* (Schwingle and Blanchette, 2008).

**Thesis Objectives**

Through this thesis, the ecological role of *Phytophthora* species in red alder dominated riparian ecosystems was investigated. Although *Phytophthora* species were found in association with red alders experiencing dieback, pathogenicity tests have not previously been completed. High levels of mortality for red alder were not observed during the WORE survey, which suggests that these *Phytophthora* species are not aggressive pathogens of red alder. Red alder trees have adapted to survive in riparian ecosystems, which is where these *Phytophthora* species have been
recovered. We tested the hypothesis: Red alder is not susceptible to the
Phytophthora species recovered from western Oregon riparian ecosystems. If the
Phytophthora species identified are also native to these riparian ecosystems, we
would expect significant disease to result from artificial inoculation. Through these
tests, it will be demonstrated that the Phytophthora species from the WORE survey
do not cause significant disease on red alder.

Twelve species of Phytophthora were selected from the Phytophthora species
recovered from the WORE survey for pathogenicity testing. Of the twelve
Phytophthora species, five species are from ITS clade 6, two are known pathogens of
other alder species, and two are pathogens of other forest trees with wide host
ranges (Brasier, 2009; Brasier et al., 2004, 2003). In addition to the Phytophthora
species isolated from the WORE survey, P. lateralis was included for the
pathogenicity tests of this study. As a host specific pathogen, P. lateralis is
recovered frequently from waterways in western Oregon, but only where dying
Port-Orford-cedar (Chamaecyparis lawsoniana) are present streamside (Hansen et
al., 2000). The range of Phytophthora species utilized included both a negative
control, P. lateralis, and positive controls, P. siskiyouensis and P. alni subsp. alni
(Brasier et al., 2004; Rooney-Latham et al., 2009).
The following pathogenicity tests were selected to test all thirteen *Phytophthora* species in order to achieve the thesis objective:

1.) Stem inoculation test on red alder seedlings under two different environmental conditions.
2.) Soil infestation test utilizing an inoculated media source mixed into the soil of red alder seedlings.
3.) Zoospore root dip test using zoospore suspensions for each *Phytophthora* species.
4.) Detached leaf test using a wound inoculation method and colonized agar media for each *Phytophthora* species.

This research serves to provide information on multiple species of *Phytophthora*, which belong to a genus that has historically caused destruction in natural systems.
**Literature Cited:**


Brasier, C., Cooke, D., Duncan, J., Hansen, E., 2003. Multiple new phenotypic taxa from trees and riparian ecosystems in Phytophthora gonapodyides-P. megsperma ITS clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. Mycological Research 107, 277-290.


CHAPTER 2. Susceptibility of Red Alder to Phytophthora Species from Western Oregon Riparian Ecosystems

Introduction

Described as one of the most destructive groups of pathogens of agricultural crops and forest trees, Phytophthora de Bary is a genus of oomycetes containing over 100 known species. They are found worldwide in many different ecological systems, where they can cause severe blight, damping off, or dieback of a broad range of plant species. Phytophthora has come to the forefront of forest health in recent decades with the introduction of several non-native species into forests around the world causing disease on the landscape level (Holdenrieder, 2004). One of these Phytophthora species of concern to forest ecosystems is P. alni, which has devastated alder trees in parts of Europe; a subspecies of this destructive pathogen was recently discovered in North America (Adams et al., 2008; Brasier et al., 2004; Sims et al., 2012). In Oregon the threat of introduced pathogens is not a new concern, as two of the most important forest diseases, P. lateralis and P. ramorum, were both introduced into the natural ecosystems and have since become established (Hansen, 2003).

New species of Phytophthora are continually being described and their ecological roles are currently unknown. Through environmental sampling, new Phytophthora species are being discovered in natural ecosystems. As reported by
Brasier (2009), 38% of the known species and taxa of *Phytophthora* identified are associated with forests and natural ecosystems. Due to increased monitoring for *Phytophthora species* worldwide, forest *Phytophthora* populations are being described, but their ecosystem roles have yet to be determined (Balci and Halmschlager, 2003; Hwang et al., 2009; Milenkovic et al., 2012; Reeser et al., 2011). Additionally, through the implementation of systematic surveys for established forest pathogens, such as with the stream monitoring in California and Oregon for the presence of *P. ramorum*, new species of *Phytophthora* have been discovered and described (Douhan and Rizzo, 2003; Reeser et al., 2007).

The ecological roles of these recently discovered *Phytophthora* species are unknown, as they were found through baiting methods and are not associated with disease symptoms on any forest trees. Of these species of *Phytophthora*, a majority belong to the ITS clade 6 and are widely found to inhabit aquatic as well as riparian ecosystems (Brasier et al., 2003). Clade 6 species are routinely isolated from fallen leaf debris in streams and submerged streamside root systems throughout Europe and North America (Brasier et al., 2003; Reeser et al., 2011; Sims and Hansen, 2012a). Although only few disease symptoms have been found in association with these clade 6 *Phytophthoras* thus far, with increasing globalization leading to the introduction of non-native organisms and shifting climates, novel forest diseases could arise (Brasier et al., 2003; Brasier, 2008).
Throughout the Pacific Northwest, riparian zones provide many important ecological functions of the forest, such as fish habitat and maintaining stream water quality (Hibbs and Bower, 2001). In riparian zones, red alders (*Alnus rubra* Bong.) act as bank enforcement, with their fibrous root system, which is typically exposed along the watercourse (Goldman, 1961). Additionally, red alders act as a high nitrogen source for other riparian plant species. A symbiotic relationship exists between *Frankia alni*, a nitrogen fixing actinomycete, and alder trees, which leads to the formation of nodules on the roots of red alders (Compton et al., 2003).

Red alder dominates the riparian ecosystems in western Oregon and have exhibited symptoms characteristic of *Phytophthora* diseases (Sims and Hansen, 2012a). Spurred by concern about the potential presence of *P. alni ssp. alni* in Oregon, a survey of western Oregon riparian ecosystems was conducted through 2010-2012 as a collaborative effort of the United States Forest Service, Oregon Department of Forestry, and Oregon State University. Known as the western Oregon riparian ecosystem (WORE) survey, eighty-eight 100 meter by 10 meter transects adjacent to waterways throughout western Oregon were systematically sampled to determine the species of *Phytophthora* present, which included sampling for the presence of *P. alni*. 
From this sampling, over 1200 individual *Phytophthora* isolates were recovered, representing 22 species and 2 taxa over 7 ITS clades, including *P. alni uniformis* (Sims and Hansen, 2012a; Sims et al., 2012). About 82% of all isolates were from clade 6 and included known pathogens of deciduous trees. While many *Phytophthora* isolates were recovered from water and streamside soil, only *Phytophthora siskiyouensis* was isolated from above ground bark samples of red alder (Sims et al., 2012).

Although many *Phytophthora* species were found in association with red alders with symptoms of dieback, pathogenicity tests have not been completed for all species. This study was conducted to investigate the ecological role of thirteen species of *Phytophthora* in relation to red alders. Red alders inhabit riparian ecosystems throughout the Pacific Northwest, with high levels of mortality not presently reported (Deal and Harrington, 2006). Twelve species of *Phytophthora* were selected from the 22 *Phytophthora* species recovered from the WORE survey for pathogenicity testing. Of the twelve *Phytophthora* species, five species are from ITS clade 6, two are known pathogens of other alder species, and two are pathogens of other forest trees with wide host ranges (Brasier, 2009; Brasier et al., 2004, 2003). In addition to the *Phytophthora* species isolated from the WORE survey, *P. lateralis* was included for the pathogenicity tests of this study. As a host specific pathogen, *P. lateralis* is recovered frequently from waterways in western Oregon, but only where
dying Port-Orford-cedar (*Chamaecyparis lawsoniana*) are present streamside (Hansen et al., 2000).

