AN ABSTRACT OF THE THESIS OF

Clare Rebecca Taylor for the degree of Master of Science in Botany and Plant Pathology presented on June 3, 2011.
Title: Comparative Epidemiology of *Phytophthora* Diseases of *Rhododendron*
Abstract approved:

_____

Niklaus J Grünwald

Rhododendron species are host to many *Phytophthora* species and are an important crop in the nursery industry. The foliar epidemiology of twelve *Phytophthora* diseases on *Rhododendron* was studied in field and growth chamber experiments on two *Rhododendron* cultivars. The relative aggressiveness of *Phytophthora* spp. was assessed using disease incidence, severity, and sporulation. Plant experiments were conducted on whole plants and on detached leaves. Growth rates of lesions and colonies for each species at different temperatures were determined in growth chambers on detached leaves and in agar culture. Whole plants were inoculated in the field in four experiments conducted at three to four month intervals. The twelve species studied appeared to fall into two groups with *P. citrophthora*, *P. foliorum*, *P. kernoviae*, *P. parasitica*, *P. plurivora* and *P. ramorum* being the most aggressive species; sporulating the most, developing the largest lesions or exhibiting the highest infection rate. *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. lateralis*, *P. nemorosa* and *P. syringae* were less aggressive on foliage under most conditions, with the exception of *P. syringae* which developed the largest lesions at lower temperatures typical of spring time in temperate climates. It is

apparent that different *Phytophthora* species are more active at different temperatures and thus surveys for *Phytophthora* infections should be concerned about different species at different times of the year. *P. kernoviae* is not known to be present in the US. *P. kernoviae* is of particular concern due to the high sporulation levels observed in detached leaf and whole plant inoculations. *Rhododendron* spp. appear to be likely candidate vectors for the introduction of novel *Phytophthora* pathogens. This work has implications for the management of *Phytophthora* pathogens and importation or movement of *Rhododendron* plants across nurseries.

Comparative Epidemiology of *Phytophthora* Diseases of *Rhododendron*

by
Clare Rebecca Taylor

A THESIS

Submitted to

Oregon State University

in partial fulfilment of
the requirements for the
degree of

Master of Science

Presented June 3, 2011
Commencement June 2012

Master of Science thesis of Clare Rebecca Taylor presented on June 3, 2011.

APPROVED:

_____

Major Professor, representing Botany and Plant Pathology

_____

Chair 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.

_____

Clare Rebecca Taylor, Author

ACKNOWLEDGEMENTS

It is a pleasure to thank those who helped make this thesis possible: Niklaus Grünwald for his support and advise and also members of the Grünwald lab, particularly; Kimberly Graham for carrying out the detached leaf inoculations on all twelve species in the growth chamber, Kenneth Rolfe who assisted with field experiment set up, Karen Fairchild for help with culture maintenance, Valerie Fieland who conducted PCR and ITS sequencing for isolate verification, and Stephanie Brewer and Rachael Workman who assisted with plant maintenance, media preparation and field data collection.

I would also like to thank the members of my graduate studies committee, Walter Mahaffee, Chris Mundt, Everett Hansen and Shawn Mehlenbacher for their time and effort in advising and proof reading manuscripts. Thanks are also due to Jennifer Parke, Everett Hansen, Paul Reeser, Nancy Osterbauer, Clive Brasier, Michael Coffey, Kurt Lamour, Matteo Garbelotto, and Jean Ristaino for providing isolates.

Financial support was provided by United States Department of Agriculture - Agricultural Research Service (USDA-ARS) CRIS 5358-22000-034-00, the USDA-ARS Northwest Nursery Research Program, the Oregon Department of Agriculture (ODA), Oregon Association of Nurseries (OAN) research program, and the USDA Floriculture Nursery Research Initiative. I am also grateful for the Larry Moore Award for Graduate Education for my research made possible through the Department of Botany and Plant Pathology.

Finally I owe my deepest gratitude to my family for the never ending support, love and encouragement I receive from them, especially my husband Curtis, my parents Peter and Jeanie, and my sister Rachel.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

DEDICATION

This thesis is dedicated to God, without whom I can achieve nothing.

Comparative Epidemiology of *Phytophthora* Diseases of *Rhododendron*

CHAPTER 1: GENERAL INTRODUCTION

The genus *Phytophthora* was first described in 1875 by Heinrich Anton de Bary (1876) and now consists of over a hundred described species, with potentially 200-600 more yet to be identified (Brasier, 2007). The genus *Phytophthora* is placed in the family Pythiaceae of the class Oomycota in the phylum Stramenopila, in the kingdom Chromalveolata (formerly Chromista) (Adl et al., 2005; Simpson and Roger, 2004). Many *Phytophthora* species have wide host ranges and are of great importance in the agriculture, nursery and forestry industries due to the devastation they can cause (Birch and Whisson, 2001; Erwin and Ribeiro, 1996; Orlikowski, 2010; Weste and Marks, 1987). For example, *P. cinnamomi* is a serious root rot pathogen of numerous woody plants, with a host list including over 1,000 species (Erwin and Ribeiro, 1996). This pathogen has become especially problematic in Western Australian forests causing Phytophthora dieback of jarrah trees (*Eucalyptus marginata*) and many other native bush land plants (Hardham, 2005; Shearer et al., 1981).

Another example of an important species is *Phytophthora ramorum* that was first described on *Rhododendron* in Europe in 2001 (Werres et al., 2001). Since then it has been identified as the pathogen causing sudden oak death (SOD) of tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) in the coastal forests of the Western United States. *P. ramorum* also causes ramorum blight on dozens of woody ornamental plants. Notable hosts include *Rhododendron* spp., red oaks, *Viburnum* spp., bay laurel, Douglas-fir, and coast redwood among many others (Davidson et al., 2003; Rizzo et al.,

2005). *P. ramorum* is an invasive species in the USA and Europe (Goss et al., 2009; Grünwald et al., 2008). *P. ramorum* threatens to alter the composition of the forests of the West coast dramatically and has had a severe impact on the nursery industry across the USA since it was first detected in California in 1994. The introduction of *P. ramorum* to California forests was traced back to nursery shipments; subsequently intensive quarantine and eradication programs have been implemented (Goss et al., 2009; Mascheretti et al., 2008; Osterbauer et al., 2004). In the nursery industry the presence of *P. ramorum* has had severe economic impacts even if the epidemic is not widespread because the quarantine regulations require all potential host plants to be destroyed and the suspension of sales until the infected nursery tests negative for the pathogen (Dart and Chastagner, 2007; Frankel, 2008; Grünwald et al., 2008). National and international trade of nursery products can result in the introduction and spread of exotic pathogens as has been observed with the invasive pathogen *P. ramorum* (Parke et al., 2004; Prospero et al., 2007; Werres et al., 2001).

Many other *Phytophthora* species are endemic to nursery environments where conditions are ideal for these 'water mold' pathogens given abundant fertilization and irrigation providing moist, dense canopies (Dart et al., 2007; Schwingle et al., 2007). Numerous woody ornamental plants, which are popular in the nursery and landscaping business, host foliar and root infecting *Phytophthora* spp. that can cause great yield losses (Yakabe et al., 2009). In the course of intensive nursery surveys to detect and eradicate *P. ramorum*, scientists and growers have gained valuable insight into the range of *Phytophthora* species that are problematic in the nursery industry. During surveys of four commercial nurseries in Oregon between 2006 and 2009 the most frequently isolated *Phytophthora* species were *P. plurivora*, *P. cinnamomi* and *P. syringae* (Grünwald et al., 2011).

It has become evident from such surveys, that at different times of the year different species are dominant, which is to be expected due to their different optimal temperatures for growth (Yakabe et al., 2009). Most species descriptions report optimal temperatures based on culture growth rates rather than *in planta* growth rates of lesion development. Optimal temperatures for growth may vary in nature compared with observations in Petri dish culture.

Since 1990 there has been a dramatic increase in the number of *Phytophthora* spp. isolated, identified and described (Brasier et al., 2005; Donahoo et al., 2006; Durán et al., 2008; Hansen et al., 2003; Jung et al., 2002; Jung et al., 2003; Mostowfizadeh-Ghalamfarsa et al., 2008; Werres et al., 2001). This is likely due to a number of factors, including: increased emphasis on disease diagnostics in agriculture, greater potential for pathogens to spread to new habitats where they can become invasive, and the development of molecular techniques for detecting and identifying potentially new species (Brasier, 2007).

Taxonomy

The fossil record of the order Peronosporomycetes, in which the genus *Phytophthora* is placed, within the Oomycetes, is sparse (Schmidt et al., 2008; Taylor and Krings, 2005). Molecular clock estimates place the first Peronosporomycetes on Earth during the Neoproterozoic period (Bhattacharya et al., 2009). Certainly by the Paleozoic period the environmental conditions on Earth could have supported Oomycetes (Dick, 2001). A handful of potential Oomycete fossils have been studied since the late 19th century with coenocytic hyphae and possible oogonia but their identification as members of the Oomycota remains questionable (Taylor and Krings, 2005). Some more recent studies have identified possible *Pythium* species in the Early Devonian Rhynie

Chert and possible *Albugo*-like spp. in carboniferous period fossils from North America (Taylor and Krings, 2005). There is little evidence of any host reaction that would indicate pathogenicity in the fossils that have been studied. The fossil record suggests most Peronosporomycetes were endophytic or saprotrophic (Krings et al., 2011). The earliest evidence for parasitism in the Oomycota dates back to the late Carboniferous Period; one sample has been observed with a haustorium like structure indicating parasitism (Strullu-Derrien et al., 2010).

Originally *Phytophthora* species were considered Fungi due to their similarities in morphology; however, recent research and advances in molecular techniques have led to significant insights into the relationships among living organisms. Cavalier-Smith (2004) put forward his six kingdom system of classification which divides the eukaryotic Protista into the Protozoa and the Chromista. According to this scheme, Oomycetes are placed in the kingdom Chromista. Phylogenetic analysis of *Phytophthora* spp. has led to the division of this genus into 10 clades based on the evolutionary relationships among species (Blair et al., 2008) (Figure 1).

Figure 1. A genus-wide phylogeny for *Phytophthora* using seven nuclear loci (~8700 nucleotides) as presented by Blair *et al.* (2008). Maximum likelihood branch lengths are shown. Numbers on nodes represent bootstrap support values for maximum likelihood (top) and maximum parsimony (middle), and Bayesian posterior probabilities presented as percentages (bottom). Nodes within clades receiving unambiguous (100%) support in all three analyses are marked with an asterisk (*). Scale bar indicates number of substitutions per site. Outlined (in green and orange) are known pathogens of *Rhododendron*, those in orange are studied in this thesis.

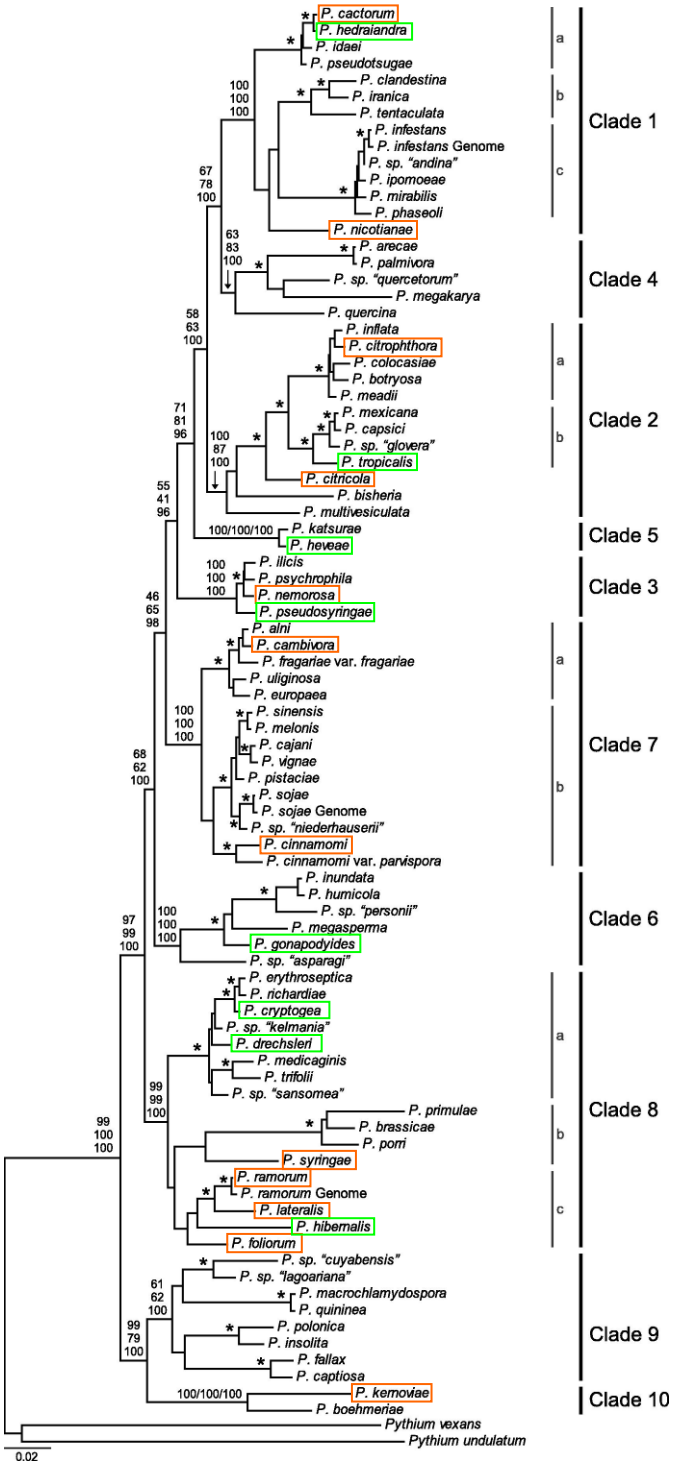
P. cactorum
P. hedraiandra
P. idaei
P. pseudotsugae
P. clandestina
P. iranica
P. tentaculata
P. infestans
P. infestans Genome
P. sp. "andina"
P. ipomoeae
P. mirabilis
P. phaseoli
P. nicotianae
P. arecae
P. palmivora
P. sp. "quercetorum"
P. megakarya
P. quercina
P. inflata
P. citrophthora
P. colocasiae
P. botryosa
P. meadii
P. mexicana
P. capsici
P. sp. "glovera"
P. tropicalis
P. citricola
P. bisheria
P. multivesiculata
P. katsurae
P. heveae
P. ilicis
P. psychrophila
P. nemorosa
P. pseudosyringae
P. alni
P. cambivora
P. fragariae var. fragariae
P. uliginosa
P. europaea
P. sinensis
P. melonis
P. cajani
P. vignae
P. pistaciae
P. sojae
P. sojae Genome
P. sp. "niederhauserii"
P. cinnamomi
P. cinnamomi var. parvispora
P. inundata
P. humicola
P. sp. "personii"
P. megasperma
P. gonapodyides
P. sp. "asparagi"
P. erythroseptica
P. richardiae
P. cryptogea
P. sp. "kelmania"
P. drechsleri
P. medicaginis
P. trifolii
P. sp. "sansomea"
P. primulae
P. brassicae
P. porri
P. syringae
P. ramorum
P. ramorum Genome
P. lateralis
P. hibernalis
P. foliorum
P. sp. "cuyabensis"
P. sp. "lagoariana"
P. macrochlamydospora
P. quininea
P. polonica
P. insolita
P. fallax
P. captiosa
P. kernoviae
P. boehmeriae
Pythium vexans
Pythium undulatum
0.02
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Clade 8
Clade 9
Clade 10
a
b
c

Figure 1.

Biology

The Oomycota (meaning egg fungi) are so named because they produce egg-like sexual spores called oospores and they share many morphological characteristics with Fungi (Dick, 2001). Oomycetes differ from Fungi in that they have coenocytic (rather than septate) hyphae, cellulosic (rather than chitin-containing) cell walls and diploid (rather than haploid) nuclei in the vegetative stage of their life cycle (Erwin and Ribeiro, 1996). Many *Phytophthora* spp. are pathogenic to plants and are favored by abundant water in soil or on foliage. The genus name *Phytophthora* comes from the Greek meaning plant destroyer.

Phytophthora species can reproduce both sexually and asexually, although some species have not been observed in the sexual phase, for example *P. citrophthora*. Sexual reproduction can be homothallic meaning that isolates are self-fertile (e.g., *P. kernoviae*) or heterothallic meaning that for sexual reproduction to occur isolates of differing mating types must interact (e.g., *P. ramorum*). Oospore formation begins with the production of an atheridium, the male organ, which fertilizes the oogonium, the female organ. A double walled sexual spore, the oospore, is formed and this can persist for long periods of time in the soil and remain viable (Fernández-Pavía et al., 2004).

Asexual reproduction occurs through the production of sporangia, which can either germinate directly or release dozens of motile zoospores. These zoospores are water-borne and move with the aid of two flagella, one whiplash and one tinsel flagellum (Dick, 2001). Subsequently a zoospore encysts, losing its flagella, and attaches to its host and germinates (Blanco and Judelson, 2005; Nogueira et al., 1977).

