

ABSTRACT

LOYD, ANDREW LEUMAS. The Risks Associated with Irrigating Woody Ornamental Plants with *Phytophthora*-Infested Water. (Under the direction of Drs. K.L. Ivors and D.M. Benson).

Dissemination of *Phytophthora* in water has been well documented. In addition, the practice of reclaiming surface runoff water for irrigation can result in the recycling of *Phytophthora* propagules. The potential for *Phytophthora*-infested water to cause disease on susceptible, ornamental plants was evaluated. A two-year field study in which containerized *Rhododendron* and *Pieris* bait plants were irrigated with water naturally infested with various species of *Phytophthora* was conducted at two nurseries to assess the plant health risk associated with this practice. *Phytophthora* was consistently recovered from irrigation water at every sampling time, but was only detected on two bait plants (e.g. <1% of plants) over the timeframe of this study. Pathogenicity assays demonstrated that the most commonly recovered taxa in the irrigation water at these two nurseries, *P. hydropathica* and *P. taxon* PgChalmydo, caused foliar infection on leaves of *Rhododendron* and *Pieris*, but were unable to cause root rot on these same hosts. Overall, *Phytophthora*-infested irrigation water did not act as a primary source of infection on *Rhododendron* and *Pieris* even though foliar pathogenic species of *Phytophthora* were present in irrigation water.

The Risks Associated with Irrigating Woody Ornamental Plants with *Phytophthora*-Infested
Water

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DEDICATION

To my parents, Mike and Kathy Loyd.

BIOGRAPHY

Andrew Leumas Loyd was born and raised in Baton Rouge, LA. He received his B.S. degree in Plant and Soils Systems from Louisiana State University in Baton Rouge, LA in the Spring of 2010. As a student at LSU, he became fascinated in the natural sciences and was a member of the Louisiana Naturalists Club. Entomology was his favorite subject in undergraduate studies, but chose plant pathology as a graduate program. He entered into his Master of Science (M.S.) program at North Carolina State University in Raleigh, NC in Fall of 2010. During his tenure as an M.S. student, Andrew was a radio DJ at the college radio station WKNC 88.1 FM. He hosted an old-time, folk music morning radio program entitled “The Church of Bluegrass and the Truer Sounds” under the handle “The Cosmic Cowboy”. In his free time Andrew enjoys playing a myriad of instruments including: banjo, guitar, fiddle, dulcimer, spoons, kazoo, accordion, and ukulele.

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TABLE OF CONTENTS

List of Tables	vii
List of Figures.....	viii

Chapter 1. Comprehensive literature review on recycling irrigation water and *Phytophthora*

ABSTRACT	1
RECYCLING IRRIGATION WATER.....	2
THE GENUS <i>PHYTOPHTHORA</i>	5
<i>PHYTOPHTHORA</i> SPP. DETECTED IN WATER	9
METHODS FOR DETECTING <i>PHYTOPHTHORA</i> IN WATER	11
Water filtration	12
Baiting	13
Immunodetection.....	13
Molecular detection.....	14
MANAGING THE WATERBORNE STAGE OF <i>PHYTOPHTHORA</i>	15
RESEARCH OBJECTIVES	18
Observing the impact of <i>Phytophthora</i> -infested water on susceptible plants ..	18
Conducting pathogenicity assays with <i>Phytophthora</i> taxa commonly recovered from irrigation water.....	19
LITERATURE CITED.....	21

Chapter 2. The risks associated with irrigating woody ornamental plants with *Phytophthora*-infested water

ABSTRACT	28
INTRODUCTION	29
MATERIALS AND METHODS	30
RESULTS	40
DISCUSSION.....	46
ACKNOWLEDGEMENTS	52
LITERATURE CITED.....	53

APPENDICES

Appendix A. DNA sequences and deposited GenBank sequence accession nos. ..	68
Appendix B. Experimental Designs and water properties.....	75

LIST OF TABLES

Chapter 2. The risks associated with irrigating woody ornamental plants with *Phytophthora*-infested water.