In order to test the hypothesis that red alder is not susceptible to the *Phytophthora* species recovered from the western Oregon riparian ecosystem, a variety of pathogenicity tests were conducted. Red alder trees have adapted to survive in riparian ecosystems, which is where these *Phytophthora* species have been recovered. High levels of mortality for red alder were not observed during the WORE survey, which indicates that these *Phytophthora* species are not pathogenic to red alder. Through the pathogenicity tests conducted for this study, it will be demonstrated that the *Phytophthora* species from the WORE survey do not cause significant disease on red alder. The pathogenicity tests were selected in order to test a wide range of *Phytophthora* species and their effects on different organs of red alder seedlings. The range of *Phytophthora* species utilized included both a negative control, *P. lateralis*, and positive controls, *P. siskiyouensis* and *P. alni* subsp. *alni* (Brasier et al., 2004; Rooney-Latham et al., 2009).

This research serves to provide information on multiple species of *Phytophthora*, which belong to a genus that has historically caused destruction in natural systems. Taking a proactive approach to determine the pathogenicity of these organisms will significantly benefit forest health in western Oregon riparian
ecosystems. These newly discovered *Phytophthora* species have the potential to cause disease if introduced into new natural ecosystems. By conducting more research on these new species, forest managers and researchers are better able to respond to emerging forest *Phytophthora* diseases.
Materials and Methods

Four different pathogenicity tests were conducted, which targeted different plant organs: the stem, roots, and leaves. Each test was designed to determine the pathogenicity of thirteen different Phytophthora species to red alder. Seedlings of red alder were used for all pathogenicity tests performed in this study.

Materials

Phytophthora species and isolates- *P. alni uniformis*, *P. cambivora*, *P. gonapodyides*, *P. lacustris*, *P. lateralis*, *P. pini*, *P. plurivora*, *P. pluvialis*, *P. pseudosyringae*, *P. riparia*, *P. siskiyouensis*, *taxon Oaksoil*, and *taxon Pgchlamydo* were used for each of the pathogenicity experiments in this study (Table 2). From the western Oregon riparian ecosystem survey conducted in 2010 to 2012, these species were isolated from root, soil, and water samples (Sims and Hansen, 2012a). Species identification was confirmed through morphological characteristics as well as DNA sequencing of the cytochrome c oxidase spacer region of the mitochondrial DNA (Sims and Hansen, 2012a). Three isolates of each species were utilized, with the exceptions of *P. lateralis* and *P. pluvialis* where only one isolate was used, and *P. lacustris*, for which four isolates were used. Additionally, *P. alni* subsp. *uniformis* was not recovered in time for the summer stem inoculation trial and was thus not utilized, but was used for all other pathogenicity experiments. Isolates were
maintained on CMAβ (corn meal agar amended with 20 ppm β-sitosterol) at 17.5°C in the dark.
Table 2. *Phytophthora* isolates used in pathogenicity testing.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate ID</th>
<th>Isolated by</th>
<th>Isolated from</th>
<th>Oregon waterway</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. alni</em> subsp. uniformis</td>
<td>118-R-1K.1</td>
<td>Sims</td>
<td>R</td>
<td>Cape Creek</td>
<td>2011</td>
</tr>
<tr>
<td><em>P. alni</em> subsp. uniformis</td>
<td>118-R-1J.3*</td>
<td>Sims</td>
<td>R</td>
<td>Cape Creek</td>
<td>2011</td>
</tr>
<tr>
<td><em>P. alni</em> subsp. uniformis</td>
<td>118-R-1J.4</td>
<td>Sims</td>
<td>R</td>
<td>Cape Creek</td>
<td>2011</td>
</tr>
<tr>
<td><em>P. cambivora</em></td>
<td>31-14-S.4</td>
<td>Sims</td>
<td>S</td>
<td>Coast Fork Willamette River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. cambivora</em></td>
<td>200-4-R.1*</td>
<td>Sims</td>
<td>R</td>
<td>Mill Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. cambivora</em></td>
<td>104-1-R.1</td>
<td>Sims</td>
<td>R</td>
<td>Upper Siletz River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. gonapodyides</em></td>
<td>209-W-1.1</td>
<td>Sims</td>
<td>W</td>
<td>Burnt Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. gonapodyides</em></td>
<td>15-6-R.1*</td>
<td>Sims</td>
<td>R</td>
<td>Willamina Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. lacustris</em></td>
<td>110-W-1.3*</td>
<td>Sims</td>
<td>W</td>
<td>Upper Smith River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. lateralis</em></td>
<td>PL3*</td>
<td>Mallams</td>
<td>ST</td>
<td>Onion Creek</td>
<td>2006</td>
</tr>
<tr>
<td><em>P. pini</em></td>
<td>5-W-2.10</td>
<td>Sims</td>
<td>W</td>
<td>Soap Creek at the Beef Barn</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. pini</em></td>
<td>210-W-2.1</td>
<td>Sims</td>
<td>W</td>
<td>Middle Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. pini</em></td>
<td>112-W-1.1*</td>
<td>Sims</td>
<td>W</td>
<td>Lower Smith River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. plurivora</em></td>
<td>3-W-1.34*</td>
<td>Sims</td>
<td>W</td>
<td>Oak Creek West Lower</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. plurivora</em></td>
<td>221-W-2.3</td>
<td>Sims</td>
<td>W</td>
<td>Laying Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. plurivora</em></td>
<td>115-W-1.6</td>
<td>Sims</td>
<td>W</td>
<td>North Fork Siuslaw River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. plurivialis</em></td>
<td>19-W-2.3*</td>
<td>Sims</td>
<td>W</td>
<td>Clear Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. pseudosyringae</em></td>
<td>125-W-2.12</td>
<td>Sims</td>
<td>W</td>
<td>North Fork Trask River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. pseudosyringae</em></td>
<td>121-W-1.17</td>
<td>Sims</td>
<td>W</td>
<td>Yachats River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. pseudosyringae</em></td>
<td>117-W-1.1*</td>
<td>Sims</td>
<td>W</td>
<td>Cape Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. riparia</em></td>
<td>32-W-1.9</td>
<td>Sims</td>
<td>W</td>
<td>Coast fork Willamette River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. riparia</em></td>
<td>208-W-2.6</td>
<td>Sims</td>
<td>W</td>
<td>Rogue River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. riparia</em></td>
<td>12-W-2.1*</td>
<td>Sims</td>
<td>W</td>
<td>Thomas Creek Bottom</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. siskiyouensis</em></td>
<td>201-36-R.2*</td>
<td>Sims</td>
<td>R</td>
<td>Squaw Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. siskiyouensis</em></td>
<td>119-W-2.5</td>
<td>Sims</td>
<td>W</td>
<td>Yachats River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. siskiyouensis</em></td>
<td>107-W-2.12</td>
<td>Sims</td>
<td>W</td>
<td>East Beaver Creek</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Oaksoil</em></td>
<td>122-W-2.5</td>
<td>Sims</td>
<td>W</td>
<td>Elk River</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Oaksoil</em></td>
<td>10-W-2.1</td>
<td>Sims</td>
<td>W</td>
<td>Thomas Creek Upper</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Oaksoil</em></td>
<td>10-W-1.13*</td>
<td>Sims</td>
<td>W</td>
<td>Thomas Creek Upper</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Pgchlamydo</em></td>
<td>5-12-R.4*</td>
<td>Sims</td>
<td>R</td>
<td>Soap Creek at the Beef Barn</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Pgchlamydo</em></td>
<td>3-W-1.25</td>
<td>Sims</td>
<td>W</td>
<td>Oak Creek West Lower</td>
<td>2010</td>
</tr>
<tr>
<td><em>P. taxon Pgchlamydo</em></td>
<td>130-10-R.1</td>
<td>Sims</td>
<td>R</td>
<td>Alsea River</td>
<td>2010</td>
</tr>
</tbody>
</table>

* = used for zoospore production, abbreviations: R=Roots, S=Soil, ST=stem, W=Water
**Red alder seedlings** - Three sets of red alder seedlings were used for the various pathogenicity experiments. The first set of seedlings contained containerized and bare-root red alder seedlings purchased from Seven Oaks Native Nursery in Albany, Oregon in February 2011; they are referred to hereafter as the 2011 containerized seedlings and 2011 bare root seedlings (Figure 2A). The containerized seedlings and bare-root seedlings were transferred to Oregon State University (OSU) and stored outside adjacent to the east greenhouse facilities. The seed source for both seedling types was Linn County, Oregon, east of Corvallis. The two-year-old containerized seedlings were purchased and maintained in trade 1-gallon containers. The one-year-old bare root seedlings were planted into 656 mL planting tubes (Stuewe and Sons, Inc., Tangent, Oregon) using MetroMix 840 PC “Professional Growing Mix” (Sun Gro Horticulture Canada Ltd, Vancouver, British Columbia). Seedlings were watered by natural rainfall until April 2011, after which a sprinkler system was used twice daily to water them during the dry summer season until they were utilized for experiments.