Zoospores are spread in water, through rain-splash, wind-blown rain or run-off into water bodies. There is evidence of wind dispersal of sporangia in the forests of the Pacific Northwest USA (Davidson et al., 2002; Denman et al.,

2006). Some *Phytophthora* species can also produce asexual chlamydospores which are a kind of resting spore, able to persist in unfavorable conditions for longer periods than sporangia or zoospores (Erwin and Ribeiro, 1996). Soil contaminated with sporangia, oospores or chlamydospores is another mode of dispersal for *Phytophthora* spp. in the forest which can be spread on muddy vehicle tires or boots (Hansen et al., 2000).

Similarly, in the nursery environment inoculum can persist if pots and planting media are not sanitized properly (Shishkoff, 2007). Standing water on nursery beds creates the ideal environment for infection of roots or plants with foliage lodged in these water puddles. With the growing ease with which plants and plant products are shipped around the world, and the popularity of exotic and new varieties of plants in the landscape, humans have created favorable circumstances for the emergence of new pathogens.

There are various mechanisms for the emergence of plant pathogens in agriculture. The transition from wild populations of a host plant to intensively cultivated crops alters the environment in which the host and pathogen interact and can facilitate the emergence of pathogens through adaptation, mutation, hybridization, and host switching. The agricultural environment generally consists of increased host density, host genetic uniformity, and more even nutrient and water distribution, compared with the natural environment. This is likely to favor pathogen establishment, survival and transmission (Grünwald and Goss, 2011; Stukenbrock et al., 2007). This artificial environment can enable pathogen adaptation for increased virulence or resistance to fungicides. Migration of plants to new environments with unfamiliar pathogens may result in novel epidemics. Similarly, the migration of plant pathogens to new environments can lead to encounters with new susceptible host species. The movement of plants (and their associated pathogens) around the world increases

the frequency of encounters between pathogen species. Close encounters between pathogen species can result in hybridization events, which could yield novel species or variant strains (Brasier et al., 2004; Grünwald and Goss, 2011; Stukenbrock et al., 2007). Hybridization can lead to novel combinations of genes providing pathogenic traits that could lead to the emergence of new hyper-virulent plant pathogens (Brasier, 2000). Since geographically isolated species may lack natural genetic barriers to interspecific hybridization, the more plants and their pathogens are transported around the globe the greater the chances that two such species could meet (Brasier, 2008). An example of an emerging hybrid pathogen is *P. alni* which has shown itself to be a destructive pathogen of alder trees (*Alnus* sp.) in the UK (Brasier et al., 2004).

Species

P. cactorum, *P. cambivora*, *P. cinnamomi*, *P. citrophthora*, *P. foliorum*, *P. kernoviae*, *P. lateralis*, *P. nemorosa*, *P. parasitica*, *P. plurivora*, *P. ramorum* and *P. syringae* were selected for study in this thesis for their pathogenicity to *Rhododendron*. These species are of particular interest to the nursery and forestry industries in the Pacific Northwest (Table 1). Included are species, which have been isolated from Oregon and California nurseries (Grünwald et al., 2011; Yakabe et al., 2009). These species were selected to include a range of clades within the genus *Phytophthora* (Blair et al., 2008). *P. kernoviae* has not been found in the USA but it is a pathogen of *Rhododendron* and was included for this reason (Brasier et al., 2005). Some of these species are established in forests in Oregon, such as *P. lateralis*, and are less frequently isolated from nurseries (Grünwald et al., 2011). Wherever possible isolates were selected that were originally isolated from *Rhododendron* spp. or similar woody ornamental hosts.

Table 1. Morphological characteristics of species studied in this thesis.

species	clade[1]	sporangiophore	sporangia shape	papillate	caducity	homothallic	heterothallic	amphygynous	paragynous	hyphal swellings	chlamydospores
P. cactorum[2]	I	simple sympodial	ovoid-obpyriform	p	+	+	-	-	+	-	+
P. cambivora[2]	VII	simple sympodial	ovoid-ellipsoid	np	-	-	+	+	-	+	-
P. cinnamomi[2]	VII	simple sympodial	ovoid-ellipsoid	np	-	-	+	+	-	+	+
P. citrophthora[2]	II	loose sympodial	variable	p	-	-	-	-	-	-	+
P. foliorum[3]	VIII	terminal	ovoid	sp	+	+	-	+	+	-	-
P. kernoviae[4]	X	sympodial	limoniform	p	+	+	-	+	-	-	-
P. lateralis[2]	VIII	simple sympodial	ovoid-obpyriform	np	-	+	-	-	+	+	+
P. nemorosa[5]	III	terminal sympodial	ovoid	sp	+	+	-	+	-	+	-
P. parasitica[2]	I	loose sympodial	ovoid-ellipsoid	p	-	-	+	+	-	+	+
P. plurivora[6]	II	simple sympodial	obpyriform	sp	-	+	-	+	+	-	-
P. ramorum[7]	VIII	simple sympodial	elongated ovoid	sp	+	-	+	+	-	-	+
P. syringae[2]	VIII	sympodial	ovoid-obpyriform	sp	-	+	-	+	+	+	-

[1]Blair et al., 2008, [2]Erwin and Ribeiro, 1996, [3]Donahoo et al., 2006, [4]Brasier et al., 2005, [5]Hansen, et al., 2003, [6]Jung and Burgess, 2009, [7]Werres et al., 2001.

P. cactorum (Lebert and Cohn) Schröeter (1886) was first described and isolated from rotting cacti by Lebert and Cohn in 1870. *P. cactorum* causes root, stem and fruit rots, leaf blights and wilts most commonly in temperate regions. Hundreds of plants in dozens of families are hosts to this pathogen including apple, pear, strawberry, dogwood, and Persian walnut (Erwin and Ribeiro, 1996). *P. cactorum* is a pathogen of *Rhododendron catawbiense, R. maximum* and *R.* x sp. (Farr et al., 1996). *P. cactorum* belongs in clade 1a (Blair et al., 2008). This species produces ovoid to obpyriform sporangia, which are papillate, caducous, and borne on a simple sympodial sporangiophore. *P. cactorum* is homothallic (self-fertile) and produces paragynous oospores. It does not produce hyphal swellings but does make chlamydospores (Erwin and Ribeiro, 1996).

P. cambivora (Petri) Buisman (1927) is best known for causing ink disease of chestnut but is also a pathogen of many other woody hosts such as *Prunus* spp. on which is causes root and crown rot (Erwin and Ribeiro, 1996). *P. cambivora* is a pathogen of *Rhododendron ponticum* (Farr et al., 1996). It is placed in clade 7a of the genus *Phytophthora* (Blair et al., 2008). This species produces ovoid to ellipsoid sporangia, which are non-papillate, non-caducous, and are borne on a simple sympodial sporangiophore. *P. cambivora* is heterothallic and produces amphygynous oospores. It does not produce chlamydospores but does make hyphal swellings (Erwin and Ribeiro, 1996).

P. cinnamomi Rands (1922) is a serious root rot pathogen of numerous woody plants, with a host list including over 1,000 species (Erwin and Ribeiro, 1996). This pathogen has become especially problematic in Australian forests causing jarrah dieback on *Eucalyptus marginata* Sm. (Hardham, 2005; Shearer et al., 1981). *P. cinnamomi* is a pathogen of *Rhododendron carolinianum, R.*

catawbiense, R. indicum, R. macrophyllum, R. mucronatum, R. obtusum, R. occidentale, R. ponticum and *R.* x sp. (Farr et al., 1996). It is placed in clade 7b of the genus *Phytophthora* (Blair et al., 2008). This species produces ovoid to ellipsoid sporangia, which are non-papillate, non-caducous, and are borne on a simple sympodial sporangiophore. *P. cambivora* is heterothallic and produces amphygynous oospores. It produces hyphal swellings and chlamydospores (Erwin and Ribeiro, 1996).

P. citrophthora (R.E. Smith and E.H. Smith) Leonian (1925) was originally isolated from rotten lemons. It causes crown rot, brown rot of fruit, root rot and gummosis. It is also an important pathogen of several woody ornamental plants including *Rhododendron* sp. and *Pieris japonica* (Osterbauer et al., 2004; Yakabe et al., 2009). *P. citrophthora* belongs in clade 2a (Blair et al., 2008). This species produces papillate sporangia, which are variable in shape and non-caducous, and are borne on a loose sympodial sporangiophore. This species has not been observed in the sexual phase in nature, but in certain pairings on carrot agar oospores have been produced. *P. citrophthora* does not produce hyphal swellings but it does make chlamydospores (Erwin and Ribeiro, 1996).

P. foliorum Donahoo and Lamour (2006) was first described causing leaf blight of azalea (*Rhododendron* sp.) in California and Tennessee. This pathogen seems not to cause significant mortality of azaleas, only developing leaf spot symptoms which reduces growth rates and crop value (Donahoo et al., 2006). *P. foliorum* is placed in clade 8c (Blair et al., 2008). *P. foliorum* produces semi-papillate, ovoid sporangia which are caducous and are borne on a sympodial sporangiophore. This species is homothallic, producing both amphygynous and paragynous oospores. *P. foliorum* does not produce hyphal swellings or chlamydospores (Donahoo et al., 2006).

P. kernoviae Brasier, Beales and Kirk (2005) was first discovered in the UK and has since been found in New Zealand (Ramsfield et al., 2007). It is an invasive species in the UK causing bleeding stem lesions on beech trees and stem necrosis of *R. ponticum* (Brasier et al., 2005). It is not clear whether this species originated in New Zealand or if it has been imported there (Ramsfield et al., 2007). *P. kernoviae* belongs in clade 10 (Blair et al., 2008). *P. kernoviae* produces papillate, limoniform sporangia which are caducous and are borne on a sympodial sporangiophore. This species is homothallic, producing amphygynous oospores. *P. kernoviae* does not produce hyphal swellings or chlamydospores.

P. lateralis Tucker and Milbrath (1942) was identified as the cause of root rot of Port Orford cedar (*Chamaecyparis lawsoniana)* in 1942 and threatens Port Orford cedar across its natural range. It was reported as a pathogen of *Rhododendron* spp. by Hoitink et al. (1974) but this work was based solely on morphology. It is thus not clear if *P. lateralis* is a pathogen on *Rhododendron*. *P. lateralis* has also been reported on hinoki cypress (*C. obtusa*), kiwi fruit (*Actinidia chinensis*) and Pacific yew (*Taxus brevifolia*) (Erwin and Ribeiro, 1996). *P. lateralis* is placed in clade 8c of the genus *Phytophthora* (Blair et al., 2008). *P. lateralis* produces non-papillate, ovoid to obpyriform sporangia which are non-caducous and are borne on a simple sympodial sporangiophore. This species is homothallic, producing paragynous oospores. *P. lateralis* produces both hyphal swellings and chlamydospores (Erwin and Ribeiro, 1996).

P. nemorosa Hansen and Reeser (2003) has been isolated from necrotic foliage of myrtlewood and California bay laurel, coast redwood and manzanita. This species resembles *P. ilicis* in morphology and physiology and produces disease symptoms similar to those of *P. ramorum* but with lower mortality rates

(Hansen et al., 2003). *P. nemorosa* is a pathogen of *Rhododendron* (Yakabe et al., 2009). *P. nemorosa* falls in clade 3 of the genus *Phytophthora* (Blair et al., 2008). *P. nemorosa* produces semi-papillate, obpyriform to ovoid sporangia which are caducous and are borne terminally on sympodial sporangiophores. This species is homothallic, producing amphygynous oospores. *P. nemorosa* does produce hyphal swellings but not chlamydospores (Hansen et al., 2003).

P. parasitica Dastur (1913) (*P. nicotianae* Breda de Haan, 1896) causes root and crown rot disease of many crops. Some isolates are host specific while others are not. Notable diseases caused by this *Phytophthora* species include black shank of tobacco, brown rot and root rot of citrus and crown rot and wilt of passion fruit (Erwin and Ribeiro, 1996). *P. parasitica* is a pathogen of *Rhododendron* spp. (Farr et al., 1996). It is placed in clade 1 of the genus *Phytophthora* (Blair et al., 2008). This species produces ovoid to ellipsoid sporangia, which are papillate, non-caducous, and are borne on a loose sympodial sporangiophore. *P. parasitica* is both heterothallic and homothallic, producing amphygynous oospores. It produces chlamydospores and hyphal swellings (Erwin and Ribeiro, 1996).

P. plurivora Jung and Burgess (2009) was recently split from the *P. citricola* complex (Jung and Burgess, 2009). *P. plurivora* is highly aggressive and threatens forests and natural ecosystems in Europe and North America. *P. plurivora* differs morphologically from *P. citricola sensu stricto* by its smaller oogonia which have thinner walls and sporangia that have lower length to breadth ratios with more frequent rates of lateral attachment to the sporangiophore. Colony growth patterns of *P. plurivora* are distinct on PDA, and radial colony growth rates are higher for *P. plurivora* on MEA and PDA at 20°C. Across the three genic regions sequenced (ITS, cox1, and β-tubulin) by Jung and Burgess (2009) there are 18 fixed polymorphisms separating *P.*

plurivora from *P. citricola* s. str. *P. plurivora* is a pathogen of *Rhododendron* spp. and has been isolated from soil samples associated with 32 other genera (Jung and Burgess, 2009). It falls in clade 2 of the genus *Phytophthora* (Blair et al., 2008). This species produces obpyriform sporangia, which are semi-papillate, non-caducous, and are borne on a simple sympodial sporangiophore. *P. plurivora* is self-fertile, producing both amphygynous and paragynous oospores. It does not have chlamydospores or hyphal swellings (Jung and Burgess, 2009).

P. ramorum Werres (2001) causes sudden oak death of tanoak (*Lithocarpus densiflorus*) and coast live oak (genus and species) as well as ramorum blight on numerous woody plants including *Rhododendron* spp., red oaks, *Viburnum* spp., bay laurel, Douglas-fir, and coast redwood (Davidson et al., 2003; Werres et al., 2001). *P. ramorum* belongs in clade 8c of the genus *Phytophthora* (Blair et al., 2008). This species produces ovoid to elongated ovoid sporangia that are semi-papillate, caducous, and are borne on a sympodial sporangiophore. *P. ramorum* is heterothallic, producing amphygynous oospores. It lacks hyphal swellings but can produce chlamydospores (Werres et al., 2001).

P. syringae Klebahn (1905) is an important pathogen of apple trees in temperate climates causing collar rot on both young and mature trees. It severely stunts growth, and reduces crop yields before killing the host. The symptoms are very similar to those of *P. cactorum* (Harris, 1979). *P. syringae* is a pathogen of *Rhododendron* spp. (azalea) (Yakabe et al., 2009). It is placed in clade 8b of the genus *Phytophthora* (Blair et al., 2008). This species produces ovoid to obpyriform sporangia, which are semi-papillate, non-caducous, and are borne on a sympodial sporangiophore. *P. syringae* is self-fertile, producing both amphygynous and paragynous oospores. It doesn't

produce chlamydospores and but can have hyphal swellings (Erwin and Ribeiro, 1996).

Goals

The aim of the research presented in this thesis was to compare the epidemiology of *Phytophthora* disease caused by twelve *Phytophthora* species known to infect *Rhododendron*. We investigated aggressiveness on *Rhododendron* and the response of *Phytophthora* species to temperature. The relative aggressiveness of *Phytophthora* species was assessed as severity or incidence. Incidence is defined here as the number of successful infections given a certain inoculum concentration. Severity is defined here as a measure of the intensity of disease measured either as the proportion (or percent) of the area of a plant or plant part affected by disease, or as an actual measure of the area diseased. Sporulation was assessed as the number of sporangia produced per unit lesion area. Finally, pathogen response to temperature was quantified as the radial growth rates of colonies on agar plates or of lesions on leaves determined at different temperatures.

References

1. Adl S.M., Simpson A.G.B., Farmer M.A., Andersen R.A., Anderson O.R., Barta J.R., Bowser S.S., Brugerolle G.U.Y., Fensome R.A., Fredericq S., James T.Y., Karpov S., Kugrens P., Krug J., Lane C.E., Lewis L.A., Lodge J., Lynn D.H., Mann D.G., McCourt R.M., Mendoza L., Moestrup Ø., Mozley-Standridge S.E., Nerad T.A., Shearer C.A., Smirnov A.V., Spiegel F.W., Taylor M.F.J.R. (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of Protists. Journal of Eukaryotic Microbiology 52:399-451.

2. Bhattacharya D., Yoon H.S., Hedges S.B., Hackett J.D. (2009) Eukaryotes (Eukaryota). The Timetree of Life, Hedges, S. B. and Kumar, S. (Eds.) Oxford University Press.

3. Birch P.R.J., Whisson S.C. (2001) *Phytophthora infestans* enters the genomics era. Molecular Plant Pathology 2:257-263. DOI: 10.1046/j.1464-6722.2001.00073.x.

4. Blair J.E., Coffey M.D., Park S.-Y., Geiser D.M., Kang S. (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genetics and Biology 45:266-277. DOI: 10.1016/j.fgb.2007.10.010.

5. Blanco F.A., Judelson H.S. (2005) A bZIP transcription factor from *Phytophthora* interacts with a protein kinase and is required for zoospore motility and plant infection. Molecular Microbiology 56:638-648. DOI: 10.1111/j.1365-2958.2005.04575.x.

6. Brasier C.M. (2000) Plant pathology: The rise of the hybrid fungi. Nature 405:134-135.

7. Brasier C.M. (2008) The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology 57:792-808. DOI: 10.1111/j.1365-3059.2008.01886.x.