Table 2.1. <i>Phytophthora</i> propagule concentrations in irrigation water at two ornamental nurseries in 2011 and 2012	58
Table 2.2. <i>Phytophthora</i> isolates recovered from irrigation water at two ornamental nurseries in 2011 and 2012	59
Table 2.3. Morphological characteristics of taxa recovered from irrigation water and respective GenBank accession numbers	60
Table 2.4. Assessment of four isolates representing unique taxa of <i>Phytophthora</i> recovered from irrigation water to cause root rot on <i>Rhododendron</i>	61
Table 2.5. Recovery of <i>Phytophthora</i> isolates with leaf baits and direct root plating from roots of inoculated, asymptomatic plants.....	62
Table 2.6. Assessment of four isolates representing unique taxa of <i>Phytophthora</i> recovered from irrigation water to cause foliar infection of <i>Rhododendron</i> and <i>Pieris</i>	63
Table 2.7. Average lesion area of plants inoculated with three unique isolates of <i>Phytophthora</i> using the Tjosvold foliar clip inoculation method	64
Table 2.8. Average lesion area of wounded sites of <i>Rhododendron</i> leaves in a detached leaf assay.....	65

LIST OF FIGURES

Chapter 2. The risks associated with irrigating woody ornamental plants with *Phytophthora*-infested water.

- Fig. 1. Morphological characteristics of representative isolates of the five most commonly recovered taxa from this study 66
- Fig. 2. Foliar symptoms observed in zoospore and mycelium spray inoculation method, reisolation of *Phytophthora* from lesion, and viability of rice grain inoculum..... 67

Chapter 1. Comprehensive literature review on recycling irrigation water and *Phytophthora*

ABSTRACT

Drought, urban competition for water resources, and legislation regarding water use are causing plant producers to modify their irrigation management strategies. To better conserve water, preserve the surrounding ecosystems and groundwater, and conform to stringent water regulations, plant producers are beginning to recycle or reclaim surface water for irrigation purposes. The major benefits of recycling water include conservation of water and reduction of pesticide and nutrient runoff, but the major deterrent is the potential for the dispersal of plant pathogens in water (51). The two most common genera of oomycete plant pathogens commonly found in water are *Pythium* and *Phytophthora* (5). Members within the genus *Phytophthora* have been described as some of the most economically important pathogens in agriculture and forestry due to their ability to cause root rots, crown rots, foliar blights, fruit rots, and damping-off diseases of a wide array of hosts (20). Many detection assays have been developed to detect *Phytophthora* in irrigation water, including filtration, baiting, immunodetection (e.g. ELISA), and molecular methods. The aquatic stage of *Phytophthora* is commonly managed with rapid filtration coupled with chlorination, but other tactics include application of chemicals (e.g. algacides), ozonation, copper ionization, and ultra-violet radiation (39). To evaluate the risks associated with irrigating ornamental crops with water containing *Phytophthora*, *in situ* detection and pathogenicity assays with taxa commonly recovered from water were conducted on *Rhododendron* sp. and *Pieris japonica*.

RECYCLING IRRIGATION WATER AT ORNAMENTAL NURSERIES

The estimated wholesale value of ornamental crops in the United States was \$9.16 billion in 2003. North Carolina is a leading producer of ornamental crops, accounting for 8.5% of the production nationally (17). Plants belonging to numerous different families are often cultivated within an individual ornamental nursery, so plant health management can be quite complex. Ornamental plants are typically grown in containers with soilless media as the substrate. A typical substrate in the southeastern U.S. would contain primarily pine bark along with lime and micronutrient amendments. Pine bark medium is high in porosity and plants grown in this substrate require frequent irrigation in summer months due to high rates of evapotranspiration. As irrigation volume increases, the amount of runoff also increases, which can carry fertilizer salts and pesticide residue; this results in pollution of the adjacent natural ecosystems.