The second set of red alder seedlings were purchased from Seven Oaks Native Nursery in January 2012 (Figure 2B). This set contained one-year-old bare root seedlings with a seed source of Linn County, Oregon and are referred to hereafter as the 2012 bare root seedlings. These seedlings were planted into 656 mL tubes with the MetroMix 840 PC. The a sample of the potting mix was plated
onto *Phytophthora* selective media, CARP (corn meal agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, 10 ppm rifamycin-SV), to test for presence of *Phytophthora* species.

The third set of red alder seedlings were obtained as seeds from a private property located 3.3 miles east of Corvallis, Oregon (Figure 2C). The seeds were brought into the OSU greenhouse facilities in early 2010 and first planted into a plastic planting tray and then transplanted into 262 mL planting tubes (Stuewe and Sons, Inc., Tangent, Oregon) using the MetroMix 840 PC. In fall 2010, nodules of *Frankia alni* were applied to the topsoil of each of the seedlings. Seedlings were stored in the greenhouse until April 2012, at which point they were relocated outside adjacent to the OSU east greenhouse facilities. In the greenhouse the seedlings were watered once daily, while outside they were maintained by natural rainfall and a sprinkler system.
Methods

**Stem inoculation test**- Two trials of the stem inoculation experiment were conducted over the course of two seasons, summer and winter. The summer trial took place over 5 weeks starting in August 2011 under greenhouse conditions (average 66 °F) using the 2011 containerized seedlings with an average height and diameter of 1500 mm and 6 mm, respectively. The winter trial took place over 10
weeks starting in February 2012 under outdoor conditions (35 to 51 °F) using the 2011 bare root seedlings with an average height and diameter of 860 mm and 7 mm, respectively. The seedlings for the summer trial had fully developed leaves and the winter trial seedlings had dormant buds.

A 6 to 10 mm long transverse cut was made into the cambial bark layer at 30 cm above the soil line for each seedling. One Phytophthora isolate was inoculated into an individual seedling by inserting a colonized 2 mm CMAβ agar plug into the cut. The cut was then wrapped in moist cheesecloth followed by aluminum foil. Sterile technique was utilized for each seedling to prevent cross contamination of isolates. Three replicates for each isolate were inoculated, in addition to control inoculations, which consisted of an uncolonized plug of CMAβ. Immediately following the inoculations, red alder seedlings were randomly arranged on the greenhouse bench for the summer trial and randomly arranged in plant tube racks for the winter trial (Figure 3A,B). The summer trial seedlings were watered once daily in the greenhouse, while the winter seedlings received natural rainfall outside adjacent to the OSU east greenhouses.
Figure 3. Experimental setup for the stem inoculation test. **A.** Red alder seedlings randomly arranged on greenhouse bench following inoculation with *Phytophthora* isolates. **B.** Close up of inoculation point on red alder seedling stems.

Following an incubation period of 5 or 10 weeks (depending on the trial), the outer bark at the inoculation point was scraped away and lesion length and width were measured with the aid of calipers. Stem discoloration and the presence of callused stem tissue were noted for each seedling. The scalpel and forceps used for this procedure were flame sterilized between each tree in order to prevent cross contamination. The lesion length and width measurements were then used to calculate lesion area (length x width/2). Bark pieces surrounding the lesion margin were plated onto CARP agar to confirm the presence of the *Phytophthora* species used for inoculation.
**Soil infestation test**- One soil infestation test was conducted starting in December 2012 in the OSU east greenhouse facilities. The red alder seedlings directly grown from seed at OSU were utilized for this experiment. The seedlings were brought into the greenhouse seven days prior to the start of the experiment to ensure actively growing roots were present on each seedling and to allow the soil temperature to equilibrate for the infestation with the *Phytophthora* isolates. Additionally, each seedling's root system was inspected for the presence of *Frankia alni* nodules before inoculation. The seedlings had an average height and diameter of 160 mm and 20 mm, respectively. The experiment was started before the seedlings initiated bud break and concluded after true leaves had developed.

Long grain white rice was colonized by the *Phytophthora* isolates and was used as the inoculum source for this pathogenicity experiment (Holmes and Benson, 1994). One 125 mL capped glass bottle was used to culture each isolate on the rice grains. Three 2 mm CMAβ plugs from a 7-day-old culture of each isolate were placed into the bottle with 10 g of long grain white rice and 7.2 mL of deionized water (autoclaved twice). The glass bottles were stored in the dark at 17.5 °C for 2 weeks with the caps slightly opened and shaken to loosen the rice grains every three days. Following infestation into the soil, a rice grain for each isolate was plated onto CARP agar to confirm viability of the inoculum.
The red alder seedlings were transferred from their 262 mL planting tubes into 656 mL tubes and then supplemented with pasteurized MetroMix 840PC potting soil. After the seedlings were repotted, a glass stir rod was inserted in the planting medium to make depressions to insert the rice grains on opposite sides of the seedling. To infest the soil, the rice grains were knocked out of the glass bottle onto a sterile Petri dish and then separated into individual grains. Approximately 3.3 g of rice was inserted into the two depressions made in the medium and then covered with potting mix. A total of three replicate seedlings for each isolate were inoculated. Each isolate was inoculated by this method; a fresh set of vinyl gloves used for each isolate in addition to a new sterile Petri plate to prevent no cross contamination occurred. Uninoculated sterile rice grains were inoculated into three tubes with seedlings, to serve as an experimental control.

Following the infestation of all of the seedlings, a biocontrol, Scanmask (BioLogic Company Inc., Willow Hill, Pennsylvania) for root weevils was applied to the topsoil of each seedling. Root weevils had been noted on other plant material stored nearby seedlings, which prompted the use of a biocontrol at the beginning of this experiment. 473 mL of Scanmask was mixed with 4.7 L of vermiculite and 1.9 L of tap water, after which 100 ml was directly applied to the topsoil of each tube.
Once inoculated, seedlings were randomly placed into tube racks and stored in the greenhouse for 14 weeks, where the average temperature was maintained at 66 °C (Figure 4A). Greenhouse staff watered seedlings daily for the duration of the experiment. Every three weeks seedlings were flooded for 72 hours by submerging tubes individually into 1 L plastic cups with water.

After 14 weeks in the greenhouse, seedlings were destructively sampled to observe the damage to the root systems. Seedlings were extracted from the potting tubes and then dipped into individual plastic cups with water, to removed excess soil (Figure 4B). Rhododendron leaf discs where floated on top of the water in the plastic cups for 48 hours and then plated onto CARP agar to confirm the presence of the Phytophthora species used for infestation (Figure 4C). Each seedling was then thoroughly rinsed under running water and then the root system was examined. For each seedling the following results were recorded: the presence of leaves and white roots, percentage of blackened fine roots, number of larger roots with visible lesions, number of broken roots, and presence of blackened F. alni nodules. Following the visual observations, a small sample from the top and bottom of the root system was plated onto CARP agar to confirm the presence of Phytophthora. Additionally, the root system for each seedling was dried at 40 °C for 5 days, after which the mass was recorded.
Figure 4. Experimental setup and teardown of the soil infestation test. **A.** Red alder seedlings after inoculation with rice grains and the Scanmask mixture. **B.** Red alder seedlings placed into plastic containers to wash root systems at the conclusion of the soil infestation test. **C.** Rhododendron leaf discs floated on the surface of water used to wash the red alder roots in order to bait for *Phytophthora*.

**Zoospore root dip test** - This experiment began in March 2013 using the 2012 bare root seedlings and one isolate of each *Phytophthora* species (Table 2). One month prior to the start of the experiment, the red alder seedlings were
brought into the greenhouse, in order to ensure active root growth. The
seedlings remained in the greenhouse for the duration of the experiment. Seedling
root systems were inspected at the time to ensure the presence of *F. alni* and overall
health. The seedlings had an average height and diameter of 980 mm and 80 mm,
respectively.