8. Brasier C.M. (2007) *Phytophthora* Biodiversity: How Many *Phytophthora* Species Are There? Phytophthoras in forests and natural ecosystems. Proceedings of the 4th Meeting of IUFRO Working Party 07.02.09, August 26–31, 2007, Monterey, CA. General Technical Report PSW-GTR-221, Albany, CA:101–115.

9. Brasier C.M., Beales P.A., Kirk S.A., Denman S., Rose J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research 109:853-859.

10. Brasier C.M., Kirk S.A., Delcan J., Cooke D.E.L., Jung T., Man In't Veld W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research 108:1172-1184. DOI: 10.1017/s095375620400 1005.

11. Buisman C.J. (1927) Root rots caused by Phycomycetes. Meded. Phytopathology Laboratory, University of Utrecht, Reviews in Applied Mycology 6:380.

12. Cavalier-Smith T. (2004) Only six kingdoms of life. Proceedings of the Royal Society B 271:1251-1262.

13. Dart N.L., Chastagner G.A. (2007) Estimated economic losses associated with the destruction of plants due to *Phytophthora ramorum* quarantine efforts in Washington State. Plant Health Progress, Online. DOI: 10.1094/PHP-2007-0508-02-RS.

14. Dastur J. (1913) *Phytophthora parasitica* n. sp., a new disease of the castor oil plant. Memoirs of the Department of Agriculture, India 22:177-231.

15. Davidson J.M., Rizzo D.M., Garbelotto M., Tjosvold S., Slaughter G.W. (2002) *Phytophthora ramorum* and sudden oak death in California: II. Transmission and survival, General Technical Report, USDA Forest Service. pp. 741-749.

16. Davidson J.M., Werres S., Garbelotto M., Hansen E.M., Rizzo D.M. (2003) Sudden oak death and associated diseases caused by *Phytophthora ramorum*, Plant Health Progress, Online. DOI: 10.1094/PHP-2003-0707-01-DG.

17. de Bary A. (1876) Researches into the nature of the potato fungus, *Phytophthora infestans*. Journal of the Royal Agricultural Society of England. Series 2 12:239-269.

18. Denman S., Kirk S., Whybrow A., Orton E., Webber J.F. (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin 36:373-376. DOI: 10.1111/j.1365-2338.2006.01014.x.

19. Dick M.W. (2001) Straminipilous fungi: systematics of the Peronosporomycetes, including accounts of the marine straminipilous protists, the plasmodiophorids, and similar organisms. Kluwer Academic Publishers.

20. Donahoo R., Blomquist C.L., Thomas S.L., Moulton J.K., Cooke D.E.L., Lamour K.H. (2006) *Phytophthora foliorum* sp. nov., a new species causing leaf blight of azalea. Mycological Research 110:1309-1322.

21. Durán A., Gryzenhout M., Slippers B., Ahumada R., Rotella A., Flores F., Wingfield B.D., Wingfield M.J. (2008) *Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile. Plant Pathology 57:715-727. DOI: 10.1111/j.1365-3059.2008.01893.x.

22. Erwin D.C., Ribeiro O.K. (1996) *Phytophthora* Diseases Worldwide, APS Press, St. Paul, Minnesota.

23. Farr D.F., Esteban H.B., Palm M.E. (1996) Fungi on *Rhododendron*: a world reference. Parkway Publishers, Inc., Boone, NC.

24. Fernández-Pavía S.P., Grünwald N.J., Díaz-Valasis M., Cadena Hinojosa M., Fry W.E. (2004) Soil-borne oospores of *Phytophthora infestans* in central Mexico survive winter fallow and infect potato plants in the field. Plant Disease 88:29-33.

25. Frankel S.J. (2008) Sudden oak death and *Phytophthora ramorum* in the USA: a management challenge. Australasian Plant Pathology 37:19-25. DOI: 10.1071/AP07088.

26. Goss E.M., Larsen M., Chastagner G.A., Givens D.R., Grünwald N.J. (2009) Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathogens 5:e1000583.

27. Grünwald N.J., Goss E.M. (2011 in press) Evolution and population genetics of exotic and re-emerging pathogens: Novel tools and approaches. Annual Review of Phytopathology 49.

28. Grünwald N.J., Kitner M., McDonald V., Goss E.M. (2008) Susceptibility in *Viburnum* to *Phytophthora ramorum*. Plant Disease 92:210-214.

29. Grünwald N.J., Martin F.N., Larsen M.M., Sullivan C.M., Press C.M., Coffey M.D., Hansen E.M., Parke J.L. (2011) Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342. DOI: 10.1094/PDIS-08-10-0609.

30. Hansen E.M., Goheen D.J., Jules E.S., Ullian B. (2000) Managing Port Orford cedar and the introduced pathogen *Phytophthora lateralis*. Plant Disease 84:4-10.

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

32. Hardham A.R. (2005) *Phytophthora cinnamomi*. Molecular Plant Pathology 6:589-604.

33. Harris D.C. (1979) The occurrence of *Phytophthora syringae* in fallen apple leaves. Annals of Applied Biology 91:309-312. DOI: 10.1111/j.1744-7348.1979.tb06505.x.

34. Hoitink H.A.J., Schmitthenner A.F. (1974) Relative prevalence and virulence of *Phytophthora* species involved in *Rhododendron* root rot. Phytopathology 64:1371-1374.

35. Jung T., Burgess T.I. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. Persoonia - Molecular Phylogeny and Evolution of Fungi 22:95-110. DOI: 10.3767/003158509x442612.

36. Jung T., Hansen E.M., Winton L., Oswald W., Delatour C. (2002) Three new species of *Phytophthora* from European oak forests. Mycological Research 106:397-411. DOI: 10.1017/s0953756202005622.

37. Jung T., Nechwatal J., Cooke D.E.L., Hartmann G., Blaschke M., Oßwald W.F., Duncan J.M., Delatour C. (2003) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. Mycological Research 107:772-789. DOI: 10.1017/ s0953756203008074.

38. Klebahn H. (1905) A new fungal disease of *Syringae.* (In German). Zentralblatt fur Bakteriologie. 15:335-336.

39. Krings M., Taylor T.N., Dotzler N. (2011) The fossil record of the Peronosporomycetes (Oomycota). Mycologia 103:445-457. DOI: 10.3852/10-278.

40. Lebert H., Cohn F. (1870) On the rot of cactus stems (in German). Beitraege zur Biologie Pflanzen. 1:51-57.

41. Leonian L. (1925) Physiological studies on the genus *Phytophthora.* American Journal of Botany 12:444-498.

42. Mascheretti S., Croucher P.J.P., Vettraino A., Prospero S., Garbelotto M. (2008) Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology 17:2755-68.

43. Mostowfizadeh-Ghalamfarsa R., Cooke D.E.L., Banihashemi Z. (2008) *Phytophthora parsiana* sp. nov., a new high-temperature tolerant species. Mycological Research 112:783-794. DOI: 10.1016/j.mycres.2008.01.011.

44. Nogueira M.L.B., Silva P.P.d., Bartnicki-Garcia S. (1977) Membrane fusion during secretion: A hypothesis based on electron microscope observation of *Phytophthora palmivora* zoospores during encystment. The Journal of Cell Biology 73:161-181.

45. Orlikowski L.B. (2010) Occurrence and harmfulness of *Phytophthora* spp. in Polish hardy ornamental nursery stock. *Acta Horticulturae* (ISHS):243-248.

46. Osterbauer N.K., Griesbach J.A., Hedberg J. (2004) Surveying for and eradicating *Phytophthora ramorum* in agricultural commodities. Plant Health Progress Online. DOI: 10.1094/PHP-2004-0309-02-RS.

47. Parke J.L., Linderman R.G., Osterbauer N.K., Griesbach J.A. (2004) Detection of *Phytophthora ramorum* blight in Oregon nurseries and completion of Koch's postulates on *Pieris*, *Rhododendron*, *Viburnum*, and *Camellia*. Plant Disease 88:87-87.

48. Prospero S., Hansen E.M., Grünwald N.J., Winton L.M. (2007) Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. Molecular Ecology 16:2958-2973.

49. Ramsfield T., Dick M., Beever R., Horner I., McAlonan M., Hill C. (2007) *Phytophthora kernoviae* in New Zealand, Fourth Meeting of IUFRO Working Party S07.02.09 Phytophthoras in Forests and Natural Ecosystems, Monterey, California. pp. P47-53.

50. Rands R. (1922) Stripe canker of cinnamon caused by *Phytophthora cinnamomi* n. sp. (In Dutch). Mededeelingen van het Instituut voor Plantenziekten 54:41pp.

51. Rizzo D.M., Garbelotto M., Hansen E.M. (2005) *Phytophthora ramorum*: Integrative research and management of an emerging pathogen in California and Oregon forests. Annual Review of Phytopathology 43:309-335. DOI: 10.1146/annurev.phyto.42.040803.140418.

52. Schmidt A.R., Dorfelt H., Perrichot V. (2008) *Palaeoanellus dimorphus* gen. et sp. nov. (Deuteromycotina): a Cretaceous predatory fungus. American Journal of Botany 95:1328-1334.

53. Schwingle B.W., Smith J.A., Blanchette R.A. (2007) *Phytophthora s*pecies associated with diseased woody ornamentals in Minnesota nurseries. Plant Disease 91:97-102.

54. Shearer B., Shea S., Fairman R. (1981) Infection of the stem and large roots of *Eucalyptus marginata* by *Phytophthora cinnamomi*. Australasian Plant Pathology 10:2-3. DOI: 10.1071/app9810002.

55. Shishkoff N. (2007) Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Disease 91:1245-1249. DOI: 10.1094/PDIS-91-10-1245.

56. Simpson A.G.B., Roger A.J. (2004) The real 'kingdoms' of eukaryotes. Current Biology 14:R693-R696.

57. Strullu-Derrien C., Kenrick P., Rioult J.P., Strullu D.G. (2010) Evidence of parasitic Oomycetes (Peronosporomycetes) infecting the stem cortex of the Carboniferous seed fern *Lyginopteris oldhamia*. Proceedings of the Royal Society B 278:675-680.

58. Stukenbrock E.H., Banke S., Javan-Nikkhah M., Mcdonald B.A. (2007) Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Biology and Evolution 24:398-411.

59. Taylor T.N., Krings M. (2005) Fossil microorganisms and land plants: Associations and interactions. Symbiosis 40:119-135.

60. Tucker C.M., Milbrath J.A. (1942) Root rot of *Chamaecyparis* caused by a new species of *Phytophthora*. Mycologia 34:94-103.

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

62. Weste G., Marks G.C. (1987) The biology of *Phytophthora cinnamomi* in Australasian forests. Annual Review of Phytopathology 25:207-229.

63. Yakabe L.E., Blomquist C.L., Thomas S.L., MacDonald J.D. (2009) Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Plant Disease 93:883-890. DOI: 10.1094/PDIS-93-9-0883.

CHAPTER 2: MANUSCRIPT

Comparative Epidemiology of *Phytophthora* Diseases of *Rhododendron*

Clare R Taylor and Niklaus J Grünwald

Introduction

Phytophthora species are well known as important or emerging pathogens. Notable examples include the jarrah dieback pathogen *P. cinnamomi* (Hardham, 2005), the sudden oak death pathogen *P. ramorum* (Grünwald et al., 2008), and the potato late blight pathogen *P. infestans* (Fry, 2008). *P. cinnamomi* threatens the jarrah tree as well as over half of the native bush land plants in Western Australia. *P. ramorum* is an emerging exotic pathogen of tan oak and many other woody ornamental hosts in the forests of the Western US. *P. infestans* is the pathogen of potato which led to the Great Famine in Ireland, and this pathogen continues to have an impact on potato production to this day. Some plants are host to multiple *Phytophthora* spp., thus increasing the probability of inadvertently becoming vectors for the movement of novel species.

Movement of pathogens could occur on asymptomatic plants or with pathogen propagules in potting media. This is thought to have been the case with the import of ornamental plants such as *Rhododendron, Pieris, Virburnum* and *Camellia* into the USA, and hypothesized to be involved in the introduction of *P. ramorum* into California (Mascheretti et al., 2008) and the Pacific Northwest (Goss et al., 2009; Goss et al., 2011). Transport of ornamental plants is likely a major vector for the movement of *Phytophthora* species. *Rhododendron* plants are unusual among most agricultural crops because they are host to many *Phytophthora* spp. (Benson and Hoitink, 1986; Linderman,

1986). *Phytophthora* spp. infecting *Rhododendron* are typically characterized as causing root rot, shoot dieback or foliar blight. Typical root rotting *Phytophthora* spp. include *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. gonapodyides*, *P. heveae*, *P. lateralis*, *P. megasperma*, *P. parasitica*, and *P. plurivora* (formerly *P. citricola*) (Daughtrey and Benson, 2001). Species typically causing dieback or foliar blight symptoms include *P. cactorum*, *P. heveae*, *P. kernoviae*, *P. parasitica*, *P. plurivora*, *P. ramorum* and *P. syringae* (Brasier et al., 2005; Daughtrey and Benson, 2001; Werres et al., 2001). The ability to infect roots and or foliage is not mutually exclusive. Some species are able to infect both roots and foliage while others are more or less specialized to either foliage or roots. This effort measures foliar disease development but includes both root and foliar *Phytophthora* pathogens of *Rhododendron*.

The genus *Rhododendron* is of considerable importance to plant regulatory agencies because it is host to many *Phytophthora* species, most notably, *P. ramorum* and *P. kernoviae* (Brasier et al., 2005; Grünwald et al., 2008; Werres et al., 2001). We currently know very little about the epidemiology of different *Phytophthora* diseases on *Rhododendron*, which is an important crop in the nursery industry. *Rhododendron* is known to host at least a dozen *Phytophthora* spp. (Erwin and Ribeiro, 1996; Farr et al., 1996; Hoitink and Schmitthenner, 1974), yet no study exists that has compared these *Phytophthora* spp. on *Rhododendron* in a systematic effort. Understanding the epidemiology of each *Phytophthora* species will contribute to the design of more targeted disease management strategies as well as inform regulatory agencies on the risks associated with both *Rhododendron* plants and particular *Phytophthora* spp. associated with *Rhododendron*.

Few studies have directly contrasted the epidemiology of different *Phytophtora* spp. on a given host (Brasier and Kirk, 2001; Jeger and Pautasso, 2008; Linderman et al., 2006; Wilcox and Mircetich, 1985). Pathogens can be compared based on measures of fitness or aggressiveness. Here aggressiveness was determined by assessing severity and intensity of disease caused by *Phytophthora* spp. on *Rhododendron*. Incidence is defined here as the number of successful infections given a certain inoculum concentration. Severity is defined here as a measure of the intensity of disease measured either as the proportion (or percent) of the area of a plant or plant part affected by disease, or as an actual measure of the area diseased, or area under the lesion expansion curve (AULEC). Sporulation was assessed as the number of sporangia produced per unit lesion area.

We investigated the epidemiology of twelve important *Phytophthora* diseases on *Rhododendron*. We studied aggressiveness on *Rhododendron* and the response of *Phytophthora* species to temperature. We tested two specific hypotheses: First, there are significant differences in aggressiveness among different *Phytophthora* spp. infecting *Rhododendron*. Second, the temperature range for disease development varies among *Phytophthora* spp. Pathogen response to temperature was quantified as the radial growth rates of colonies on agar plates or of lesions on leaves determined at different temperatures. Where possible, isolates were evaluated in detached leaf, whole plant and field studies. Understanding the temperature requirements and aggressiveness of these *Phytophthora* species to *Rhododendron* will help improve disease management enabling growers to adapt agricultural practices to target different *Phytophthora* species at different temperatures.

Materials and Methods

Species and isolates. The *Phytophthora* species used in this study were *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citrophthora*, *P. foliorum*, *P. kernoviae*, *P. lateralis*, *P. nemorosa*, *P. parasitica*, *P. plurivora*, *P. ramorum* and *P. syringae* (Table 2). Isolates were maintained at 20°C in the dark on 10% V8 agar (100 ml V8 juice; 2 g $CaCO_3$; 30 mg/L β-sitosterol (EMD Chemicals, Inc., San Diego, CA); 15 g agar; 900 ml deionized water) in Petri plates and transferred monthly. Isolates were identified to species by sequencing of the internal transcribed spacer (ITS) region as described by Grünwald et al. (2011). PCR amplicons of the ITS region were sequenced in forward and reverse at the Center for Genome Research and Biocomputing (CGRB, Oregon State University, Corvallis) on an ABI 3730 capillary sequencer using standard reagents (Applied Biosystems Inc.). Species were identified by BLAST search against www.Phytophthora-ID.org (Grünwald et al., 2011).

Table 2. *Phytophthora* species and isolates used in this study.