The Clean Water Act set stringent standards for water management in order to reduce the amount of pollution from fertilizers and pesticides into natural systems (66). Skimina (66) recommended that recycling irrigation water was the most efficient means of conforming to the regulatory standards. Kabashima (49) documented irrigation water usage at two ornamental nurseries in the mid 1980s as the Ground Water Protection Program and California Safe Drinking Water and Toxic Enforcement Act were beginning to be implemented. Nursery I voluntarily installed a water recycling system to reduce costs, and Nursery II was forced to install a recycling system to comply with government

regulations (49). Within 4 years, Nursery I and II were able to reduce their annual water usage (AWU) by 70 and 46%, respectively (49).

Many growers use a retention basin to collect total runoff and then utilize this water as the main source for irrigation. Typically, excess irrigation water is channeled through French drains, canals, pipes or by passive movement downhill into a retention basin where the water is sometimes mixed with an additional source (e.g. well, river, stream, lake, etc) (11). The recycled water is then filtered and sometimes also sanitized before utilized for irrigation (68). It is estimated that 35% of water applied as irrigation can be captured and reused, significantly reducing the cost and reliance on such a limiting resource (70). Many issues can arise from the practice of recycling water including changes in water chemistry, redistribution of water-soluble pesticides, weed seed dispersal, and pathogen spread and survival (51, 52, 75).

Fox *et al.* (23) found that total dissolved salts increased due to recycling nursery effluent, which resulted in foliar salt damage on susceptible plants such as *Rhododendron* spp., *Fagus grandifolia* and *Tsuga canadensis* (6, 23). Water with concentrated salts can be mitigated by diluting the water with a potable water source, and furthermore, eliminating its contact with the foliage by utilizing drip irrigation as opposed to overhead irrigation (23). When surface water is recycled, the water is subjected to fluctuations in pH from the time of application to the time of redistribution. If the pH of the water is too low or high, direct and indirect plant health problems could arise. For example, irrigation water with a low pH (i.e. <5) applied to a containerized plant can quickly dissolve dolomitic limestone as the water percolates through the container, possibly resulting in

calcium and magnesium deficiencies (75). Whitcomb (75) suggested using less soluble forms of limestone with large particle sizes to mitigate the effects of low water pH. Using water with high pH (i.e. >7.5) can result in foliar damage due to high levels of bicarbonates present in the water (75). Acid injections can successfully lower the pH of the water applied to the crop.

Water soluble pesticides applied at a nursery that reclaims surface water can potentially result in the future redistribution of the pesticide onto non-target plants exposed to this water through irrigation (75). For example, ornamental nurseries growing containerized plants often apply water-soluble herbicides to reduce weed pressure. Keese *et al.* (50) found that the potential for herbicide-residues to be captured in a retention basin was greater when plants were grown on ground cloth compared to gravel. Weed problems are inevitable in an ornamental plant production setting. Weeds vigorously produce vegetative and reproductive propagules that compete for nutrients, light and water with desired ornamental crops. In addition, large numbers of weeds can impact the local microclimate by reducing air movement between plants. Evolutionarily, weeds are successful because of their abundant production of seed and their long-distance seed dispersal mechanisms. At nurseries that recycle irrigation water, seed can be carried in runoff, held in a retention basin, and potentially redistributed back to the crop. For example, prostrate spurge has small seeds that can be carried in runoff water to a retention basin and remain viable in the basin for an extended period of time (75). These seeds are small enough to pass through an orifice of a sprinkler head, and thus be redistributed across the irrigation zone (75). To contain weed seeds, filtration could be

implemented in the recycling system, where the water is filtered through fine sand (0.15-0.35 mm pores) before applied to the crop (68). This treatment is often used for removal of pathogen propagules from water, but is also a viable means for removing weed seeds (75).