Zoospores were produced based on the protocols described in Oh and Hansen (2007) for *P. alni uniformis, P. gonapodyides, P. lacustris, P. lateralis, P. pini, P. plurivora, P. pluvialis, P. pseudosyringae, P. taxon Oaksoil, P. taxon Pgchlamydo; the protocol in Saavedra et al. (2007) for *P. cambivora; the protocol in Reeser et al. (2007) for *P. cambivora.* Following Oh and Hansen (2007), the isolates were plated onto V8S agar (V8 agar amended with 20 ppm β-sitosterol) and incubated at 17.5 °C in the dark for 5 days. After 5 days, nine 2 mm agar plugs were taken from the actively growing colony margin and placed onto a 90 mm Petri dish containing 10 mL of pea broth (50 g of split peas in 1 L of deionized water autoclaved for 4 min and filtered with 20 ppm β-sitosterol). The pea broth plates were incubated for three days at 20 °C in the dark. After three days, the pea broth was replaced with 10 mL of 5 μm filtered soil extract water (10 g soil with 1 L of deionized water) and then allowed to incubate for an additional day or two days, depending on the species, in order to produce abundant sporangia. The *P. cambivora* isolate was grown on carrot agar (CA) for seven days and then the above pea broth
incubation method was followed. The plugs were allowed to incubate for an extended period of five days in the soil water extract before zoospore release was initiated. For *P. riparia* and *P. siskiyouensis*, nine 2 mm agar plugs were taken from the actively growing margins and placed onto 90 mm Petri plates containing 10 mL of soil extract water. After two days, abundant sporangia were produced and zoospore release was induced. In order to release zoospores, the isolate plates were incubated at 4 °C for one hour and then room temperature for one hour. Once released, a 500 μL aliquot of zoospores was encysted by vortexing and then quantified using a hemacytometer. The concentration of the zoospore solution was adjusted to $10^4$ zoospores/mL for each species using soil water extract following quantification.

For each isolate, three red alder seedlings were used as experimental replicates. Each seedling was dipped into a 30 mL of the zoospore solution that was gently poured into the bottom of a 1 L plastic cup and a seedling was then placed into the zoospore solution. In order to ensure root submersion into the zoospore solution, 100 mL of deionized water was added into each plastic cup. The seedlings were left for two days in the zoospore solution, after which the seedlings were randomly arranged into tube racks in the greenhouse.
Following inoculation with the zoospore solution, seedlings were flooded with water every two weeks using the same method described for the soil infestation experiment. Two weeks following the zoospore dip, the red alder seedlings were treated for an aphid infestation. The seedlings were sprayed for aphids using a combination of Avid® (Syngenta Crop Protection, Inc. Greensboro, North Carolina) and Ornazin® (SePRO Corporation, Carmel, Indiana).

After 6 weeks in the greenhouse, seedlings were destructively sampled to observe the damage to the root systems. Seedlings were extracted from the potting tubes and then dipped into individual plastic cups with water, to remove excess soil. Rhododendron leaf discs were floated on top of the water in the plastic cups for 48 hours and then plated onto CARP agar to confirm the presence of the Phytophthora species used for infestation. Each seedling was then rinsed under running water and the root system was then observed. For each seedling the following results were recorded: the presence of white roots, percentage of fine root necrosis, number of larger roots with visible lesions, number of broken roots, and presence of blackened F. alni nodules. Following the visual observations, a small sample from the top and bottom of the root system was plated onto CARP agar to confirm the presence of Phytophthora.
**Detached leaf test** - This experiment was conducted in April 2013 and was run for 10 days in a growth chamber at OSU. Each *Phytophthora* isolate used for this experiment was grown on CMAβ for 7 days prior to inoculation onto red alder leaves. For this experiment, two-month-old leaves were collected the day of inoculation from red alder seedlings that were stored outside adjacent to the east greenhouse facilities. Moisture boxes were constructed using 27-liter plastic boxes filled with 2 cm of deionized water at the bottom (Figure 5A). Thirty-seven red alder leaves were placed into a single box in order to inoculate each *Phytophthora* isolate and an experimental control, which consisted of uninoculated CMAβ. To inoculate the leaves, a 2mm inoculated agar plug was taken from the growing margin of each *Phytophthora* isolate and the control and then placed onto a red alder leaf directly adjacent to the midrib with a size 1 insect pin (Figure 5B). The pin was used in order to wound the leaf and secure the inoculum source (Figure 5C). Five moisture boxes were utilized for this experiment, which represented five experimental replications. All five boxes were stored in a growth chamber for the duration of this experiment. The growth chamber had a 12 hour light cycle and an average temperature of 20.1 °C. Moisture boxes were sprayed daily with a fine mist of deionized water in order to maintain leaf moisture.

At the conclusion of 10 days, the red alder leaves were inspected for lesion development. Leaves with a visible lesion were photographed for lesion area
calculation using Assess (APS Press, St. Paul, MN). After the leaves were photographed, samples were taken from the growing lesion margin on the leaf and then plated onto CARP for reisolation of the Phytophthora species used for the test.

Figure 5. Experimental setup for the detached leaf test. A. Moisture box construction for detached leaf test, which included a layer of paper towels, a platform made from wire mesh, poster board, and a final layer of paper towels. B. The 37 leaves arranged in the moisture box. C. Close up of a leaf inoculated with an isolate of Phytophthora.
**Data analysis** - Statistical analysis of the data collected from each experiment was performed using the R statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA) tests were run on each data set from the stem inoculation test following a natural log transformation because the mean lesion area of each *Phytophthora* species did not fit a normal distribution. In order to compare the pathogenicity of each species of *Phytophthora* used for the stem inoculation test, Tukey’s test for the multiple comparison of means was carried out following the ANOVA. Additionally, the statistical groupings for each species were obtained using the agricolae package in R (agricolae: Statistical Procedures for Agricultural Research) based on the results of Tukey’s test. A non-parametric analysis of variance test, the Kruskal-Wallis test, was used for the soil infestation, zoospore root dip, and detached leaf tests because the data did not exhibit a normal distribution following multiple transformation attempts. Statistical significance for all statistical tests was interpreted by the calculated p-value being below 0.05. Graphical representation of the analysis was generated using the ggplot2 package in R (ggplot2: elegant graphics for data analysis).
Results

Four different pathogenicity tests were conducted from August 2011 to May 2013. Two trials of the stem inoculation test were performed on red alder seedlings over the course of two seasons, summer and winter. One trial each of the soil infestation, zoospore root dip, and detached leaf tests were completed for this study.

Stem inoculation test- For the summer trial, stem symptoms ranged from a slight brown discoloration to a blackening of the stem surrounding the inoculation wound (Figure 6B,C). Control inoculations resulted in no discoloration of the stem (Figure 6A). New white roots were observed just above the inoculation point for one of the replicates of *P. lacustris* (Figure 6D). Each *Phytophthora* isolate used for this test was successfully reisolated from each stem lesion. For the summer trial, *P. siskiyouensis* produced the largest average lesion area of all *Phytophthora* species tested, while *P. pluvialis* resulted in the smallest average lesion area of the *Phytophthora* species tested (Table 3, Figure 7). Analysis of variance indicated a significant difference in mean lesion area among *Phytophthora* species including the control inoculations (p-value < 0.0001, Table 4) With the exception of *P. pluvialis* *P. lateralis, P. pseudosyringae*, and *P. taxon Oaksoil*, the mean lesion areas caused by the *Phytophthora* species tested during the summer trial were found to be significantly different from the control inoculations (Table 3).
Figure 6. Stem lesions caused by *Phytophthora* species. **A.** Control inoculation with no stem discoloration. **B.** Dark brown stem lesion caused by *P. gonapodyides*. **C.** Black stem lesion caused by *P. siskiyouensis*. **D.** A new white root growing under the cheesecloth and foil wrapping above the stem lesion caused by *P. lacustris*. **E.** Control inoculation during the winter trial, where callus tissue grew over the inoculation point.
Table 3. Mean lesion area for each *Phytophthora* species from both stem inoculation trials.