Species	Isolate ID	Host genus or environment of origin	Origin	Isolated by
P. cactorum	PCC-07-001*	*Fragaria*	OR	P. Reeser[1]
P. cactorum	PCC-07-003	*Panax*	OR	P. Reeser[1]
P. cactorum	PCC-07-004	*Fragaria*	OR	P. Reeser[1]
P. cambivora	PCM-07-001*	*Fagus*	OR	P. Reeser[1]
P. cambivora	PCM-07-002	*Photinia*	OR	P. Reeser[1]
P. cambivora	PCM-07-003	*Ulmus*	OR	P. Reeser[1]
P. cinnamomi	PCN-07-002*	*Vaccinium*	OR	P. Reeser[1]
P. cinnamomi	JP-06-0006	*Pinus*	OR	J. Parke[2]/N. Grünwald[3]
P. cinnamomi	JP-06-0217	soil/substrate	OR	J. Parke[2]/N. Grünwald[3]
P. citrophthora	PCR-07-001	*Picea*	OR	P. Reeser[1]
P. citrophthora	PCR-07-003	*Rhododendron*	OR	P. Reeser[1]
P. citrophthora	PCR-07-005*	*Pieris*	OR	P. Reeser[1]
P. foliorum†	JP-08-070	*Pieris*	OR	J. Parke[2]/N. Grünwald[3]
P. foliorum†	P-04-001*	azalea	TN	K. Lamour[4]
P. kernoviae†	PK-05-001	unknown	UK	C. Brasier[5]
P. kernoviae†	PH-05-005	*Annona*	NZ	M. Coffey[6]
P. kernoviae†	PK-05-002*	unknown	UK	C. Brasier[5]
P. lateralis†	PL-0X-001	unknown	CA	E. Hansen[1]
P. lateralis†	PL-05-004	*Chamaecyparis*	OR	M. Garbelotto[7]
P. lateralis†	PL-06-009	*Chamaecyparis*	WA	E. Hansen[1]
P. lateralis†	PL-06-003*	*Chamaecyparis*	CAN	E. Hansen[1]
P. nemorosa†	PN-07-002	*Lithocarpus*	OR	E. Hansen[1]
P. nemorosa†	P-07-034	*Camellia*	OR	J. Parke[2]/N. Grünwald[3]
P. nemorosa†	P-06-010*	*Pieris*	OR	N. Osterbauer
P. parasitica	PNI-07-001	*Forsythia*	OR	P. Reeser[1]
P. parasitica	PNI-07-002*	*Malva*	OR	P. Reeser[1]
P. parasitica	PNI-07-005	*Daphne*	OR	P. Reeser[1]
P. plurivora	PCT-07-002*	nursery	OR	P. Reeser[1]
P. plurivora	JP-07-0251	*Kalmia*	OR	J. Parke[2]/N. Grünwald[3]
P. plurivora	JP-06-0160	soil/substrate	OR	J. Parke[2]/N. Grünwald[3]
P. ramorum†	PR-05-186	*Rhododendron*	OR	N. Osterbauer[8]
P. ramorum†	PR-07-113	*Rhododendron*	OR	N. Osterbauer[8]

Table 2 (Continued)

Species	Isolate ID	Host genus or environment of origin	Origin	Isolated by
P. ramorum†	PR-07-166*	*Rhododendron*	OR	J. Ristaino[9]
P. syringae	PSY-07-001	*Malus*	OR	P. Reeser[1]
P. syringae	PSY-07-002*	*Zelkova*	OR	P. Reeser[1]
P. syringae	PSY-07-003	*Pyrus*	OR	P. Reeser[1]

* denotes isolates used in the temperature inoculation experiment.
† denotes species evaluated in containment in compliance with active USDA APHIS PPQ permits.
[1] Department of Botany and Plant Pathology, Oregon State University
[2] Department of Crop and Soil Science, Oregon State University
[3] USDA ARS HCRL, Corvallis, OR
[4] Department of Entomology and Plant Pathology, The University of Tennessee
[5] Forestry Commission, Forest Research Station, Farnham, Surrey, UK
[6] The College of Natural and Agricultural Sciences, UC Riverside
[7] Department of ESPM, UC Berkley
[8] Oregon Department of Agriculture, Salem, OR
[9] Department of Plant Pathology, NC State University
Abbreviations: CA = California, USA; CAN = Canada; NZ = New Zealand; OR = Oregon, USA; TN = Tennessee, USA; UK = United Kingdom; WA = Washington, USA,

Plant maintenance. The highly susceptible *Rhododendron catawbiense* 'Boursault' (CB) and the moderately susceptible *Rhododendron catawbiense* × unknown 'Lee's dark purple' (LDP) (McDonald and Grünwald, unpublished) were maintained in #1 trade gallon containers of 'Professional Growing Mix' (Sun Gro Horticulture Canada Ltd, Vancouver, British Columbia) in a greenhouse with natural lighting and watered as needed. The plants were fertilized every 2 months with slow release pellets of 'Apex 20-10-10/20-4.3-8.3 Evergreen Nursery Fertilizer' (J. R. Simplot Company, Professional Products, Boise, ID) and twice a year with a foliar application of 'Liquid Iron' (Fertilome, Voluntary Purchasing Groups Inc., Bonham, TX).

Inoculum production. Petri plates with 10 ml of 10% V8 juice broth were inoculated with three 0.5 cm diameter mycelial plugs of a *Phytophthora* isolate. Broth plates were incubated for 4 days in the dark at 20°C. The broth was then decanted without dislodging the mycelial mats and replaced with 10 ml of either sterile distilled water, filter sterilized pond water or non-sterile filtered soil extract to create starvation conditions and stimulate sporangia production (Ahonsi et al., 2007). The plates were then incubated in the dark at 20°C for three days. Zoospore release was induced by incubating plates at 4°C for one hour and then placing them at room temperature on the lab bench for one hour. The concentration of zoospores was estimated using a hemacytometer and adjusted to the desired concentration by adding deionized H_2O.

Aggressiveness and sporulation: detached leaf experiments. Disease severity and sporulation were assessed in detached leaf assays using all isolates including quarantine and non-quarantine isolates (Table 2). Experiments

followed a factorial 12 × 2 (species × cultivar) randomized complete block design with six replications and ten subsamples (individual leaves) per replicate (Appendix, Figure 1). There were only two isolates of *P. foliorum* available, and there were four isolates of *P. lateralis* used. Three isolates were used in mixture for all other species (Table 2). An experimental unit consisted of ten leaves of a cultivar inoculated with a species (mixed isolates) and placed together in a plastic box. Leaves were detached from *Rhododendron* plants and placed in plastic boxes (23 × 33 × 11.5 cm) on a plastic mesh over moist vermiculite. Leaves were wound inoculated by abrading a point in the center of each leaf to one side of the mid-vein with a sterile hypodermic needle and then placing 20 µl of zoospore solution (5×10^3 zoospores ml^{-1}) onto the wound. A fine mist of sterile water was applied immediately after inoculation and every 48 hours thereafter and leaves were incubated at 18°C with a 16 h day length. After 10 days, leaves were photographed and digitally processed for lesion area using Assess (version 2.0, APS Press, St. Paul, MN). The leaves from each experimental unit having developed lesions were then placed together in a 50 ml tube with 20 ml $CuSO_4$, vortexed to dislodge the sporangia, and centrifuged at 290 × g (1,200 rpm) for 5 minutes. Pellets were re-suspended in 100 µl of $CuSO_4$ and the number of sporangia was estimated using a hemacytometer. This experiment was conducted twice.

Aggressiveness and sporulation: whole plant experiments. Disease severity, incidence and sporulation of eight *Phytophthora* species was assessed on whole plants without wounding. Only eight species yielded enough zoospore inoculum to inoculate the whole plants. This experiment was conducted twice with six replicates each. An experimental unit consisted of a single plant. Small (10 cm container/570 ml) *Rhododendron* cv. *catawbiense*

'Boursault' plants were dip-inoculated in zoospore suspension at a concentration of 3×10^3 zoospores ml^{-1} for 10 minutes. The plants were then wrapped in plastic bags to maintain humidity and leaf wetness and incubated at 18°C with a 16 h day length for 7 days. The leaves were then removed and lesion area was estimated as described above (Appendix, Figure 1). Three infected leaves were selected from each plant to assess sporulation of the lesions as described above.

Effect of temperature: detached leaf and agar culture experiments. The effect of temperature on colony and lesion growth rates was investigated. Isolates were inoculated on detached leaves and agar plates and placed in growth chambers randomly assigned to temperatures at 5°C increments from 5°C to 35°C with a 16 hour day length. Experiments followed a factorial 7 × 7 (temperature × species) randomized complete block design with five replicates per experiment. An experimental unit consisted of a single detached leaf or Petri plate (Appendix, Figure 1). Detached *Rhododendron* cv. *catawbiense* 'Boursault' leaves were placed in moist tubs and wound inoculated with a single mycelial plug. Petri plates of 10% V8 agar were inoculated using a mycelial plug taken from the Petri plates the species were maintained on. Two perpendicular measurements (length and width) of lesion or colony diameter were taken using calipers every other day for 12 days. Lesion area was estimated using the average of these two measurements to represent the diameter of a circle and area was then calculated as colony area = πr^2 where r = ½ diameter. Experiments were conducted three times and temperature treatments were randomized among chambers between experiments. *P. parasitica* and *P. syringae* were included as positive controls in both the quarantine and non-quarantine species experiments. Actual temperature was

measured using HOBO RH Temp Light External Sensor Logger H09-004-02 (Onset Computer Corporation, Pocasset, MA).

Aggressiveness and effect of temperature: field experiments. To assess the effect of environmental temperature variation on *Phytophthora* epidemics whole plants of both *Rhododendron* cultivars in #1 trade gallon containers were wound inoculated in a nursery like setting. A factorial 7 × 2 (species × cultivar) randomized complete block design was used with six replicates (Appendix, Figure 2). The experiment was conducted four times, roughly every three to four months of the year. The plants were inoculated on three leaves (sub-samples) by wounding each leaf (as above), and clipping an Eppendorf cap containing three agar plugs (one plug for each isolate of the same *Phytophthora* species) and a drop of water to the leaf (Table 2). Sterile agar plugs were used on control plants. The plants were inoculated in a greenhouse and kept there for one week for infection to take place, after which inoculum caps were removed and the plants were moved to the field for lesion development for a period of six weeks. Perpendicular measurements (length and width) of lesions were taken using calipers each week for six weeks and this was used to estimate lesion area (as described above). Area under the lesion expansion curve (AULEC) in mm^2 × days was calculated from these measurements using the trapezoid method. Lesions were destructively sampled six weeks after inoculation and the *Phytophthora* species were re-isolated from the lesion margin onto V8 PARP agar to confirm identity of the re-isolated strain by morphology on V8 100 agar. Weather data was collected using a Campbell Scientific CR1000 data logger with sensors recording the temperature (CR500) for the duration of the experiments. The mean temperature over the six weeks of each experiment was calculated using hourly temperature recordings from

the data logger. Supplemental overhead irrigation was applied for 15 minutes, six times a day during experiments 1 and 2, which occurred during the dry season to maintain conditions conducive to epidemic development.

Statistical analysis. Lesion area (mm^2), % lesion area, the area under the lesion expansion curve (AULEC, mm^2 × days), and sporulation (sporangia/mm^2 lesion area) were subjected to analysis of variance. Sporulation, % lesion area and AULEC (field experiments) were square-root transformed for homogeneity of variance and to normalize the data. A log transformation was used for % lesion area from the field experiments and for AULEC from growth chamber temperature experiments for homogeneity of variance and to normalize the data. Analyses of variance using the general mixed linear model and mean separation using Tukey's test were conducted using Minitab (State College, PA) (Tukey, 1991). Fixed effects were *Rhododendron* cultivar, *Phytophthora* species and where applicable, temperature; replicate was considered a random effect.

Results

Aggressiveness and sporulation: detached leaf experiments. There was a significant species × cultivar interaction for lesion area (mm^2) and % lesion area ($P < 0.001$); however, there was no significant species × cultivar interaction for sporulation (Table 3). The three species with significantly higher % lesion area were *P. parasitica*, *P. ramorum, and P. foliorum* in both experiments ($P < 0.01$), although they were not ranked in the same order on both cultivars (Figure 2, A, C, E and G). Sporulation per mm^2 lesion area in both experiments was higher for *P. kernoviae* than any of the other species (Figure 2, B, D, F and H).

Aggressiveness and sporulation: whole plant experiments. Of the twelve *Phytophthora* species studied, only eight yielded sufficient concentrations of zoospores to carry out whole plant dip inoculation experiments. *P. foliorum*, *P. nemorosa*, *P. cambivora* and *P. cinnamomi* either failed to produce sporangia in culture or did not release zoospores in sufficient concentrations to be used in these experiments. In both experiments there was a significant difference in the percentage of leaves infected per plant among the eight species ($P < 0.001$) with *P. ramorum, P. kernoviae* and *P. citrophthora* exhibiting the highest infection rate (Figure 3, A, B). There were significant differences in lesion area (%) among the *Phytophthora* species tested ($P \leq 0.002$) (Figure 3, C, D). *P. ramorum*, *P. kernoviae*, *P. citrophthora*, *P. parasitica* and *P. cactorum* showed comparable levels of sporulation (Figure 3, E, F). *P. plurivora*, *P. lateralis*, and *P. syringae* did not sporulate under these conditions (Figure 3, E, F).

Figure 2. Detached leaf inoculation of twelve *Phytophthora* species. **A**, and **E**, show lesion area (%) on *Rhododendron* cv. *catawbiense* 'Boursault' in experiments 1 and 2, respectively. **B** and **F** show sporulation (sporangia/mm^2) on *Rhododendron* cv. *catawbiense* 'Boursault' in experiments 1 and 2, respectively. **C**, and **G**, show lesion area (%) on *Rhododendron* cv. 'Lee's dark purple' in experiments 1 and 2, respectively. **D** and **H** show sporulation (sporangia/mm^2) on *Rhododendron* cv. 'Lee's dark purple' in experiments 1 and 2, respectively. Lower case letters in each panel represent statistically significant differences among *Phytophthora* spp. within each cultivar based on Tukey's test at the 95% confidence level carried out on square root transformed data. The data presented are not transformed. Bars show standard deviation from the mean of replicates (based on non-transformed data).

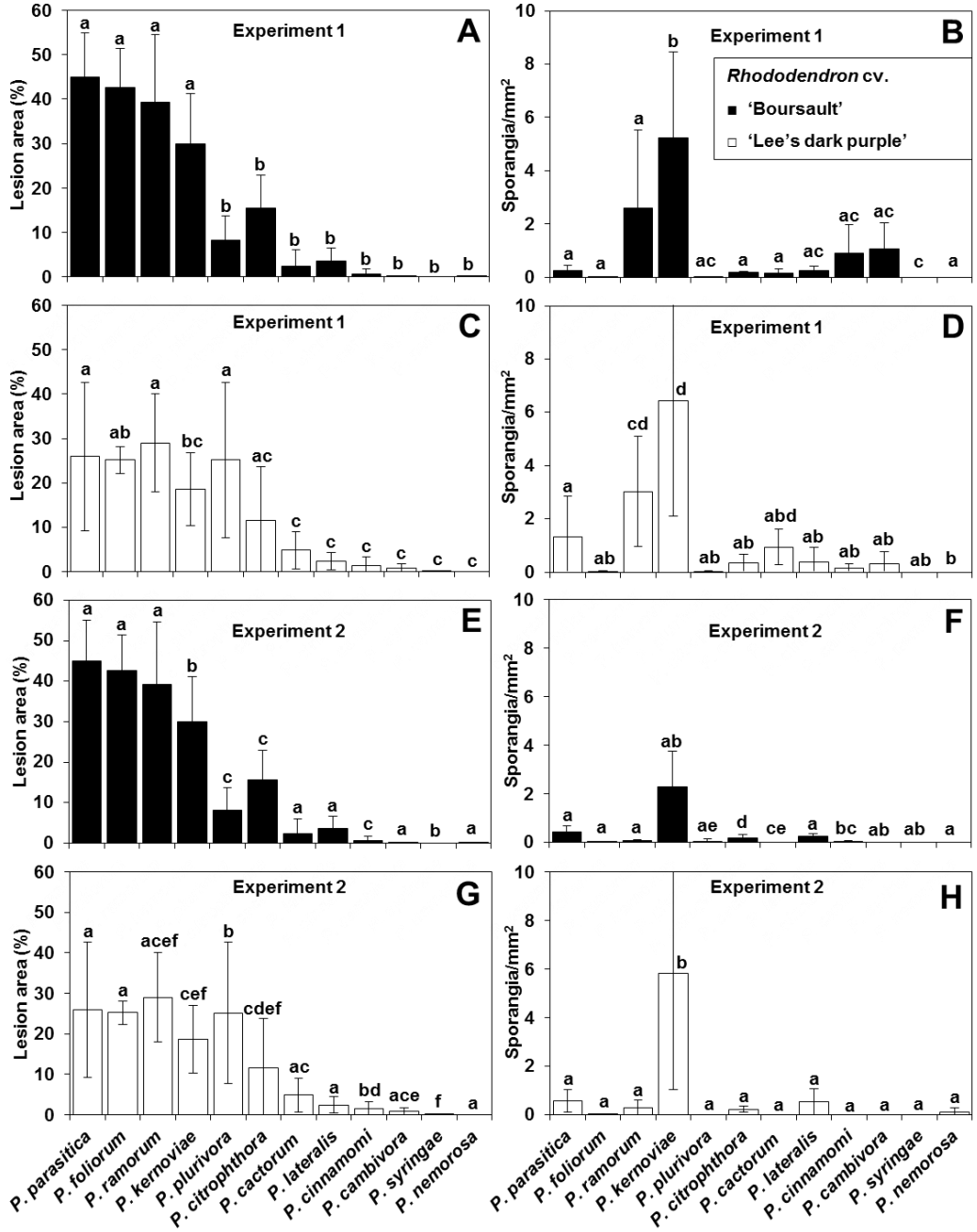
Experiment 1
A
Lesion area (%)
Experiment 1
B
Sporangia/mm²
Rhododendron cv.
'Boursault'
'Lee's dark purple'
Experiment 1
C
Lesion area (%)
Experiment 1
D
Sporangia/mm²
Experiment 2
E
Lesion area (%)
Experiment 2
F
Sporangia/mm²
Experiment 2
G
Lesion area (%)
Experiment 2
H
Sporangia/mm²
P. parasitica
P. foliorum
P. ramorum
P. kernoviae
P. plurivora
P. citrophthora
P. cactorum
P. lateralis
P. cinnamomi
P. cambivora
P. syringae
P. nemorosa

Figure 2

Table 3. Analysis of variance of lesion area and sporulation for detached leaf inoculation of twelve *Phytophthora* species.