The dispersal of waterborne plant pathogens associated with recycling water is the major disincentive for implementing an irrigation water recycling regime in crop production (11). Irrigation water typically comes in contact with plants, plant debris, organic material and soil. The effluent can then serve as an inoculum reservoir with the potential to disperse pathogens by way of irrigation (1, 8, 26, 39, 62, 65, 72).

Plant pathogen-infested water used as a source of irrigation was first noted as a plant health risk early in the last century (8). Bewley and Buddin (1921) observed widespread damping off of tomato seedlings in a glass house setting, and investigated the water as a possible source of inoculum. *Phytophthora cryptogea* was identified as the causal agent, and the irrigation water was identified as the source of infective propagules (i.e. zoospores) (8). Since the report of Bewley and Buddin, all major groups of plant pathogens have been detected in irrigation water including nematodes (39, 67), bacteria (13, 39, 67), fungi (8, 16, 39, 71), and viruses (39), but the zoosporic “fungi” are the most commonly detected plant pathogens in water (1, 5, 8, 9, 11, 12, 25-27, 32, 37-39, 45, 48, 51, 54, 58, 65, 68, 69, 74). These “fungal-like” organisms are well adapted to aquatic ecosystems due to the production of abundant motile zoospores, previously referred to as “swarm spores” (44). The two most important genera of plant pathogens within this

group are *Pythium* and *Phytophthora* (5). This review will focus on summarizing the major findings of *Phytophthora* in water.

THE GENUS *PHYTOPHTHORA*

The genus *Phytophthora* has been described as one of the most economically important pests in agriculture and forestry (20), containing numerous species of global significance, such as *Phytophthora infestans*, the causal agent of the Irish potato famine, *Phytophthora ramorum*, the causal agent of sudden oak death, and *Phytophthora cinnamomi*, the causal agent of Eucalypt dieback. Members of this genus can cause root rots, crown rots, fruit rots, pod rots, foliar blights and diebacks of a wide array of hosts representing many plant families (20). The host specificity of various species of *Phytophthora* can range from broad to very narrow (20). Host range varies from host-specific species as in *Phytophthora idaei*, a pathogen of *Rubus*, to a very broad host range with thousands of host species as in *P. cinnamomi* (20). As a genus, *Phytophthora* is usually associated colonizing living plant tissue; however, *P. gonapodyides* has been reported to colonize living and dead leaf tissue in streams, implying saprobic behavior (20, 30, 48). Although the ecosystem function of *P. gonapodyides* is still unclear, it is ubiquitous in forest streams and has been documented as a weak pathogen of oaks (30, 48).

As of 2011, the genus *Phytophthora* was comprised of 114 described species (31). The taxonomic status of *Phytophthora* has changed many times since the original classification by Anton de Bary in 1876 (20). Historically, *Phytophthora* was placed in the class phycomycete (along with zygomycetes) within the subclass oomycete, and

collectively in the kingdom Fungi (11). The current taxonomic status places both genera in the kingdom Stramenopila, which makes them more related to golden-brown algae (53). Members of the genus *Phytophthora* can produce many types of propagules, including sporangia, zoospores, oospores, chlamydospores and hyphal swellings (20). Juxtaposing true fungi and *Phytophthora* reveals major biochemical, genetic, and biological differences between the two groups. The cell walls of *Phytophthora* and other members of the Oomycota are composed of β -glucan and cellulose, as opposed to the chitin cell walls of true fungi (20); oomycetes do not synthesize sterols, while fungi do (11); *Phytophthora* mainly exists in a diploid state, while fungi exist predominately in a haploid state (20); although some fungi, primarily in the phylum Chytridiomycota, produce motile zoospores with a single opisthokont, whiplash-like flagellum, *Phytophthora* produces biflagellate, motile zoospores with no cell wall and a posterior whiplash-like flagellum and an anterior tinsel flagellum similar to that of the heterokont algae (20, 63); the flagella allow the zoospore to swim.