<table>
<thead>
<tr>
<th>Species</th>
<th>Summer Trial</th>
<th>Winter Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean lesion area (mm$^2$)$^1$</td>
<td>Mean lesion area (mm$^2$)$^1$</td>
</tr>
<tr>
<td>Control</td>
<td>38.6</td>
<td>d</td>
</tr>
<tr>
<td><em>P. alni</em> subsp. uniformis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. cambivora</em></td>
<td>145.8</td>
<td>abc</td>
</tr>
<tr>
<td><em>P. gonapodyides</em></td>
<td>98.7</td>
<td>bc</td>
</tr>
<tr>
<td><em>P. lacustris</em></td>
<td>161.3</td>
<td>abc</td>
</tr>
<tr>
<td><em>P. lateralis</em></td>
<td>38.3</td>
<td>d</td>
</tr>
<tr>
<td><em>P. pini</em></td>
<td>151.7</td>
<td>abc</td>
</tr>
<tr>
<td><em>P. plurivora</em></td>
<td>218.1</td>
<td>ab</td>
</tr>
<tr>
<td><em>P. pluvialis</em></td>
<td>36.4</td>
<td>d</td>
</tr>
<tr>
<td><em>P. pseudosyringae</em></td>
<td>86.4</td>
<td>cd</td>
</tr>
<tr>
<td><em>P. riparia</em></td>
<td>100.5</td>
<td>bc</td>
</tr>
<tr>
<td><em>P. siskiyouensis</em></td>
<td>370.6</td>
<td>a</td>
</tr>
<tr>
<td><em>P. taxon Oaksoil</em></td>
<td>78.3</td>
<td>cd</td>
</tr>
<tr>
<td><em>P. taxon Pgclamydo</em></td>
<td>119.9</td>
<td>bc</td>
</tr>
</tbody>
</table>

$^1$Mean lesion area followed by lower case letters represent statistically significant differences based on Tukey’s test performed on the log-transformed data.
Figure 7. Mean lesion area on red alder seedlings caused by twelve different *Phytophthora* species at the conclusion of the summer trial of the stem inoculation test. The black bars represent standard error for the mean lesion area. Letters above bars represent statistically significant differences based on Tukey’s test performed on the log-transformed data.

Table 4. Analysis of variance for *Phytophthora* species effect (including the control as a species) on the log transformation of mean lesion area of red alder seedlings from the summer trial of stem inoculations.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean square</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>13</td>
<td>36.395</td>
<td>2.7996</td>
<td>13.577</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residuals</td>
<td>98</td>
<td>20.208</td>
<td>0.2062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>56.603</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The winter trial resulted in *P.* taxon Pgchlamydo causing the largest mean lesion area among the *Phytophthora* species used (Table 3, Figure 8). Overall, mean lesion area was smaller for each *Phytophthora* species at the conclusion of the winter trial compared to the summer trial. One control inoculation resulted in the formation of callus tissue, which healed the inoculation cut made in the stem (Figure 6E). Each *Phytophthora* isolate used for this test was successfully reisolated from each stem lesion. *P.* pluvialis resulted in the smallest average lesion area of the *Phytophthora* species tested (Table 3, Figure 8). Analysis of variance indicated a significant difference in mean lesion area among *Phytophthora* species including the control inoculations (p-value<0.0001, Table 3). For the winter trial, the mean lesion areas caused by *P.* cambivora, *P.* gonapodyides, *P.* lateralis, *P.* pseudosyringae, *P.* taxon Pgchlamydo were found to be significantly different from the control inoculations (Table 3).
Figure 8. Mean lesion area on red alder seedlings caused by thirteen different *Phytophthora* species at the conclusion of the winter trial of the stem inoculation test. The black bars represent standard error for the mean lesion area. Lower case letters above bars represent statistically significant differences based on Tukey’s test performed on the log-transformed data.

Table 5. Analysis of variance for *Phytophthora* species effect (including the control as a species) on the log transformation of mean lesion area of red alder seedlings from the winter trial of stem inoculations.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean square</th>
<th>F-statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>13</td>
<td>26.515</td>
<td>2.03960</td>
<td>5.1696</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residuals</td>
<td>109</td>
<td>43.005</td>
<td>0.39454</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>69.52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Soil Infestation Test - At conclusion of the soil infestation test, the root system of each red alder seedling was examined and destructively sampled. For each *Phytophthora* isolate, reisolation was successful either through direct plating of the roots or indirect isolation from the leaf baits. A *Pythium* species was recovered from the direct plating of one of the control seedlings. The formation of new white roots above the root collar was observed on 15% of seedlings (Figure 9C). Stem lesions extending from the root collar were observed on 12% of the red alder seedlings (Figure 9B); three seedlings of *P. taxon Pgchlamydo* had stem lesions and two each of *P. siskiyouensis* and *P. lacustris*. The blackening of *Frankia alni* nodules was observed on 10% of the seedlings at the conclusion of the soil infestation test (Figure 9E). Leaf emergence was recorded at the conclusion of the experiment, as all seedlings were dormant at the beginning of the experiment (Figure 10). Fine root necrosis was rated using percentages based on the entire root system, with careful washing of the roots conducted in order to keep intact all necrotic fine roots (Figure 9A, 11). Larger roots of the seedling root system with black necrotic lesions were recorded as a percentage compared to the total number of large roots and were used for statistical analysis. The Kruskal-Wallis rank sum test did not demonstrate a significant difference in percentage of roots with lesions between *Phytophthora* species (p-value = 0.06, Figure 12). Despite a lack of significance, a difference in the mean percentage of roots with lesions was demonstrated along
with a high variance by the standard errors (Figure 12). Broken roots were
the result of advanced root necrosis, which led to the breaking of the larger roots
above the end of the root system (Figure 9D). Broken roots were statistically
analyzed as a percentage of broken roots compared to healthy roots. The Kruskal-
Wallis rank sum test did not indicate a significant difference in the percentage of
broken roots between *Phytophthora* species (p-value = 0.16, Figure 13).

Figure 9. Examples of disease symptoms on the red alder seedlings’ root systems
resulting from the soil infestation test. **A.** Fine root necrosis observed on a red alder
seedling inoculated with *P.* taxon Oaksoil. **B.** A stem lesion extending from the root
collar down one of the large roots on a red alder seedling inoculated with *P.* taxon
Pgchlamydo. **C.** White roots growing from above the root collar of a seedling
inoculated with *P.* lacustris. **D.** Broken and necrotic large roots caused by *P.* lacustris.
**E.** Blackened nodule of *Frankia alni* on a seedling inoculated with *P.* riparia.
Figure 10. Leaf emergence of red alder seedlings for each species of *Phytophthora* used for the soil infestation test. Percentage of red alder seedlings within each leaf development category was calculated. Test was initiated when all seedlings were dormant and had not experienced bud burst.
Figure 11. Fine root necrosis observed at the conclusion of the soil infestation test for the red alder seedlings. Percentage of red alder seedlings within each fine root necrosis category was calculated. Visual examination of the roots was performed after the root system was thoroughly washed of debris.
Figure 12. Mean percentage of roots with lesions resulting from the soil infestation test conducted on red alder seedlings using thirteen different *Phytophthora* species. The black bars represent standard error for the mean percentage of roots with lesions.
Figure 13. Mean percentage of broken roots at the conclusion of the soil infestation test conducted on red alder seedlings using thirteen different *Phytophthora* species. The black bars represent standard error for the mean percentage of broken roots.