Experiment; effect	**Source**	**DF**	**F**	***P***
Experiment 1; % lesion area†				
	Species	11	121.99	< **0.001**
	Cultivar	1	7.97	**0.005**
	Species*Cultivar	11	8.11	< **0.001**
	Block	4	0.50	0.733
Experiment 1; % lesion area on *Rhododendron* cv. CB				
	Species	11	30.46	< **0.001**
	Block	4	0.69	0.606
Experiment 1; % lesion area on *Rhododendron* cv. LDP				
	Species	11	8.54	< **0.001**
	Block	4	0.75	0.566
Experiment 1; sporulation/mm^2 †				
	Species	11	23.67	< **0.001**
	Cultivar	1	1.07	0.303
	Species*Cultivar	11	1.10	0.373
	Block	4	0.17	0.953
Experiment 1; sporulation/mm^2 † on *Rhododendron* cv. CB				
	Species	11	10.60	< **0.001**
	Block	4	0.46	0.766
Experiment 1; sporulation/mm^2 † on *Rhododendron* cv. LDP				
	Species	11	14.05	< **0.001**
	Block	4	0.55	0.701
Experiment 2; % lesion area†				
	Species	11	163.69	< **0.001**
	Cultivar	1	70.48	< **0.001**
	Species*Cultivar	11	8.20	< **0.001**
	Block	4	0.05	0.996
Experiment 2; % lesion area on *Rhododendron* cv. CB				
	Species	11	76.55	< **0.001**
	Block	4	0.43	0.785
Experiment 2; % lesion area on *Rhododendron* cv. LDP				
	Species	11	23.76	< **0.001**
	Block	4	0.57	0.686

Table 3 (Continued)

Experiment; effect	Source	DF	F	*P*
Experiment 2; sporulation/mm^2 †				
	Species	11	38.47	< **0.001**
	Cultivar	1	4.94	**0.029**
	Species*Cultivar	11	1.90	0.050
	Block	4	3.36	**0.013**
Experiment 2; sporulation/mm^2 † on *Rhododendron* cv. CB				
	Species	11	27.17	< **0.001**
	Block	4	1.39	0.253
Experiment 2; sporulation/mm^2 † on *Rhododendron* cv. LDP				
	Species	11	20.04	< **0.001**
	Block	4	3.82	**0.009**

† data were square root transformed for analysis of variance
CB = *Rhododendron* cv. *catawbiense* 'Boursault'
LDP = *Rhododendron* cv. 'Lee's dark purple'

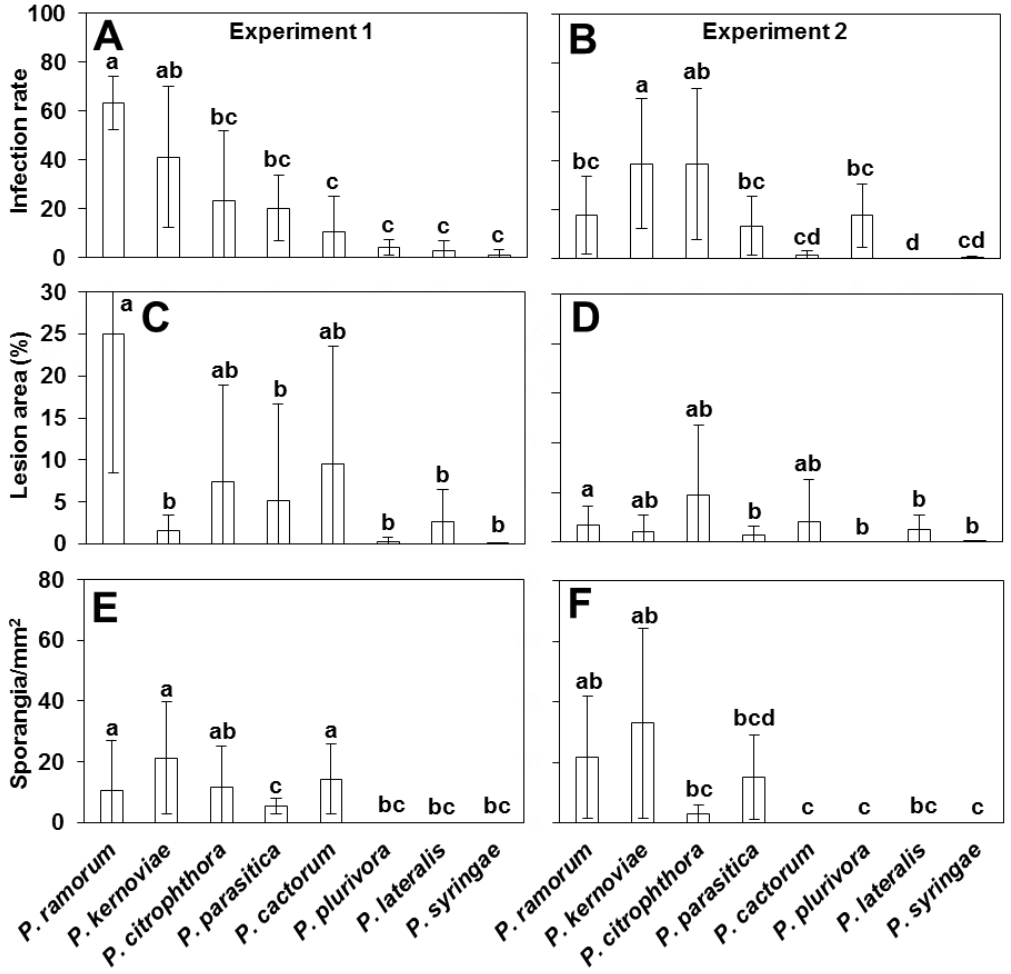

Figure 3. A, **B**, Infection rate at 18°C (% of leaves infected), **C**, **D**, lesion area (%), and **E**, **F**, sporulation (sporangia/mm^2), on non-wounded dip-inoculated *Rhododendron* cv. *catawbiense* 'Boursault' plants in experiments 1 and 2, respectively. Bars show standard deviation from the mean of replicates (non-transformed data). Lower case letters in each panel represent statistically significant differences based on Tukey's test at the 95% confidence level (based on transformed data).

Effect of temperature: detached leaf and agar culture experiments. There was a significant species × temperature interaction for colony growth rate, lesion growth rate and AULEC in all experiments ($P < 0.001$) (Figure 4, Figure 5). Three independent experiments showed similar trends for the colony growth rate response of *Phytophthora* spp. to different temperatures (Figure 4). Notably, *P. cambivora*, *P. cinnamomi*, *P. citrophthora* and *P. parasitica* had similar responses to temperature with the ability to grow at 30°C (Figure 4). *P. parasitica* stood out with the ability to grow well at high temperatures and is the only species that could grow at or potentially above 35°C. Similarly, *P. syringae*, *P. kernoviae*, *P. lateralis*, *P. nemorosa*, and *P. ramorum*, stood out as requiring lower temperatures for growth and their inability to grow at ≥ 30°C (Figure 4).

Detached leaf inoculations with seven (non-quarantine) *Phytophthora* spp. showed patterns similar to the Petri dish assays (Figure 5). *P. parasitica*, *P. plurivora* and *P. citrophthora* all had significantly higher AULEC than the other four species across all temperatures in all three experiments ($P < 0.05$) except in experiment 1 where there was no significant difference between these three species and *P. syringae* ($P > 0.05$) (Figure 5). In all experiments *P. syringae* developed highest AULEC at 5°C than any other species ($P < 0.01$). At 35°C *P. parasitica* showed significantly higher AULEC in all experiments ($P < 0.01$). At both 15 and 20°C *P. plurivora* was the species with the highest AULEC in all experiments ($P < 0.01$).

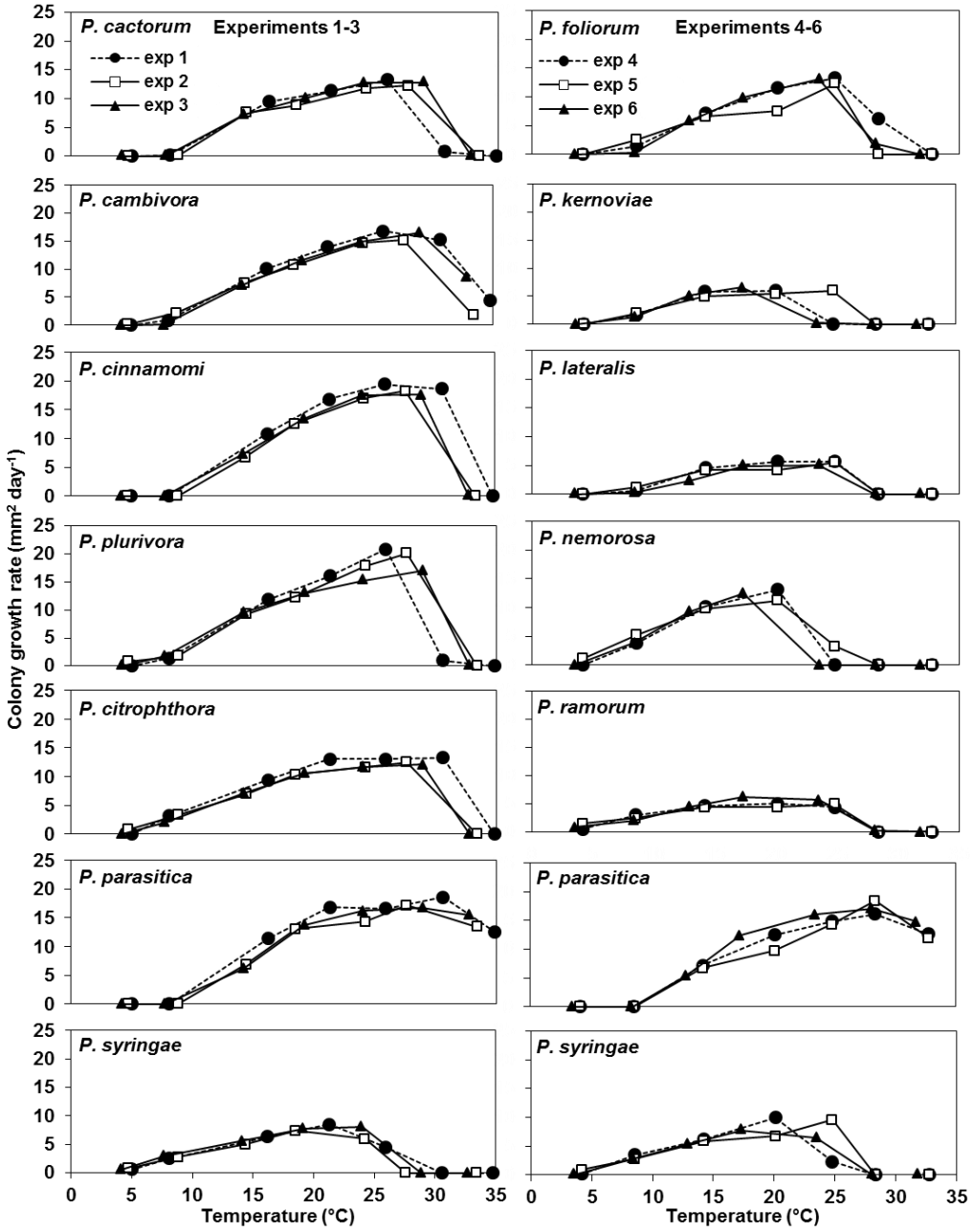

Figure 4. Colony growth rates (mm day^{-1}) 4 days post-inoculation for each *Phytophthora* species grown at seven temperatures on V8 100 agar. Two sets of three experiments were conducted, one with all non-quarantine species (left column) and one with all quarantine species (right column) including *P. syringae* and *P. parasitica* as positive controls.

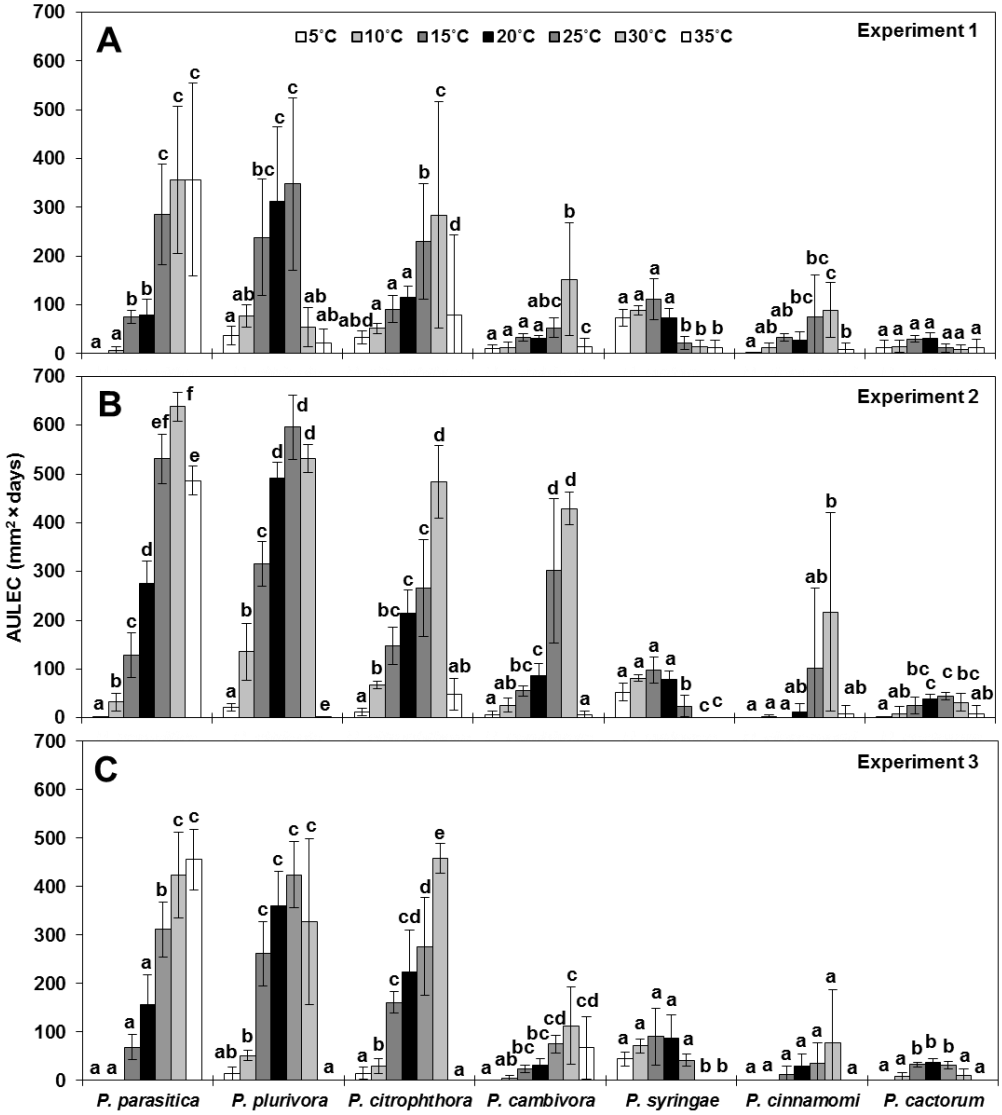

Figure 5. Area under the lesion expansion curve (AULEC) in mm^2 × days, at seven temperatures for the seven non quarantine species on detached *Rhododendron* cv. *catawbiense* 'Boursault' leaves in experiments **A**, 1, **B**, 2 and **C**, 3, respectively. Lower case letters in each panel represent statistically significant differences among temperatures within species based on Tukey's test at the 95% confidence level (based on transformed data). Bars show standard deviation.