The zoospore accounts for 95% of propagules of *Phytophthora* recovered from irrigation water (14) because it has the capability of swimming, while other propagules (e.g. mycelium, chlamydospores, etc) tend to sink to the bottom of water (71). Zoospores exhibit a negative geotropic behavior by swimming against the force of gravity (20), hence the highest species diversity is found in the upper layer (1-1.5 m) of water (12). Zoospores swim at a rate of about 150 micrometers per second in a random, corkscrew-like pattern, and can be carried in water for long distances (14). Tomato seedlings were infected with *Phytophthora nicotianae* 50 meters upstream from a source of inoculum

and *P. ramorum* has been baited with rhododendron leaves approximately 1km from a known inoculum source (44). Zoospores of *P. infestans* have been shown to survive in water, especially with soil, for periods of 0-16 and 0-20 days in nonshaded and shaded conditions, respectively (62). In addition to swimming long distances, zoospores can respond to environmental stimuli, such as chemical gradients, electrical currents, oxygen, and light (44). Plant root exudates released from wounds or natural openings create chemical and electrical gradients in the soil solution, which acts as an attractant for a swimming zoospore (44). Several studies have shown zoospore concentration thresholds associated with plant infections (7, 55), where infection does not occur until a certain concentration of zoospores is present. Before zoospores infect plant tissue, the flagella are shed, adhesive molecules are released and the zoospore encysts on host tissue (20).

In a production system, *Phytophthora* spp. can exist as chlamydospores in the soil, sporangia living on host material, oospores in dead roots in media or soil, or as motile zoospores in water (20); all stages can pose a direct risk to plant health. How does *Phytophthora* enter an ornamental production system? Parke and Grünwald (60) identified five potential contamination points within a nursery, which include incoming plants, potting media, water, pots and bare ground. Once *Phytophthora* has entered the production system, susceptible plants can become infected, ultimately increasing the inoculum density. By reclaiming runoff water in a retention basin, the grower risks recycling *Phytophthora* and other waterborne pathogens, potentially redistributing *Phytophthora* across the irrigation zone (39). Parke and Grünwald (60) suggest disinfecting irrigation water with an approved method or to use deep wells or municipal

sources of water to avoid waterborne pathogens. In addition, preventing standing water by avoiding overwatering, promoting drainage and raising plants off the ground can reduce the potential for splash dispersal (60).

Phytophthora root rot is one the most commonly diagnosed diseases of ornamental plants (17). When scouting for disease, it is important to understand the biology of the pathogen and what environmental conditions are most conducive for disease development. For example, in the case of Phytophthora root rot, where water is essential for disease development, diseased plants show up in “hot spots” where drainage may be poor or tends to accumulate, or where plants are overwatered (21). Poor drainage directly around the root zone is often a result of poor cultural practices that often leads to root rot. Cultural practices that yield poor drainage, and ultimately a saturated root zone, include overwatering, placing plants at low points in the nursery, using broken down media with few macropores, and maintaining root-bound plants in pots. Phytophthora root rot is often more severe and widespread in areas with poor drainage (i.e. saturated root zone). Low matric potential of soil (i.e. saturated root zone) enhances disease development (24); this is because matric potentials near 0 kPa stimulate zoospore release from sporangia, resulting in many secondary infections (64). Shew (64) showed that saturating the soil of tobacco seedlings inoculated with *Phytophthora parasitica* var. *nicotianae* for 24 hr resulted in higher disease incidence when compared to the non-saturated control. Feld *et al.* (21) demonstrated that furrow and drip irrigation in citrus groves saturate the root zone, and as a result, created a conducive environment for root infection by the soilborne pathogen *P. nicotianae* (21). By allowing a drying period

between irrigation events, disease incidence was lessened significantly compared to the treatment with no drying period (21). Controlling the matric potential of the rhizosphere through managing irrigation duration could be an effective way to limit the amount of disease in a field setting.