**Zoospore root dip test**- In order to obtain results from the zoospore root dip test, the root system of each red alder seedling was thoroughly examined and destructively sampled. For each *Phytophthora* isolate, reisolation was successful either through direct plating of the roots or indirect isolation from the leaf baits.
The formation of new white roots above the root collar was observed on 5% of seedlings. No stem lesions were observed on the red alder seedlings used for this test. The blackening of *Frankia alni* nodules was observed on 5% of the seedlings at the conclusion of the zoospore root dip test. Fine root necrosis was rated using percentages based on the entire root system, with careful washing of the roots conducted in order to keep intact all necrotic fine roots (Figure 14). Larger roots with black necrotic lesions were recorded and the percentage of roots with lesions compared to the total number of large roots was used for statistical analysis. The Kruskal-Wallis rank sum test demonstrated a slightly significant difference in percentage of roots with lesions between *Phytophthora* species (p-value = 0.04, Figure 15). *P. gonapodyides, P. plurivora, P. pseudosyringae, P. taxon Pgchlamydo* were found to cause statistically significant lesions on the root systems of the red alder seedlings compared to the control inoculations (p-values = 0.01, 0.01, 0.01, 0.03 respectively). Broken roots were the result of advanced root necrosis, which led to the breaking of the larger roots above the end of the root system. Broken roots were statistically analyzed as a percentage of broken roots compared to healthy roots. The Kruskal-Wallis rank sum test did not indicate a significant difference in the percentage of broken roots between *Phytophthora* species (p-value = 0.99, Figure 16).
Figure 14. Fine root necrosis observed at the conclusion of the zoospore root dip test for the red alder seedlings. Percentage of red alder seedlings within each fine root necrosis category was calculated. Visual examination of the roots was performed after the root system was thoroughly washed of debris.
Figure 15. Mean percentage of roots with lesions resulting from the zoospore root dip test conducted on red alder seedlings using thirteen different *Phytophthora* species. The black bars represent standard error of the mean percentage of roots with lesions.
Figure 16. Mean percentage of broken roots at the conclusion of the zoospore root dip test conducted on red alder seedlings using thirteen different Phytophthora species. The black bars represent standard error for the mean percentage of broken roots.

**Detached leaf test**- Following a 10 day incubation period, each of the inoculated detached red alder leaves were examined for lesions. Of the 185 leaves utilized in the test, only 15.6% leaves had visible lesions on the leaves at the conclusion of the test. Lesions were observed on the leaves inoculated with twelve
of the *Phytophthora* species, with *P. lateralis* not causing any lesions (Figure 17). After plating the lesion margins of each of the 29 leaves with lesions onto *Phytophthora* selective media, only *P. cambivora*, *P. gonapodyides*, *P. lacustris*, *P. plurivora*, *P. taxon Oaksoil*, and *P. taxon Pgchlamydo* were reisolated. *P. cambivora* caused the largest lesion (13.49 cm$^2$) and percent lesion area compared to the other species of *Phytophthora* (Figure 17). The Kruskal-Wallis rank sum test did not indicate a significant difference in the percentage of lesion area between *Phytophthora* species (p-value= 0.08).
Figure 17. Mean percent lesion area caused by thirteen different *Phytophthora* species on detached red alder leaves. The black bars represent standard error for the mean percent lesion area.
Discussion

The genus *Phytophthora* is known to cause disease in many forest ecosystems worldwide, with many non-native species of *Phytophthora* responsible for causing landscape level mortality of forest trees. In western Oregon riparian ecosystems (WORE), a survey was conducted in order to determine if *P. alni*, a known forest pathogen, was present and whether the organism was responsible for causing reported alder dieback. During this survey, many other species of *Phytophthora* were isolated in association with riparian red alder trees with symptoms of dieback. Four different pathogenicity tests were conducted with thirteen species of *Phytophthora* in order to determine if they are the causal organisms of the observed decline of red alder in western Oregon riparian ecosystems. Each pathogenicity test utilized a different inoculation method in order to observe the effect of each of the *Phytophthora* species on different organs of the red alder seedlings: the stem, roots, and leaves. Through the comparison of all the pathogenicity tests conducted, the hypothesis was supported; red alder is not susceptible to the *Phytophthora* species recovered from the WORE survey. The results of this study demonstrate that the twelve species of *Phytophthora* recovered from the WORE survey do not cause significant disease on red alders (Table 6). As the twelve *Phytophthora* species are not the causal organism responsible for the noted decline of red alder and have
previously been recovered throughout western Oregon, it can be inferred that these *Phytophthora* species are native to riparian ecosystems in western Oregon.
Table 6. A summary of disease symptom results caused by the thirteen species of *Phytophthora* from four pathogenicity tests on red alder seedlings.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stem Inoculation</th>
<th>Soil Infestation</th>
<th>Zoospore Root Dip</th>
<th>Detached Leaf$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer trial$^1$</td>
<td>Winter Trial$^1$</td>
<td>Roots with lesions$^2$</td>
<td>Broken Roots$^3$</td>
</tr>
<tr>
<td>Control</td>
<td>38.6</td>
<td>13.3</td>
<td>16.7</td>
<td>0.0</td>
</tr>
<tr>
<td><em>P. alni</em> subsp. <em>uniformis</em></td>
<td>-</td>
<td>39.4</td>
<td>26.3</td>
<td>9.4</td>
</tr>
<tr>
<td><em>P. cambivora</em></td>
<td>145.8</td>
<td>27.6</td>
<td>27.1</td>
<td>23.6</td>
</tr>
<tr>
<td><em>P. gonapodyides</em></td>
<td>98.7</td>
<td>49.1</td>
<td>32.9</td>
<td>1.9</td>
</tr>
<tr>
<td><em>P. lacustris</em></td>
<td>161.3</td>
<td>51.8</td>
<td>41.1</td>
<td>22.2</td>
</tr>
<tr>
<td><em>P. lateralis</em></td>
<td>38.3</td>
<td>35.3</td>
<td>41.7</td>
<td>33.3</td>
</tr>
<tr>
<td><em>P. pini</em></td>
<td>151.7</td>
<td>39.4</td>
<td>36.9</td>
<td>10.4</td>
</tr>
<tr>
<td><em>P. plurivora</em></td>
<td>218.1</td>
<td>30.2</td>
<td>36.2</td>
<td>18.8</td>
</tr>
<tr>
<td><em>P. pluvialis</em></td>
<td>36.4</td>
<td>25.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>P. pseudosyringae</em></td>
<td>86.4</td>
<td>50.9</td>
<td>10.4</td>
<td>2.2</td>
</tr>
<tr>
<td><em>P. riparia</em></td>
<td>100.5</td>
<td>29.3</td>
<td>8.5</td>
<td>0.0</td>
</tr>
<tr>
<td><em>P. siskiyouensis</em></td>
<td>370.6</td>
<td>26.9</td>
<td>25.0</td>
<td>16.9</td>
</tr>
<tr>
<td><em>P. taxon Oaksoil</em></td>
<td>78.3</td>
<td>33.3</td>
<td>34.1</td>
<td>11.5</td>
</tr>
<tr>
<td><em>P. taxon Pgchlamydo</em></td>
<td>119.9</td>
<td>79.6</td>
<td>50.3</td>
<td>16.8</td>
</tr>
</tbody>
</table>

$^4$Greyed numbers indicate a statistically significant difference from control inoculation

$^1$Mean lesion area (mm$^2$)

$^2$Mean percentage of roots with lesions (%)

$^3$Mean percentage of broken roots (%)

$^4$Mean percent lesion area (%)
The first pathogenicity test conducted for this study was the stem inoculation test, which involved the creation of an artificial wound in the stem of red alder seedlings and which was then inoculated with one of the thirteen species of Phytophthora. Two trials of this pathogenicity test were completed over the course of two different seasons, the summer and winter. For this pathogenicity test, disease development was measured as the area of the stem lesion caused by a Phytophthora species. The stem inoculation test resulted in significant disease development compared to control inoculations on the stems of red alder seedlings from both the summer and winter trials. Both of the trials resulted in lesion development for each Phytophthora species tested. However, the largest average lesions in each trial were caused by different species of Phytophthora; P. siskiyouensis and P. taxon Pgchlamydo caused the largest average lesions at the conclusion of the summer and winter trials, respectively. P. siskiyouensis was the only species directly isolated from a stem lesion on a red alder (Sims and Hansen, 2012a). P. siskiyouensis has been found to cause stem lesions on Italian alders (Alnus cordata) in the United States, however the trees were planted in urban areas and not in natural riparian ecosystems (Rooney-Latham et al., 2009). Although P. taxon Pgchlamydo has been recovered abundantly in the riparian ecosystem through water sampling, it has not been found associated with stem symptoms on red alder. However, Schwingle and Blanchette (2008) determined that P. taxon Pgchlamydo was capable of causing stem lesions on other tree species. Using bur oak (Quercus macrocarpa) and northern red oak (Quercus rubra) seedlings, P. taxon Pgchlamydo was found to
cause significant stem lesions compared to control inoculations (Schwingle and Blanchette, 2008). The results of this test further confirm the potential for both *P. siskiyouensis* and *P. taxon Pgchlamydo* to cause disease symptoms on the stems of trees, specifically red alder.