Aggressiveness and effect of temperature: field experiments. *Phytophthora* aggressiveness on *Rhododendron* species was evaluated in four field studies with average temperatures of 4, 8, 15, and 18°C (Figure 6, C). In each of the four experiments there was a significant species by cultivar interaction (P = 0.041, 0.038, and < 0.001) (Table 4). Results were similar in nature to detached leaf inoculations and radial growth assays in Petri dish culture (Figure 4, Figure 5). On *Rhododendron* cv. *catawbiense* 'Boursault' *P. parasitica* had significantly higher AULEC than all the other species at 18°C (Figure 6, A). On *Rhododendron* cv. 'Lee's dark purple' *P. parasitica* had significantly higher AULEC than all species except *P. citrophthora* at 18°C (Figure 6, B). *P. plurivora* and *P. citrophthora* were the most aggressive species at 15°C (Figure 6, A and B). On *Rhododendron* cv. *catawbiense* 'Boursault' *P. plurivora* and *P. citrophthora* had significantly higher AULEC than any other species (Figure 6, A). On *Rhododendron* cv. 'Lee's dark purple' there was no significant difference between these two species and *P. parasitica* (Figure 6, B). *P. syringae* was more aggressive at moderate temperatures and caused most disease at 8°C (Figure 6). There was no significant difference between *P. syringae* and *P. plurivora* and *P. citrophthora* at 8°C on either cultivar (Figure 6, A and B). At 4°C *P. syringae* and *P. plurivora* had the highest AULEC on *Rhododendron* cv. *catawbiense* 'Boursault' (Figure 6, A). However, on *Rhododendron* cv. 'Lee's dark purple' *P. plurivora* and *P. citrophthora* developed the highest AULEC (Figure 6, B).

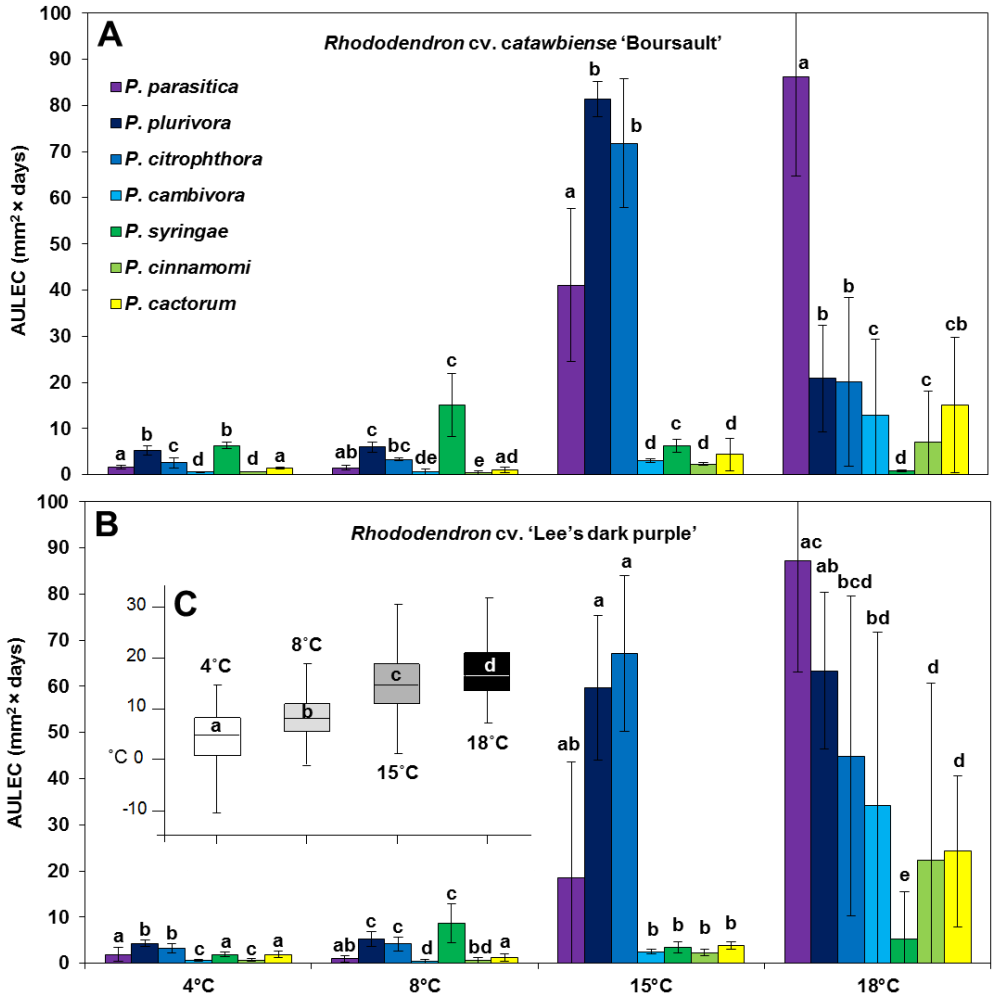

Figure 6. Whole plant field inoculation of seven *Phytophthora* species on two *Rhododendron* cultivars using wound inoculation. Graphs show area under the lesion expansion curve (AULEC) in mm^2 × days for each *Phytophthora* spp. at each seasonally observed temperature (inset **C**) of 4, 8, 15, and 18°C, on **A**, *Rhododendron* cv. *catawbiense* 'Boursault', and **B**, *Rhododendron* cv. 'Lee's dark purple. Inset, **C**, shows the temperatures (°C) measured during each of the four experiments. Bars show standard deviation, lower case letters represent statistically significant differences based on Tukey's test at the 95% confidence level (based on transformed data).

Table 4. Analysis of variance of AULEC from whole plant field inoculation of seven *Phytophthora* spp. on two *Rhododendron* cultivars. Analysis of variance of AULEC (mm^2 × days) (square root transformed).

Experiment	Source	DF	F	*P*
1 (18°C)	Species	6	58.81	< **0.001**
	Cultivar	1	38.20	< **0.001**
	Species*Cultivar	6	2.81	**0.012**
	Block	5	2.91	0.060
	Leaf(Block)	12	0.71	0.737
CB	Species	6	39.24	< **0.001**
	Block	5	5.86	**0.006**
	Leaf(Block)	12	0.36	0.975
LDP	Species	6	23.92	< **0.001**
	Block	5	1.97	0.156
	Leaf(Block)	12	0.74	0.705
2 (15°C)	Species	6	125.12	< **0.001**
	Cultivar	1	18.92	< **0.001**
	Species*Cultivar	6	3.54	**0.002**
	Block	5	0.39	0.845
	Leaf(Block)	12	1.15	0.322
CB	Species	6	183.79	< **0.001**
	Block	5	0.75	0.600
	Leaf(Block)	12	0.98	0.472
LDP	Species	6	31.63	< **0.001**
	Block	5	0.27	0.922
	Leaf(Block)	12	0.65	0.799
3 (5°C)	Species	6	130.58	< **0.001**
	Cultivar	1	4.85	**0.029**
	Species*Cultivar	6	14.68	< **0.001**
	Block	5	6.01	**0.005**
	Leaf(Block)	12	0.53	0.892
CB	Species	6	120.04	< **0.001**
	Block	5	2..92	0.059
	Leaf(Block)	12	0.26	0.994
LDP	Species	6	45.37	< **0.001**
	Block	5	7.37	**0.002**
	Leaf(Block)	12	0.60	0.834

Table 4 (Continued)

Experiment	Source	DF	F	*P*
4 (8°C)	Species	6	53.66	< **0.001**
	Cultivar	1	4.41	**0.037**
	Species*Cultivar	6	1.67	0.130
	Block	5	41.49	< **0.001**
	Leaf(Block)	12	0.13	1.000
CB	Species	6	30.45	< **0.001**
	Block	5	12.50	< **0.001**
	Leaf(Block)	12	0.42	0.951
LDP	Species	6	25.34	< **0.001**
	Block	5	16.77	< **0.001**
	Leaf(Block)	12	0.15	1.000

CB = *Rhododendron* cv. *catawbiense* ‘Boursault’
LDP = *Rhododendron* cv. ‘Lee’s dark purple’

Discussion

Our work revealed some interesting results in regard to temperature and aggressiveness responses among *Phytophthora* spp. *P. parasitica* was consistently able to grow at higher temperatures (≥ 35°C) than any of the other *Phytophthora* spp. *P. parasitica* showed significantly higher aggressiveness (in terms of AULEC) than any of the other species when studied at 18°C in the field and at 35°C in the growth chamber. The ability of *P. parasitica* to grow at high temperatures has been used as a diagnostic characteristic to identify this species relative to other *Phytophthora* spp. as developed by Tucker (1931). *P. cactorum*, *P. cambivora*, *P. citrophthora*, and *P. plurivora* prefer moderate to high temperatures (20 – 30°C). *P. kernoviae*, *P. nemorosa*, *P. ramorum* and *P. syringae* preferred lower temperature regimes (< 25°C).

P. cinnamomi and *P. cactorum*, typically thought of as root-infecting species, were able to cause as much foliar disease (lesion area or AULEC) as *P. syringae* (a foliar pathogen) indicating that the common categorization of *Phytophthora* pathogens as either root or foliar species may be misleading. It should be noted that the inoculation techniques used here may have facilitated infection of plants or plant parts that would not naturally get infected. We deliberately created an environment conducive to infection and disease development to facilitate the study of the epidemiology of these twelve species. In wound inoculating in this way we may have observed more disease than would naturally occur. Due to the challenging nature of working with multiple *Phytophthora* species it was necessary to conduct some experiments with wounding as an inoculation technique in order to reduce the likelihood of inoculation failure; however, infection rate cannot be measured in experiments conducted in this manner. With non-wounded inoculations there can be

considerably greater variation in the data due to higher failure rates of inoculation. It is generally accepted that non-wounded inoculation methods more closely reflect the natural infection process, but it should be noted that in the nursery setting plants may get damaged by tipping over or being overcrowded and these wounds may provide the opportunity for infection to occur.

P. lateralis is rarely isolated from nurseries in Oregon and when it is, it is usually from soil substrate rather than from infected plants. It has only once been reported on *Rhododendron* (Hoitink and Schmitthenner, 1974) and is considered to have a narrow host range including Port Orford cedar and Pacific yew (Erwin and Ribeiro, 1996). Here we observed disease development on *Rhododendron* in both wounded and non-wounded inoculations.

P. syringae stood out as it appeared to be a relatively weak pathogen, showing no sporulation and low levels of disease severity, except at low temperatures. In Oregon it was among the species isolated most frequently from different nursery crops, mostly *Rhododendron*, *Viburnum*, *Pieris*, and *Kalmia* spp., after *P. plurivora* and *P. cinnamomi* (Grünwald et al., 2011). Thus, our results might indicate that *P. syringae*, although not an aggressive pathogen in warmer climates, is well adapted to the cool climate of the Pacific Northwest. Temperatures in the Pacific Northwest typically average between 9 and 11°C annually, with an average high of 16°C and low of 5°C (Mote, 2003).

During the fourth field experiment, when the mean temperature was 8°C, *P. syringae* infection spread to other leaves on the inoculated plants (Appendix, Figure 3). This type of spread was not observed with any of the other species or in any other season. Usually the inoculated leaf developed a second lesion at the tip where water pooled and dripped from the leaf, and frequently lesions developed on leaves below the inoculated leaf (Appendix, Figure 3). Leaves

above and adjacent to the inoculated leaf were also seen to develop secondary lesions which are likely the result of rain splash (Davidson et al., 2005; Ristaino and Gumpertz, 2000). This type of spread of *P. syringae* was observed in both 2009 and 2010 during spring time in Oregon (data not shown). Further experiments to assess sporulation at different temperatures may shed light on why *P. syringae* is such a frequently isolated species in the Pacific Northwest despite its relatively slow growth rate and small lesion area. The ability of *P. syringae* to spread quickly at lower temperatures further supports our inference that *P. syringae* is adapted to the climate of the Pacific Northwest.

P. parasitica was the only other species we observed spreading beyond the inoculated leaf during the field experiments. This occurred as vascular spread to the stem and then to adjacent leaves rather than by rain splash or drip spread (Appendix, Figure 3). This type of spread was only seen during the field experiments carried out at 15 and 18ºC. Considering how problematic *Phytophthora* spp. are in the nursery industry it is surprising that disease spread was so rarely observed in our field experiments. By spacing out the inoculated plants in the field experiment to avoid cross contamination of treatments, the plant canopy is broken up and this may have altered the microenvironment sufficiently to reduce sporulation and the potential for infection.

P. kernoviae should be of particular concern to regulatory agencies. In our studies, *P. kernoviae* was consistently among the most aggressive *Phytophthora* species (with large lesion area and high AULEC) and the highest sporulator. This is of note due to the threat *P. kernoviae* could pose to the nursery and forestry industries in the USA if it were introduced (Brasier et al., 2005). This species causes bark necrosis and bleeding cankers of European beech (*Fagus* sylvatica) and foliar necrosis and shoot dieback of *Rhododendron ponticum* in the woodland of Cornwall, UK and has been found in the nursery

industry in the UK (Brasier et al., 2005). It is thought that the spread of *P. ramorum* and *P. kernoviae* is in part due to their ability to sporulate on asymptomatic plants (Denman et al., 2006). Our inferences are based solely on controlled growth chamber experiments because, due to quarantine restrictions, we could not study *P. kernoviae* in controlled field experiments.

Further studies on infection efficiency and sporulation at different temperatures would provide more valuable insights into the potential threat of these species for disease development at different times of the year. In this paper sporulation and infection efficiency were assessed at 18°C only. This was due to containment constraints and the availability of growth chamber space for different temperatures. The issue of producing sufficient quantities of zoospore inoculum at the desired concentrations simultaneously also makes non wounded inoculations more challenging. It is likely that the species which did not sporulate in these experiments may do so at different temperatures, such as *P. syringae* which is favored by lower temperatures.

The field experiments were conducted at different times of the year and this not only altered the mean temperatures of those experiments but it also affected the quantity and types of precipitation experienced by the Rhododendrons. These effects were to some extent controlled for by the application of overhead irrigation during the drier experiments, however, these precipitation differences will likely have an effect on the lifecycle of the *Phytophthora* species inoculated. There is also a seasonal effect on the susceptibility of Rhododendrons as these evergreen plants go dormant in the winter, their leaves develop a thicker waxy layer enabling them to withstand the colder weather (Meyer, 1928). This may confer resistance to infection by *Phytophthora* spp. as well as cold resistance. This increased resistance to infection was observed during whole plant dip and detached leaf inoculation

experiments where we found that infection rates dropped around October each year (data not shown). This limited our ability to conduct non-wounded inoculations during the winter months even using greenhouse grown plants. We suggest that *P. syringae* may have found a way to circumnavigate this host resistance so that it can infect during the colder months.

Few studies have compared the severity of multiple *Phytophthora* spp. on a single host (Jeger and Pautasso, 2008; Wilcox and Mircetich, 1985). This might be because working simultaneously with several *Phytophthora* species provides unique challenges. One major challenge is the difficulty of producing sporangial or zoospore inoculum for all taxa, appropriately timed for inoculation on a given date (Ahonsi et al., 2007; Erwin and Ribeiro, 1996). Another challenge is capturing variation within a species, which can be accomplished by using several isolates per species. This obviously increases the logistical constraints. Brasier and Kirk (2001) compared the aggressiveness of several variants of alder Phytophthoras alongside 7 other species; *P. cambivora, P. cinnamomi, P. citricola, P. cryptogea, P. fragariae* var. *rubi, P. gonapodyides,* and *P. megasperma*. Their study consisted of wounded inoculation of logs using agar plugs and aggressiveness was assessed by measuring lesion area. This hybrid *Phytophthora* was found to be relatively host specific and more aggressive on alder when compared with *P. cambivora,* and it has since been described as *P. alni*. (Brasier et al., 2004).

We are only aware of one study contrasting different *Phytophthora* species on *Rhododendron*. Linderman et al. (2006) contrasted *P. ramorum* with *P. cactorum, P. cinnamomi, P. citricola, P. citrophthora, P. heveae, P. parasitica* and *P. syringae* using detached leaf, wound-inoculations on *Rhododendron* cv. 'Nova Zembla'. Their results are based on a single experiment that showed that *P. cactorum, P. citricola, P. citrophthora* and *P.*

parasitica were most pathogenic at 20°C. The work was not validated with whole plant assays and non-wounded inoculations. *P. syringae* again caused very little disease at these temperatures. Our experiments also indicated the same four species were among the most aggressive and we found *P. syringae* was less aggressive at higher temperatures of 20°C and above. Interactions between *Phytophthora* species and host cultivars and between temperatures and *Phytophthora* species make it difficult to rank the pathogens in terms of aggressiveness.

Greater understanding of the differences among species and the ability to identify Phytophthoras to species level using PCR and sequencing, means control of *Phytophthora* spp. in nurseries can be targeted towards particular species. For example if *P. syringae* is known to be present in a nursery, management practices need to be focused on the colder months. Control against the spread of *Phytophthora* species which sporulate prolifically should place emphasis on drainage and irrigation management. Management of a foliar epidemic should focus on breaking up the plant canopy by spacing out the plants to alter the microenvironment. The ability for *Phytophthora* spp. to spread in soil or on asymptomatic plants means that visual inspection of plants prior to import or export is not sufficient to reduce the risk of introducing novel pathogens to the nursery industry or to new environments. These results further our understanding of the epidemiology of these important *Phytophthora* species and add to the body of information which will aid the development of more effective strategies to manage *Phytophthora* diseases in nurseries and mitigate the spread of *Phytophthora* species to new ecosystems.

References

1. Ahonsi M.O., Banko T.J., Hong C. (2007) A simple *in vitro* 'wet-plate' method for mass production of *Phytophthora nicotianae* zoospores and factors influencing zoospore production. Journal of Microbiological Methods 70:557-560.