***PHYTOPHTHORA* SPP. DETECTED IN WATER**

Irrigation is a common input among all types of ornamental production settings. While water is considered a supplement in field-grown ornamentals, it is vital for the health of greenhouse and containerized ornamentals (10). This is because the volume of water needed for optimal plant productivity is limited by the size of the container; the container must be well drained, resulting in the need for high volumes of water and frequent application (10). As an example, Florida nurseries growing containerized plants apply approximately 1.42 to 3.05 m annually (10). Approximately 75% of ornamental crops grown in the U.S. are grown in containers, which mean susceptible, ornamental crops are irrigated frequently with large volumes of water. If this water is infested with *Phytophthora* spp., susceptible plants could potentially be at risk of infection (10).

Over 25 *Phytophthora* spp. have been reported in irrigation water, including: *Phytophthora cactorum*, *P. cambivora*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *P. humicola*, *P. hydropathica*, *P. inundata*, *P. irrigata*, *P. megasperma*, *P. nicotianae* (=parasitica), *P. palmivora*, *P. pini*, *P. polonica*, *P. pseudosyringae*, *P. ramorum*, *P. tropicalis*, and many other uncharacterized *Phytophthora* spp. (1, 8, 12, 26, 34, 35, 37, 38, 41, 42, 48, 51, 52,

54, 58, 71, 73). Ghimire *et al.* (26) found 10 different species of *Phytophthora* in irrigation water over a two year period at an ornamental nursery in Virginia that recycled total runoff as a source of irrigation water. The highest concentration of *Phytophthora* propagules occurred at the point where runoff entered the retention basin and the lowest concentration occurred at the furthest point away from the point of runoff entry. The authors suggested that locating the pump inlet pipe at the farthest point away from the runoff entry could reduce the amount of inoculum redistributed through irrigation (26). Bush and Hong (12) also suggested that placing baits at depths of 1-1.5 m below the surface of the water detected a higher diversity of *Phytophthora* spp. when compared to surface baiting. Bush and Hong (12) detected seven different species in their study and concentrations ranging from 0-85 colony forming units(CFUs)/liter which was lower than previous studies (52). In a similar study, MacDonald *et al.* found *Phytophthora* CFUs/liter ranging from 0-400 (52).

Many nurserymen in North Carolina and other places throughout the U.S. utilize natural bodies of water as irrigation sources. A 'natural' body of water refers to a river, stream, spring or creek that drains from a watershed. With the national survey program for *Phytophthora ramorum*, many new species have been discovered in natural waterways. Tjosvold *et al.* (73) used pear baits to monitor a stream for *P. ramorum* in Santa Cruz County, CA and found that *P. ramorum* detection was positively correlated with periods of lowest daily temperature and highest rainfall, which occurred in the winter and spring months (73). In comparison, Bush *et al.* (12) surveyed nursery irrigation water effluent with baiting and filtration methods and found that the highest

detection of *Phytophthora* spp. correlated at times when the nursery had the most plant trading (i.e. the summer months). The authors speculated that many of the species were transient inhabitants living on plants purchased from outside propagators (12).

METHODS FOR DETECTING *PHYTOPHTHORA* SPP. IN WATER

When *Phytophthora* is detected in irrigation water, it is important to understand the limitations, or threshold, of the detection assay used (39, 68). The detection threshold is the amount of infectious propagules of a pathogen that can be detected by a particular sampling method (39). Once a detection threshold is determined, a biological threshold, or the amount of viable inoculum present related to the disease potential associated with inoculum concentration, must be assessed (39, 68). Determining detection and biological thresholds can help make projections on disease potential and help guide nurserymen if water treatment is necessary (68). In 1981 Hallet and Dick (29) proposed four general methods for detecting fungi in water, including a) detection on naturally occurring substrates, b) direct propagule trapping, c) baiting, and d) water filtration. Each detection assay differs in the ability to accurately quantify the amount of fungal propagules (29). Detection with naturally occurring substrates can be used when a single substrate is specific to a single pathogen, but this detection assay lacks the ability to estimate time of infection as well as type and quantity of infective propagule (11, 29). Propagule trapping can be used to detect spores that have distinctive morphologies, but this assay is not fit for spores that lack distinctive morphological features such as oomycete zoospores (29).