In addition to different *Phytophthora* species causing the largest average lesion, the mean lesion areas were larger at the conclusion of the summer trial than the winter trial. Differing phenological stages of the red alder seedlings could be an explanation for the decrease in mean lesion area between the winter and summer trials. For the summer trial, the red alder seedlings had developed leaves, in contrast to the winter trial, where the seedlings were dormant at the start of the trial and underwent bud burst by the conclusion. With a reduction in the transport of water and nutrients in the red alder seedlings during the winter trial, the available host tissue may not have been as conducive to stem lesion development. A study by Brasier and Kirk (2001) using *P. alni* demonstrated that mean lesion area varied with regard to the season in which inoculated logs were cut for experimental use. Larger lesions were caused by *P. alni* on logs harvested during the months of July to October compared to those cut in November to April. Additional seasonal differences have been demonstrated to be significant in the development of stem lesions in northern red oaks by *P. cinnamomi* (Robin et al., 1994). By measuring both lesion development and the relative water content of the bark, it was determined that the period of greatest susceptibility occurred during active shoot
growth (Robin et al., 1994). For this study, active shoot development would have occurred for the entire duration of the summer trial and only near the end of the winter trial, which could have led to the differences in mean lesion area between the two trials.

In order to test the pathogenicity of each Phytophthora species to the roots of a red alder seedling, a soil infestation test was conducted for this study. Each Phytophthora species was grown on rice grains and then inoculated directly into the soil with a red alder seedling, with disease development recorded after 15 weeks. Disease development was recorded using multiple measures for each seedling, which included determining the percentage of roots with lesions, percentage of broken roots, amount of fine root necrosis, and leaf emergence at the conclusion of the test. The high variability observed in the number of broken roots and roots with lesions caused by each species of Phytophthora prevented detection of significant differences in disease severity in the soil infestation test (Figure 7, 8). However, this variability between even the same isolates of each species could be an artifact of the inoculation technique used for this test. By not artificially wounding the red alder root system, each Phytophthora isolate had to colonize the roots without an initial entry point. Therefore, the soil infestation is more representative of a natural infection compared to the stem inoculation test. Of the thirteen species tested, eleven of the Phytophthora species were determined to cause greater than 50% fine root necrosis of the red alder seedlings tested (Figure 6). From the WORE survey,
root lesions were observed on about 3% of the red alder trees surveyed (Sims and Hansen, 2012a). Of those eleven species, *P. alni* subsp. *uniformis*, *P. gonapodyides*, *P. lacustris*, *P. lateralis*, *P. plurivora*, and *P. pseudosyringae* have previously been demonstrated to be root pathogens (Brasier et al., 2004, 1993; Jung et al., 2003; Orlikowski et al., 2011; Weiland et al., 2010; Zobel et al., 1985).

The final measurement of disease development for the soil infestation test was the amount of leaf emergence. Each red alder seedling was dormant at the beginning of the experiment; the number of leaves that had emerged at the conclusion of the test was recorded. From this test, it was determined that bud burst did not occur in every seedling, which could be correlated to the development of root damage caused by the *Phytophthora* species during this test (Figure 5). With the added disease pressure of the *Phytophthora* species, the seedlings may not have been capable ofexpending the energy to break bud due to the necrosis occurring in their root systems. According to Jönsson, 2004, there has been minimal research on the relationship between above ground growth and root damage.

Zoospores act as disease propagules for many species of *Phytophthora*, thus utilizing swimming spores in a pathogenicity test is necessary to determine the ecological roles of the thirteen species of *Phytophthora* to red alder. For the zoospore root dip test, zoospores of each *Phytophthora* species were produced and then inoculated directly to the root systems of red alder seedlings. At the conclusion of the zoospore root dip test, the root system of each red alder seedling was
inspected for disease development by recording the percentage of roots with lesions, the percentage of broken roots, and the amount of fine root necrosis. In contrast to the soil infestation test, four species of Phytophthora, *P. gonapodyides*, *P. plurivora*, *P. pseudosyringae*, *P. taxon Pgchlamydo* were determined to cause significant root lesions at the conclusion of the zoospore root dip test. Additionally, these species resulted in disease symptoms in the stem inoculation test for this study and in previous root pathogenicity studies (Brasier et al., 2003; Jung and Blaschke, 2004; Jung et al., 2003; Weiland et al., 2010). Red alder trees line stream banks and typically have their root systems in direct contact with waterways, thus this inoculation method may be more representative of natural infections occurring throughout western Oregon riparian ecosystems (Harrington, 2006; Sims and Hansen, 2012a). In a previous study by Reeser et al. (2011), up to 160 colonies of *Phytophthora* were isolated from one liter of filtrated stream water throughout western Oregon. Based on those colony concentrations, the concentration of $10^4$ zoospores/ml should have been a sufficient concentration for disease symptoms to develop. As motile spores, zoospores are capable of finding and infecting root systems through chemoattractants that are exuded by the roots of the plant (Judelson and Blanco, 2005). The zoospore root dip test was concluded using seedlings that had active root growth, which most likely led to the attraction of the zoospores of each *Phytophthora* species and caused fine root necrosis (Figure 9).
Roots of red alder are being infected by multiple species of *Phytophthora*, which deviates from other known forest diseases in Oregon that are caused by a single *Phytophthora* species (Sims and Hansen 2012a, Rizzo and Garbelotto, 2003; Saavedra et al., 2007; Zobel et al., 1985). Further studies testing the pathogenicity of different combinations of *Phytophthora* species could be beneficial to determine if greater disease development occurs in the root systems of red alders; these tests could be more consistent with field observations of red alder decline. Additionally, in future zoospore pathogenicity tests, leaf debris could be amended into the flooding process to aid in the sporulation of the *Phytophthora* species in order to produce more inoculum for further disease development (Sims and Hansen, 2012a). Through the utilization of the infective zoospores, the pathogenicity of four of the *Phytophthora* species tested to the roots of red alder seedlings was demonstrated in this test.

The final pathogenicity test was conducted in order to determine disease development on detached leaves of red alder using the thirteen species of *Phytophthora*. Colonized agar plugs of each *Phytophthora* species placed directly atop a wound in the leaf were utilized. The resulting lesion on the leaf was used in order to assess disease development. A preliminary detached leaf test was conducted using red alder leaves and the thirteen *Phytophthora* species. However, sufficient moisture was not maintained in the experiment boxes resulting in the premature drying out of the colonized agar plugs. Therefore, the experiment was
repeated with sufficient moisture inside the experimental boxes, which resulted in retaining the colonized plugs in contact with the red alder leaves for a longer time period. Similar to the stem inoculation test, the detached leaf test also involved the wounding of the host tissue prior to inoculation with the species of *Phytophthora*. However, the greater opportunity for disease development granted by wounding the leaves did not result in significant pathogenicity of the *Phytophthora* species to the red alder leaves. With such low disease development for the whole test, no single *Phytophthora* species caused a significant lesion on the red alder leaves. Although *P. cambivora* did result in the largest lesion produced from the test, it is not constantly associated with leaf lesions, but rather stem lesions and root rots of other tree species (Saavedra et al., 2007). During the WORE survey, foliar pathogen spots were one of the highest occurring symptoms on the red alders sampled, but were not demonstrated to be caused by the twelve *Phytophthora* species used in this study (Sims and Hansen, 2012a).

By comparing all four of the pathogenicity tests conducted for this study, *P. taxon Pgchlamydo* was found to cause significant disease on both the stem and root tissue in addition to minor lesions on red alder leaves. As a formally undescribed species, there have been few pathogenicity studies utilizing *P. taxon Pgchlamydo* (Brasier and Jung, 2006; Jung and Nechwatal, 2008). In this study, *P. taxon Pgchlamydo* did cause disease symptoms on red alder seedlings in all four tests, which is the first report for this species. Utilizing *P. taxon Pgchlamydo* in
combination with other *Phytophthora* species for pathogenicity testing could
demonstrate disease symptoms similar to field observations, as the species were
isolated simultaneously from WORE survey transects (Sims and Hansen, 2012a). *P.*
taxon *Pgchlamydo* has previously been isolated in abundance from Oregon streams
and was one of only two species of *Phytophthora* to be isolated from all sites at
every time point in a previous study of western Oregon waterways (Reeser et al.,
2011). *P.* taxon *Pgchlamydo* has not been described as causing disease symptoms on
riparian trees in Oregon at the present. Additional pathogenicity studies using *P.*
taxon *Pgchlamydo* and other riparian vegetation may further describe the ecological
role of this species in Oregon waterways.