2. Benson D.M., Hoitink H.A.J. (1986) Phytophthora dieback, in: D. L. Coyier and M. K. Roane (Eds.), Compendium of *Rhododendron* and azalea diseases, APS Press, St. Paul. MN. pp. 4-8.

3. Brasier C.M., Beales P.A., Kirk S.A., Denman S., Rose J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research 109:853-859.

4. Brasier C.M., Kirk S.A., Delcan J., Cooke D.E.L., Jung T., Man In't Veld W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research 108:1172-1184. DOI: 10.1017/s0953756204 001005.

5. Brasier C.M., Kirk S.A. (2001) Comparative aggressiveness of standard and variant hybrid alder Phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. Plant Pathology 50:218-229. DOI: 10.1046/j.1365-3059.2001. 00553.x.

6. Daughtrey M.L., Benson D.M. (2001) *Rhododendron* diseases, in: R. K. Jones Diseases of woody ornamentals and trees in nurseries., APS Press, St. Paul, MN. pp. 334-341.

7. Davidson J.M., Wickland A.C., Patterson H.A., Falk K.R., Rizzo D.M. (2005) Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. Phytopathology 95:587-596. DOI: 10.1094/PHYTO-95-0587.

8. Denman S., Kirk S., Whybrow A., Orton E., Webber J.F. (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin 36:373-376. DOI: 10.1111/j.1365-2338.2006.01014.x.

9. Erwin D.C., Ribeiro O.K. (1996) *Phytophthora* Diseases Worldwide, APS Press, St. Paul, Minnesota.

10. Farr D.F., Esteban H.B., Palm M.E. (1996) Fungi on *Rhododendron*: a world reference. Parkway Publishers, Inc., Boone, NC.

11. Fry W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology 9:385-402.

12. Goss E., Larsen M., Vercauteren A., Werres S., Heungens K., Grünwald N. (2011) *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. Phytopathology 101:166-71.

13. Goss E.M., Larsen M., Chastagner G.A., Givens D.R., Grünwald N.J. (2009) Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathogens 5:e1000583.

14. Grünwald N.J., Goss E.M., Press C.M. (2008) *Phytophthora ramorum*: a pathogen with a remarkably wide host-range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Molecular Plant Pathology 9:729-40.

15. Grünwald N.J., Martin F.N., Larsen M.M., Sullivan C.M., Press C.M., Coffey M.D., Hansen E.M., Parke J.L. (2011) Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342. DOI: 10.1094/PDIS-08-10-0609.

16. Hardham A.R. (2005) *Phytophthora cinnamomi*. Molecular Plant Pathology 6:589-604.

17. Hoitink H.A.J., Schmitthenner A.F. (1974) Relative prevalence and virulence of *Phytophthora* species involved in *Rhododendron* root rot. Phytopathology 64:1371-1374.

18. Jeger M., Pautasso M. (2008) Comparative epidemiology of zoosporic plant pathogens. European Journal of Plant Pathology 122:111-126. DOI: 10.1007/s10658-008-9289-y.

19. Linderman R.G. (1986) *Phytophthora syringae* blight., in: D. L. Coyier and M. K. Roane (Eds.), Compendium of *Rhododendron* and azalea Diseases., APS Press, St. Paul, MN. pp. 4-8.

20. Linderman R.G., Davis E.A., Marlow J.L. (2006) Response of selected nursery crop plants to inoculation with isolates of *Phytophthora ramorum* and other *Phytophthora* species. Horttechnology 16:216-224.

21. Mascheretti S., Croucher P.J.P., Vettraino A., Prospero S., Garbelotto M. (2008) Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology 17:2755-68.

22. Meyer B. (1928) Seasonal variations in the physical and chemical properties of the leaves of the pitch pine, with especial reference to cold resistance. American Journal of Botany 15:449-472.

23. Mote P.W. (2003) Trends in temperature and precipitation in the Pacific Northwest during the twentieth century. Northwest Science 77:271-282.

24. Ristaino J.B., Gumpertz M.L. (2000) New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*. Annual Review of Phytopathology 38:541-576. DOI: 10.1146/annurev.phyto.38.1.541.

25. Tucker C.M. (1931) Taxonomy of the genus *Phytophthora* de Bary., University of Missouri Agricultural Experiment Station Research Bulletin. pp. 207.

26. Tukey J.W. (1991) The Philosophy of Multiple Comparisons. Statistical Science 6:100-116.

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

28. Wilcox W.F., Mircetich S.M. (1985) Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75:221-226.

CHAPTER 3: CONCLUSION

Direct comparison of twelve *Phytophthora* spp. on *Rhododendron* revealed differences in aggressiveness of *Phytophthora* spp. and their temperature responses. Results supported the hypothesis that there would be significant differences in aggressiveness among the different *Phytophthora* spp. studied. There were also clear differences in the temperature niches occupied by each of the twelve *Phytophthora* spp.

P. citrophthora, P. kernoviae, P. parasitica, P. plurivora and *P. ramorum* were consistently the most aggressive species across *in vitro*, detached leaf, and whole plant inoculations. *P. parasitica* was able to grow well at high temperatures (> 30°C) and was the most aggressive species at the highest temperatures evaluated in this study. Several species were more aggressive at lower temperatures (< 20°C) including *P. kernoviae* and *P. syringae*. *P. syringae* did not sporulate under the conditions studied and caused relatively low levels of disease severity despite being a problematic and frequently isolated pathogen in Oregon nurseries (Grünwald et al., 2011). This pathogen seems to be particularly well adapted to the climate of the Pacific Northwest.

Further investigations into the effect of temperature on infection efficiency and sporulation will build on these findings. In this study infection efficiency was measured on whole plants, and sporulation was assessed on detached leaves and whole plants at 18°C only. It is likely that species which did not sporulate in these experiments may do so at different temperatures, such as *P. syringae* which is favored by lower temperatures.

P. kernoviae is of particular interest due to its aggressiveness and ability to sporulate well. This species is causing severe epidemics in the UK (Brasier

et al., 2005). If it were introduced in the US it could pose a significant threat to the nursery and forestry industries here in a similar fashion to the emergence of *P. ramorum* as an invasive, exotic species (Brasier et al., 2005; Grünwald et al., 2008). Like *P. ramorum*, *P. kernoviae* prolifically produces caducous sporangia, increasing the potential for spread of the pathogen. Unlike *P. ramorum*, *P. kernoviae* is homothallic (self-fertile), eliminating the need for the presence of another mating type for it to carry out sexual reproduction (Brasier et al., 2005). Both *P. ramorum* and *P. kernoviae* can sporulate on asymptomatic hosts (Denman et al., 2006).

Rhododendron is an important crop in the nursery industry and it is a host to many *Phytophthora* species which is of particular concern because this makes it a good candidate to become a vector to introduce exotic species to different ecosystems (De Dobbelaere et al., 2010). Indeed, *Rhododendron* has already been implicated in the introduction of *P. ramorum* to the West coast of North America (Goss et al., 2009; Mascheretti et al., 2008). *Phytophthora* species could also be introduced in potting media or soil if they produce chlamydospores or oospores that could survive for long periods in these environments

Developing a better understanding of these pathogens will aid regulatory agencies in managing the risks associated with the national and international movement of plants. Understanding the variation in aggressiveness of species and the different temperatures at which they are most active will help in the development of more targeted strategies for disease management in nurseries.

References

1. Brasier C.M., Beales P.A., Kirk S.A., Denman S., Rose J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research 109:853-859.

2. De Dobbelaere I., Vercauteren A., Speybroeck N., Berkvens D., Van Bockstaele E., Maes M., Heungens K. (2010) Effect of host factors on the susceptibility of *Rhododendron* to *Phytophthora ramorum*. Plant Pathology 59:301-312.

3. Denman S., Kirk S., Whybrow A., Orton E., Webber J.F. (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin 36:373-376. DOI: 10.1111/j.1365-2338.2006.01014.x.

4. Goss E.M., Larsen M., Chastagner G.A., Givens D.R., Grünwald N.J. (2009) Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathogens 5:e1000583.

5. Grünwald N.J., Goss E.M., Press C.M. (2008) *Phytophthora ramorum*: a pathogen with a remarkably wide host-range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Molecular Plant Pathology 9:729-40.

6. Grünwald N.J., Martin F.N., Larsen M.M., Sullivan C.M., Press C.M., Coffey M.D., Hansen E.M., Parke J.L. (2011) Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342. DOI: 10.1094/PDIS-08-10-0609.

7. Mascheretti S., Croucher P.J.P., Vettraino A., Prospero S., Garbelotto M. (2008) Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology 17:2755-68.

BIBLIOGRAPHY

1. Adl S.M., Simpson A.G.B., Farmer M.A., Andersen R.A., Anderson O.R., Barta J.R., Bowser S.S., Brugerolle G.U.Y., Fensome R.A., Fredericq S., James T.Y., Karpov S., Kugrens P., Krug J., Lane C.E., Lewis L.A., Lodge J., Lynn D.H., Mann D.G., McCourt R.M., Mendoza L., Moestrup Ø., Mozley-Standridge S.E., Nerad T.A., Shearer C.A., Smirnov A.V., Spiegel F.W., Taylor M.F.J.R. (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of Protists. Journal of Eukaryotic Microbiology 52:399-451.

2. Ahonsi M.O., Banko T.J., Hong C. (2007) A simple *in vitro* 'wet-plate' method for mass production of *Phytophthora nicotianae* zoospores and factors influencing zoospore production. Journal of Microbiological Methods 70:557-560.

3. Benson D.M., Hoitink H.A.J. (1986) Phytophthora dieback, in: D. L. Coyier and M. K. Roane (Eds.), Compendium of *Rhododendron* and azalea diseases, APS Press, St. Paul. MN. pp. 4-8.

4. Bhattacharya D., Yoon H.S., Hedges S.B., Hackett J.D. (2009) Eukaryotes (Eukaryota). The Timetree of Life, Hedges, S. B. and Kumar, S. (Eds.) Oxford University Press.

5. Birch P.R.J., Whisson S.C. (2001) *Phytophthora infestans* enters the genomics era. Molecular Plant Pathology 2:257-263. DOI: 10.1046/j.1464-6722.2001.00073.x.

6. Blair J.E., Coffey M.D., Park S.-Y., Geiser D.M., Kang S. (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genetics and Biology 45:266-277. DOI: 10.1016/j.fgb.2007.10.010.

7. Blanco F.A., Judelson H.S. (2005) A bZIP transcription factor from *Phytophthora* interacts with a protein kinase and is required for zoospore motility and plant infection. Molecular Microbiology 56:638-648. DOI: 10.1111/j.1365-2958.2005.04575.x.

8. Brasier C.M. (2000) Plant pathology: The rise of the hybrid fungi. Nature 405:134-135.

9. Brasier C.M. (2008) The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology 57:792-808. DOI: 10.1111/j.1365-3059.2008.01886.x.

10. Brasier C.M. (2007) *Phytophthora* Biodiversity: How Many *Phytophthora* Species Are There? Phytophthoras in forests and natural ecosystems. Proceedings of the 4th Meeting of IUFRO Working Party 07.02.09, August 26–31, 2007, Monterey, CA. General Technical Report PSW-GTR-221, Albany, CA:101–115.

11. Brasier C.M., Beales P.A., Kirk S.A., Denman S., Rose J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research 109:853-859.

12. Brasier C.M., Kirk S.A. (2001) Comparative aggressiveness of standard and variant hybrid alder Phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. Plant Pathology 50:218-229. DOI: 10.1046/j.1365-3059.2001. 00553.x.

13. Brasier C.M., Kirk S.A., Delcan J., Cooke D.E.L., Jung T., Man In't Veld W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research 108:1172-1184. DOI: 10.1017/s095375620400 1005.

14. Buisman C.J. (1927) Root rots caused by Phycomycetes. Meded. Phytopathology Laboratory, University of Utrecht, Reviews in Applied Mycology 6:380.

15. Cavalier-Smith T. (2004) Only six kingdoms of life. Proceedings of the Royal Society B 271:1251-1262.

16. Dart N.L., Chastagner G.A. (2007) Estimated economic losses associated with the destruction of plants due to *Phytophthora ramorum* quarantine efforts in Washington State. Plant Health Progress, Online. DOI: 10.1094/PHP-2007-0508-02-RS.

17. Dastur J. (1913) *Phytophthora parasitica* n. sp., a new disease of the castor oil plant. Memoirs of the Department of Agriculture, India 22:177-231.

18. Daughtrey M.L., Benson D.M. (2001) *Rhododendron* diseases, in: R. K. Jones Diseases of woody ornamentals and trees in nurseries., APS Press, St. Paul, MN. pp. 334-341.

19. Davidson J.M., Rizzo D.M., Garbelotto M., Tjosvold S., Slaughter G.W. (2002) *Phytophthora ramorum* and sudden oak death in California: II. Transmission and survival, General Technical Report, USDA Forest Service. pp. 741-749.

20. Davidson J.M., Werres S., Garbelotto M., Hansen E.M., Rizzo D.M. (2003) Sudden oak death and associated diseases caused by *Phytophthora ramorum*, Plant Health Progress, Online. DOI: 10.1094/PHP-2003-0707-01-DG.

21. Davidson J.M., Wickland A.C., Patterson H.A., Falk K.R., Rizzo D.M. (2005) Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. Phytopathology 95:587-596. DOI: 10.1094/PHYTO-95-0587.

22. de Bary A. (1876) Researches into the nature of the potato fungus, *Phytophthora infestans*. Journal of the Royal Agricultural Society of England. Series 2 12:239-269.

23. De Dobbelaere I., Vercauteren A., Speybroeck N., Berkvens D., Van Bockstaele E., Maes M., Heungens K. (2010) Effect of host factors on the susceptibility of *Rhododendron* to *Phytophthora ramorum*. Plant Pathology 59:301-312.

24. Denman S., Kirk S., Whybrow A., Orton E., Webber J.F. (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin 36:373-376. DOI: 10.1111/j.1365-2338.2006.01014.x.

25. Dick M.W. (2001) Straminipilous fungi: systematics of the Peronosporomycetes, including accounts of the marine straminipilous protists, the plasmodiophorids, and similar organisms. Kluwer Academic Publishers.

26. Donahoo R., Blomquist C.L., Thomas S.L., Moulton J.K., Cooke D.E.L., Lamour K.H. (2006) *Phytophthora foliorum* sp. nov., a new species causing leaf blight of azalea. Mycological Research 110:1309-1322.

27. Durán A., Gryzenhout M., Slippers B., Ahumada R., Rotella A., Flores F., Wingfield B.D., Wingfield M.J. (2008) *Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile. Plant Pathology 57:715-727. DOI: 10.1111/j.1365-3059.2008.01893.x.

28. Erwin D.C., Ribeiro O.K. (1996) *Phytophthora* Diseases Worldwide, APS Press, St. Paul, Minnesota.

29. Farr D.F., Esteban H.B., Palm M.E. (1996) Fungi on *Rhododendron*: a world reference. Parkway Publishers, Inc., Boone, NC.

30. Fernández-Pavía S.P., Grünwald N.J., Díaz-Valasis M., Cadena Hinojosa M., Fry W.E. (2004) Soil-borne oospores of *Phytophthora infestans* in central Mexico survive winter fallow and infect potato plants in the field. Plant Disease 88:29-33.

31. Frankel S.J. (2008) Sudden oak death and *Phytophthora ramorum* in the USA: a management challenge. Australasian Plant Pathology 37:19-25. DOI: 10.1071/AP07088.

32. Fry W. (2008) *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology 9:385-402.

33. Goss E., Larsen M., Vercauteren A., Werres S., Heungens K., Grünwald N. (2011) *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. Phytopathology 101:166-71.

34. Goss E.M., Larsen M., Chastagner G.A., Givens D.R., Grünwald N.J. (2009) Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathogens 5:e1000583.

35. Grünwald N.J., Goss E.M. (2011 in press) Evolution and population genetics of exotic and re-emerging pathogens: Novel tools and approaches. Annual Review of Phytopathology 49.

36. Grünwald N.J., Goss E.M., Press C.M. (2008) *Phytophthora ramorum*: a pathogen with a remarkably wide host-range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Molecular Plant Pathology 9:729-40.

37. Grünwald N.J., Kitner M., McDonald V., Goss E.M. (2008) Susceptibility in *Viburnum* to *Phytophthora ramorum*. Plant Disease 92:210-214.

38. Grünwald N.J., Martin F.N., Larsen M.M., Sullivan C.M., Press C.M., Coffey M.D., Hansen E.M., Parke J.L. (2011) Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342. DOI: 10.1094/PDIS-08-10-0609.

39. Hansen E.M., Goheen D.J., Jules E.S., Ullian B. (2000) Managing Port Orford cedar and the introduced pathogen *Phytophthora lateralis*. Plant Disease 84:4-10.

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

41. Hardham A.R. (2005) *Phytophthora cinnamomi*. Molecular Plant Pathology 6:589-604.

42. Harris D.C. (1979) The occurrence of *Phytophthora syringae* in fallen apple leaves. Annals of Applied Biology 91:309-312. DOI: 10.1111/j.1744-7348.1979.tb06505.x.

43. Hoitink H.A.J., Schmitthenner A.F. (1974) Relative prevalence and virulence of *Phytophthora* species involved in *Rhododendron* root rot. Phytopathology 64:1371-1374.