Baiting and water filtration are the most common assays used to detect oomycetes in water (11).

Water Filtration. The major advantage of using a filtration method to detect *Phytophthora* in irrigation water is the ability to estimate the propagule concentration. MacDonald *et al.* (52) developed a protocol for filtering nursery irrigation effluent to quantify pythiaceae fungi in 1994. This method has been cited in over fifty publications and been adopted and modified as the primary detection method for the national *P. ramorum* survey. In general, this method consists of six steps including: 1) water collection of usually ≥ 500 mL, 2) processing of smaller volumes ≤ 200 mL, 3) water filtration through a membranous filter with pore sizes < 10 μm , 4) plating of the filter topside down on selective media (47), 5) incubation for 24 hr, removal of filter, followed by additional incubation, and 6) counting of pythiaceae colonies to calculate colony forming units (CFUs) present by volume of water. Hong *et al.* (2003) (36) evaluated the efficiency of different filters for this method based on recovery precision and accuracy and speed of filtration. The Durapore5 filter was the most sensitive for detection of pythiaceae fungi in irrigation water, as well as having the quickest filtering time of less than 10 seconds (36). The major limitation to the filtration assay is that it is time sensitive, and water samples must be processed within 24 h to obtain accurate estimations.

Baiting. Baiting for *Phytophthora* with plant tissue, such as fresh fruit or detached leaves, or by using whole 'trap plants', is a viable means to recover many species of *Phytophthora* from water or potting media (22, 39). Ferguson and Jeffers (22)

showed that multiple species of *Phytophthora* could be detected by baiting potting media with camellia leaves and shore juniper needles. Since camellia leaves recovered more contaminants, (e.g. *Pythium* spp.), and the two leaf types differed in selectivity to certain species of *Phytophthora*, they concluded that baiting with both camellia and shore juniper leaves simultaneously was the most effective detection method (22). The selectivity of bait should always be evaluated to ensure that it can detect a diversity of *Phytophthora* species and discriminate against contaminants (39). The major limitations with using baits to detect waterborne pathogens are the inevitability of recovering fast growing contaminants such as *Pythium* and bacteria, and the inability to quantify the amount of propagules.

Immunodetection. Immunology utilizes antibodies that are specific to a particular antigen and proves to be a viable diagnostic tool, however the accuracy and ability to detect *Phytophthora* present in water using immunological methods is questionable. Pettitt *et al.* (61) assessed the performance of various immunological assays for detecting *Phytophthora* and *Pythium* in irrigation water and found that the zoospore trapping immunoassay (ZTI) was the most sensitive detection assay for *Phytophthora* and *Pythium* present in water, and baiting and immunodiagnostic dipstick assays were the most consistent *in situ* assays. Commercial ELISA kits have proven to be a viable means of detecting *Phytophthora* at the genus level (2). Although ELISA kits can detect *Phytophthora*, Ali-Shtayeh *et al.* (2) demonstrated that there is a degree of cross reaction with some species of *Pythium*. All *Phytophthora* species tested showed a positive reaction with the *Phytophthora* ELISA kits, and negative reaction with *Pythium* ELISA

kits (2). Comparatively, some of the *Pythium* species tested showed cross-reactivity with *Phytophthora* ELISA kits, and all tested positive with the *Pythium* ELISA kits (2). Thus, the major concern with using ELISA kits to detect *Phytophthora* in water is false-positive diagnosis.