Of the thirteen species tested, *P. gonapodyides, P. lacustris, P. riparia, P. taxon
Oaksoil,* and *P. taxon Pgchlamydo* are representatives of ITS clade 6, which are
abundant in streams worldwide and seem to be opportunistic pathogens rather
than aggressive ones in natural ecosystems (Brasier et al., 2003; Hansen, 2000; Jung
et al., 2011; Kroon et al., 2012; Reeser et al., 2011). From the WORE survey, ITS
clade 6 species were routinely isolated from water and root samples from 75 of the
88 transects (Sims and Hansen, 2012a; Sims et al., 2012). With such high isolation
of ITS clade 6 species in Oregon, one can speculate that these are native organisms
and could be causing the fine root necrosis observed by infecting the root systems of
streamside red alders. Additional ecological roles of ITS clade 6 *Phytophthora*
species in western Oregon riparian ecosystems could be associated with the
breakdown of green leaf litter, which would result in higher inoculum present in the streams leading to the development of root disease (Brasier et al., 2003; Sims and Hansen, 2012b).

The high variability observed in disease symptom development for each Phytophthora species in the soil infestation, zoospore root dip, and detached leaf tests, prevented the detection of statistically significant results in these pathogenicity tests. The variation between replicates for each species of Phytophthora could have been a product of the experimental methods used for each of three tests. For the soil infestation test, the seedlings were replanted into larger tubes, which could have resulted in fine root damage before the test was started. Additionally, the presence of other soil microorganisms could have led to misleading root necrosis, since the control inoculations had reported fine root necrosis as well. For the zoospore root test, the lower variability in the percentage of roots with lesions did result in significant disease development for four of the Phytophthora species, but the variability in the percentage of broken roots did not result in significant disease development. The percentage of broken roots for the red alder seedlings was a measure developed in order to quantitatively assess root damage caused by each Phytophthora species. However, this measurement of disease development proved to be highly variable between replicates of the same Phytophthora species and was thus not a reliable way to assess the red alder seedlings. Using the percentage of broken roots as part of a disease rating system in
future root pathogenicity tests could lower the variability in the results obtained between replicates of each *Phytophthora* species. Lastly, the detached leaf test did not result in significant lesion development for any of the *Phytophthora* species. The experimental methods could be the underlying cause of the lack of leaf lesions observed at the conclusion of the test. Although proper leaf moisture appeared to be obtained throughout the test, the contact between the inoculated plug and the red alder leaf could have not been sufficient to initiate lesion development. With five of the thirteen species of *Phytophthora* belonging to ITS clade 6, a higher moisture level throughout the test could have led to larger lesions produced, since ITS clade 6 Phytophthoras are adapted for aquatic environments. By evaluating and adjusting the methods for these three pathogenicity tests, they can be more reliably used in the future without such high variability in the results.

Historically, invasive species of *Phytophthora* have been known to cause more disease in natural systems than the native Phytophthoras in the same system (Hansen, 2008; Sims and Hansen, 2012a). Thus far there is no evidence that the twelve *Phytophthora* species recovered from the WORE survey are invasive to western Oregon riparian ecosystems (Sims and Hansen, 2012a). This could explain the less aggressive nature of the twelve *Phytophthora* species used in this study from the WORE survey.

Although none of these species of *Phytophthora* were overtly aggressive towards red alder seedlings throughout the pathogenicity tests, they were able to cause
minor disease symptoms. With the decline of red alder in western Oregon, these Phytophthoras could be affecting red alder trees in combination with other insects and pathogens (Sims and Hansen, 2012a). By weakening the red alders through fine root necrosis or stem lesions, the twelve species of *Phytophthora* from the WORE survey could be the initial biotic factors of the decline of red alder.

With increasing research into large scale surveys of forest Phytophthoras around the world, further research is needed in order to determine their ecological roles in native ecosystems. By conducting pathogenicity tests on the recovered species of *Phytophthora*, it can be determined if the disease symptoms observed in natural ecosystems are truly caused by the organisms. As a historically known genus of pathogens, continued research on *Phytophthora* species is necessary in order to characterize them morphologically as well as ecologically.
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CHAPTER 3: Conclusion

The genus *Phytophthora* has become known as a “plant destroyer” of agricultural crops and forest trees, however, more species of this genus are being described on a continual basis that may not play the same role in ecosystems. With more environmental sampling for *Phytophthora* in natural ecosystems being conducted worldwide, new species are discovered without being associated with any disease in those ecosystems (Hwang et al., 2009; Milenkovic et al., 2012; Reeser et al., 2011). Continued research into the ecological roles of these new species of *Phytophthora* recovered from natural ecosystems is necessary given the historical destructive nature of the genus *Phytophthora*.

In western Oregon, riparian ecosystems were surveyed to determine the presence of *Phytophthora* species and the agents responsible for the noted dieback in red alder trees (Sims and Hansen, 2012a). From this survey many species of *Phytophthora* were isolated from water, streamside soil, and the fine roots of red alders, but they could not be formally described as the causal organisms of the decline of riparian red alder. In order to determine the ecological role of these *Phytophthora* species in western Oregon riparian ecosystems (WORE), this study was conducted. Through various pathogenicity tests, this study tested the hypothesis that red alder is not susceptible to the twelve *Phytophthora* species recovered from the WORE survey. Red alder seedlings were utilized for all
pathogenicity tests in order to demonstrate that the twelve *Phytophthora* species do not cause significant disease on these riparian adapted trees.

Four pathogenicity tests were conducted, which utilized different plant organs of red alder seedlings in order to accurately describe the ecological roles of the twelve species of *Phytophthora* in riparian ecosystems where red alder trees are the dominant species. Additionally, one species, *P. lateralis*, was included in the study because of its presence in other western Oregon streams and its designation as a host-specific pathogen to Port-Orford-cedar. The pathogenicity tests demonstrated that the thirteen *Phytophthora* species from riparian ecosystems do not cause significant disease symptoms on red alder seedlings. Although found in association with red alder trees, these *Phytophthora* species are not the causal organisms of the decline described from the WORE survey.

None of the tested species of *Phytophthora* were overtly aggressive towards red alder seedlings throughout the pathogenicity tests, but they were able to cause minor disease symptoms. From the stem inoculation test, nine of the thirteen *Phytophthora* species resulted in the development of significant stem lesions in one or both of the conducted trials compared to the control inoculations (Table 6). There were no significant disease symptoms observed for the soil infestation and the detached leaf tests. However, for the zoospore root dip test, one measurement, the percentage of roots with lesions, resulted in significant disease development by four species of *Phytophthora* (Table 6). Red alder trees have evolved to inhabit and
thrive in riparian ecosystems and have probably become tolerant of these *Phytophthora* species recovered from the WORE survey (Deal and Harrington, 2006). These species of *Phytophthora* could be acting as the initial biotic factors leading to the dieback of red alder in riparian ecosystems. Once weakened, the red alders could become more susceptible to other insects and pathogens, which could then lead to the noted decline from the WORE survey.

By including the host specific pathogen, *P. lateralis*, in the pathogenicity testing, the variability in each test was demonstrated. As a known pathogen of Port-Orford-cedar, *P. lateralis* was not expected to cause disease symptoms on the red alder seedlings across the pathogenicity tests. However, inoculation with *P. lateralis* did result in variable disease symptom development, which could be explained by the difference in artificial versus natural inoculation methods.

With continued environmental sampling for species of *Phytophthora* in natural ecosystems, parallel research into their ecological roles should be conducted. Knowing the pathogenicity of *Phytophthora* species in their native ecosystems will better prepare forest managers, if they were to become an invasive forest pathogen in another natural ecosystem. *Phytophthora* species have the potential to have global impacts on forest ecosystems, which can be mitigated by conducting research on indigenous species before they become global issues of forest health.
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