44. Jeger M., Pautasso M. (2008) Comparative epidemiology of zoosporic plant pathogens. European Journal of Plant Pathology 122:111-126. DOI: 10.1007/s10658-008-9289-y.

45. Jung T., Burgess T.I. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. Persoonia - Molecular Phylogeny and Evolution of Fungi 22:95-110. DOI: 10.3767/003158509x442612.

46. Jung T., Hansen E.M., Winton L., Oswald W., Delatour C. (2002) Three new species of *Phytophthora* from European oak forests. Mycological Research 106:397-411. DOI: 10.1017/s0953756202005622.

47. Jung T., Nechwatal J., Cooke D.E.L., Hartmann G., Blaschke M., Oßwald W.F., Duncan J.M., Delatour C. (2003) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. Mycological Research 107:772-789. DOI: 10.1017/ s0953756203008074.

48. Klebahn H. (1905) A new fungal disease of *Syringae.* (In German). Zentralblatt fur Bakteriologie. 15:335-336.

49. Krings M., Taylor T.N., Dotzler N. (2011) The fossil record of the Peronosporomycetes (Oomycota). Mycologia 103:445-457. DOI: 10.3852/10-278.

50. Lebert H., Cohn F. (1870) On the rot of cactus stems (in German). Beitraege zur Biologie Pflanzen. 1:51-57.

51. Leonian L. (1925) Physiological studies on the genus *Phytophthora*. American Journal of Botany 12:444-498.

52. Linderman R.G. (1986) *Phytophthora syringae* blight., in: D. L. Coyier and M. K. Roane (Eds.), Compendium of *Rhododendron* and azalea Diseases., APS Press, St. Paul, MN. pp. 4-8.

53. Linderman R.G., Davis E.A., Marlow J.L. (2006) Response of selected nursery crop plants to inoculation with isolates of *Phytophthora ramorum* and other *Phytophthora* species. Horttechnology 16:216-224.

54. Mascheretti S., Croucher P.J.P., Vettraino A., Prospero S., Garbelotto M. (2008) Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Molecular Ecology 17:2755-68.

55. Meyer B. (1928) Seasonal variations in the physical and chemical properties of the leaves of the pitch pine, with especial reference to cold resistance. American Journal of Botany 15:449-472.

56. Mostowfizadeh-Ghalamfarsa R., Cooke D.E.L., Banihashemi Z. (2008) *Phytophthora parsiana* sp. nov., a new high-temperature tolerant species. Mycological Research 112:783-794. DOI: 10.1016/j.mycres.2008.01.011.

57. Mote P.W. (2003) Trends in temperature and precipitation in the Pacific Northwest during the twentieth century. Northwest Science 77:271-282.

58. Nogueira M.L.B., Silva P.P.d., Bartnicki-Garcia S. (1977) Membrane fusion during secretion: A hypothesis based on electron microscope observation of *Phytophthora palmivora* zoospores during encystment. The Journal of Cell Biology 73:161-181.

59. Orlikowski L.B. (2010) Occurrence and harmfulness of *Phytophthora* spp. in Polish hardy ornamental nursery stock. *Acta Horticulturae* (ISHS):243-248.

60. Osterbauer N.K., Griesbach J.A., Hedberg J. (2004) Surveying for and eradicating *Phytophthora ramorum* in agricultural commodities. Plant Health Progress Online. DOI: 10.1094/PHP-2004-0309-02-RS.

61. Parke J.L., Linderman R.G., Osterbauer N.K., Griesbach J.A. (2004) Detection of *Phytophthora ramorum* blight in Oregon nurseries and completion of Koch's postulates on *Pieris*, *Rhododendron*, *Viburnum*, and *Camellia*. Plant Disease 88:87-87.

62. Prospero S., Hansen E.M., Grünwald N.J., Winton L.M. (2007) Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. Molecular Ecology 16:2958-2973.

63. Ramsfield T., Dick M., Beever R., Horner I., McAlonan M., Hill C. (2007) *Phytophthora kernoviae* in New Zealand, Fourth Meeting of IUFRO Working Party S07.02.09 Phytophthoras in Forests and Natural Ecosystems, Monterey, California. pp. P47-53.

64. Rands R. (1922) Stripe canker of cinnamon caused by *Phytophthora cinnamomi* n. sp. (In Dutch). Mededeelingen van het Instituut voor Plantenziekten 54:41pp.

65. Ristaino J.B., Gumpertz M.L. (2000) New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*. Annual Review of Phytopathology 38:541-576. DOI: 10.1146/annurev.phyto.38.1.541.

66. Rizzo D.M., Garbelotto M., Hansen E.M. (2005) *Phytophthora ramorum*: Integrative research and management of an emerging pathogen in California and Oregon forests. Annual Review of Phytopathology 43:309-335. DOI: 10.1146/annurev.phyto.42.040803.140418.

67. Schmidt A.R., Dorfelt H., Perrichot V. (2008) *Palaeoanellus dimorphus* gen. et sp. nov. (Deuteromycotina): a Cretaceous predatory fungus. American Journal of Botany 95:1328-1334.

68. Schwingle B.W., Smith J.A., Blanchette R.A. (2007) *Phytophthora s*pecies associated with diseased woody ornamentals in Minnesota nurseries. Plant Disease 91:97-102.

69. Shearer B., Shea S., Fairman R. (1981) Infection of the stem and large roots of *Eucalyptus marginata* by *Phytophthora cinnamomi*. Australasian Plant Pathology 10:2-3. DOI: 10.1071/app9810002.

70. Shishkoff N. (2007) Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Disease 91:1245-1249. DOI: 10.1094/PDIS-91-10-1245.

71. Simpson A.G.B., Roger A.J. (2004) The real 'kingdoms' of eukaryotes. Current Biology 14:R693-R696.

72. Strullu-Derrien C., Kenrick P., Rioult J.P., Strullu D.G. (2010) Evidence of parasitic Oomycetes (Peronosporomycetes) infecting the stem cortex of the Carboniferous seed fern *Lyginopteris oldhamia*. Proceedings of the Royal Society B 278:675-680.

73. Stukenbrock E.H., Banke S., Javan-Nikkhah M., Mcdonald B.A. (2007) Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Biology and Evolution 24:398-411.

74. Taylor T.N., Krings M. (2005) Fossil microorganisms and land plants: Associations and interactions. Symbiosis 40:119-135.

75. Tucker C.M. (1931) Taxonomy of the genus *Phytophthora* de Bary., University of Missouri Agricultural Experiment Station Research Bulletin. pp. 207.

76. Tucker C.M., Milbrath J.A. (1942) Root rot of *Chamaecyparis* caused by a new species of *Phytophthora*. Mycologia 34:94-103.

77. Tukey J.W. (1991) The Philosophy of Multiple Comparisons. Statistical Science 6:100-116.

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

79. Weste G., Marks G.C. (1987) The biology of *Phytophthora cinnamomi* in Australasian forests. Annual Review of Phytopathology 25:207-229.

80. Wilcox W.F., Mircetich S.M. (1985) Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75:221-226.

81. Yakabe L.E., Blomquist C.L., Thomas S.L., MacDonald J.D. (2009) Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Plant Disease 93:883-890. DOI: 10.1094/PDIS-93-9-0883.

APPENDIX

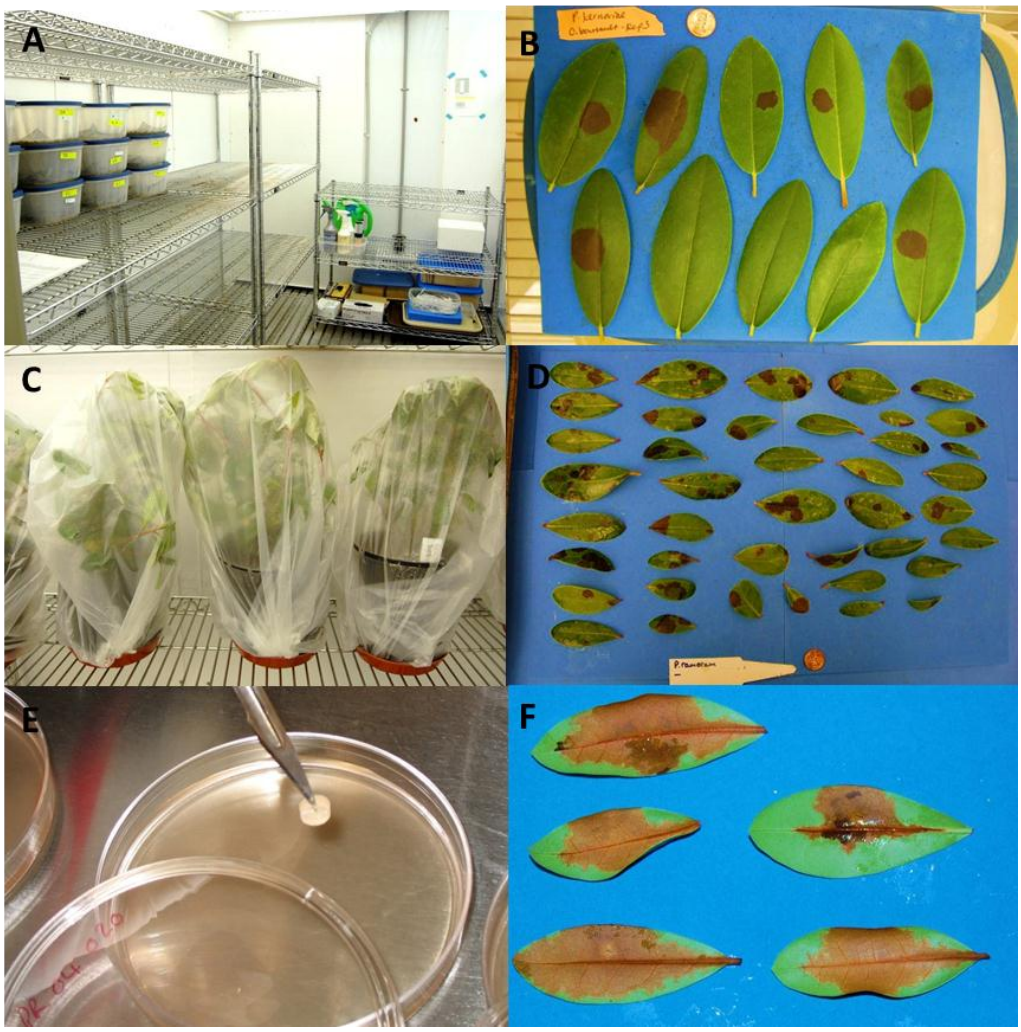

Figure 1. Illustration of methods. **A**, containment chamber with tubs of inoculated detached leaves, **B**, photographic analysis of detached leaves, **C**, whole plants incubated in bags following dip inoculation, **D**, photo analysis of leaves from whole plant inoculation, **E**, inoculation of V8 100 agar plates for temperature experiment, **F**, photo analysis of detached leaves following temperature experiment.

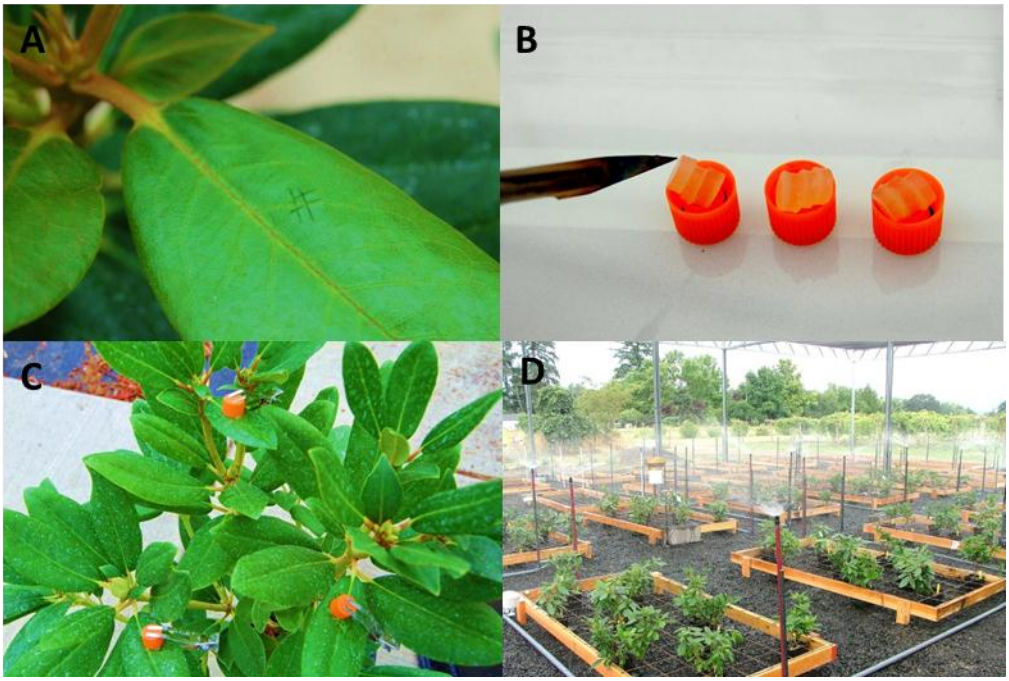

Figure 2. Illustration of field experiments. **A**, leaves were wounded with a scratch just to one side of the midrib, **B**, three mycelial agar plugs, one of each isolate of the same species were placed in Eppendorf caps with a drop of water, **C**, caps were clipped to the leaf wound sites, three subsamples per plant, **D**, plants were placed in a nursery like field setting on gravel with overhead irrigation and shade cloth. Each pair of wooden frames constitutes one block.

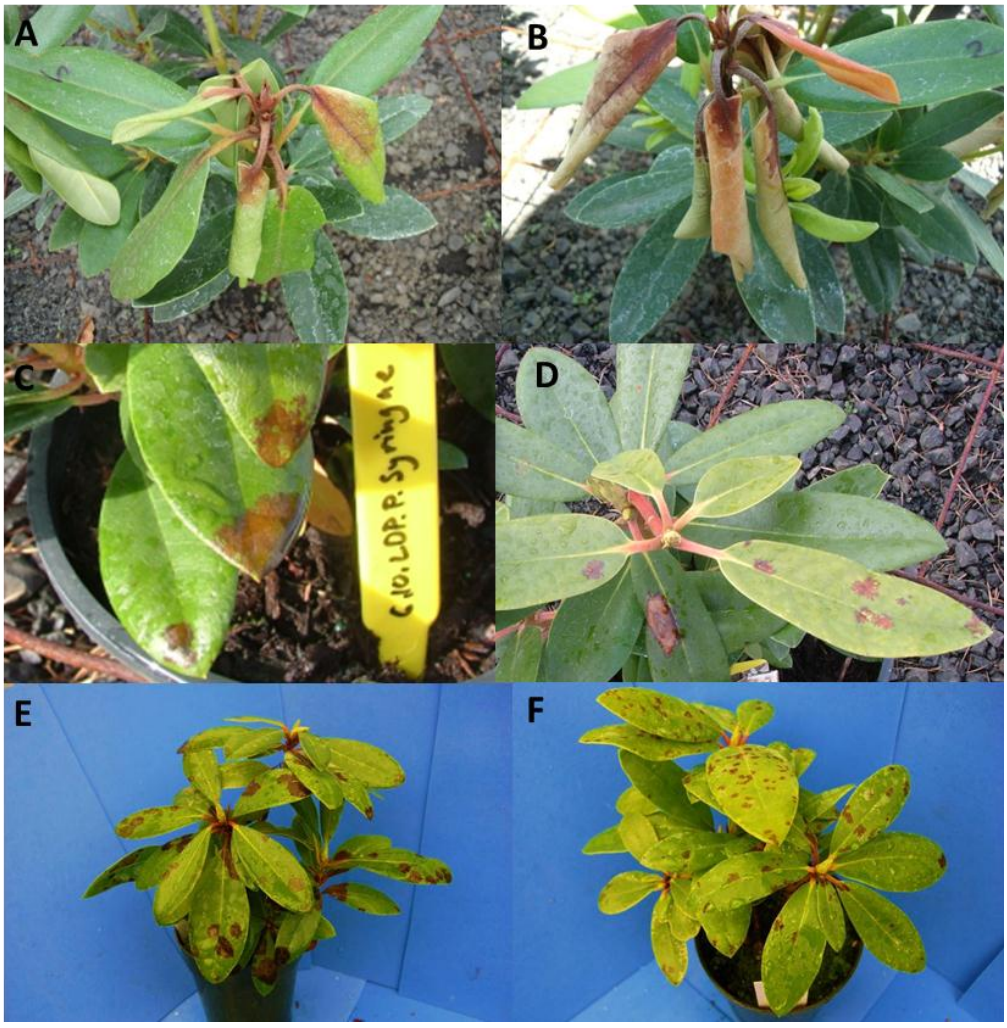

Figure 3. Photographs of results. **A**, and **B**, *P. nicotianae* disease spread on *Rhododendron* cv. 'Lee's dark purple' during 15°C experiment, 3 and 6 weeks post inoculation respectively. **C**, and **D**, *P. syringae* disease spread on *Rhododendron* cv. 'Lee's dark purple' during 8°C experiment, 6 weeks post inoculation. **E**, *P. ramorum* on *Rhododendron* cv. *catawbiense* 'Boursault', one week post dip inoculation, **F**, *P. kernoviae* on *Rhododendron* cv. *catawbiense* 'Boursault', one week post dip inoculation.