Molecular Detection. The quickest and most reliable means of detecting species of *Phytophthora* present in water is by sequencing loci of genomic DNA (39). DNA can be extracted directly from tissue obtained in pure culture, environmental samples (e.g. soil, water, etc.), or from plant material (i.e., *P. ramorum* survey protocol) (39). The polymerase chain reaction (PCR) is used to amplify specific regions (e.g. ITS I & II) the sample DNA. This process creates millions of copies of the locus, and can be sequenced fairly readily (39). The Phytophthora Database (www.phytophthoradb.org) and GenBank (<http://blast.ncbi.nlm.nih.gov>) are curated by the scientific community and are used to compare sequenced loci to a library of thousands of sequences (59, 76). These databases are utilized for sequence-based identification, comparing similarity between queried and deposited sequences. Sequencing DNA is the fastest method for identifying an unknown organism, and this method accurately identifies samples to the level of species or genera (39). For the best resolution of detection and identification, multiple methods should be used.

MANAGING THE WATERBORNE STAGE OF *PHYTOPHTHORA*

Daughtrey and Benson (17) state that for effective management of plant disease, an integration of these three principal factors need to occur: the initial plant material must be

disease-free, the environment where plants are grown should not favor pathogens, and if problems do arise, proper diagnoses and management should be expedited (17).

Nurserymen should take precautionary measures to ensure plant material, tools and land within a production facility or field are pathogen free prior to production.

Avoidance of the pathogen is the most parsimonious way to lower the risk of disease. The literature suggests that well water is most often found free of *Phytophthora* and *Pythium* (70). Placing susceptible plant species in zones irrigated with well water would reduce the disease-risk for these plants. With the majority of nurseries exceeding many acres, it is hard to rely solely on well water for production. Recycling water is the most efficient way to conserve water, but it is also a good way to preserve pathogens.

Water treatment strategies are being adopted in the ornamental nursery industry with the goal of eliminating *Phytophthora* and other pathogens from the water. Many considerations should be accounted for when evaluating methods for sanitizing water, such as the type of pathogen propagule present in the water, the short- and long-term economic feasibility of the method, the impact of the strategy on other beneficial organisms, or the potential for the pathogen population to become resistant to the particular strategy. Many methods for water disinfection are available, but all of them have different pros and cons (11).

Members within the genus *Phytophthora* are pleomorphic and exist in many different forms including zoospores, sporangia, cysts, chlamydozoospores, oospores, and mycelium. The zoospore is the only motile stage of *Phytophthora*, and thus, the stage of most importance in irrigation source water. Chlamydozoospores, oospores, cysts, sporangia

and mycelial fragments usually settle to the bottom of a pond (71). A float pump is a good way to avoid these types of propagules; however, zoospores are negatively geotropic (20), meaning they swim against the pull of gravity, and despite using a float pump this propagule still remains a concern in irrigation water. A nurseryman must choose a management strategy that is efficient in the elimination of zoospores. Water management strategies to help reduce the risk of zoospores include mechanical separation, chemical application, UV radiation, and chlorination.

In Europe, the most common practice to mechanically separate plant pathogens from irrigation water is slow sand filtration (SSF). This method has been used for over 100 years for disinfestation of municipal water, and is a reliable way to remove fungi, fungal-like organisms, bacteria, and nematodes and reduce the titer of viruses such as ToMV (68). Filtration is most efficient in removing plant pathogenic propagules when the sand is fine (0.15-0.35mm) compared to coarse (0.5-1.6mm), and when water is filtered through the sand at slower rates (100L/m²) compared to faster rates (300L/m²) (68). Slow sand filtration is a physical separation of pathogen propagules from water, but in addition to the physical separation, communities of beneficial microflora living on the surface layer of the sand called the “schmutzdecke” help suppress some types of plant pathogen propagules in the process of filtration (68). For large scale producers that require high volumes of water for irrigation, SSF could be too slow.. Filtration research has focused on enhancing the efficiency of the mechanical separation by testing different substrates including crushed brick, sand and clay at faster flow rates (56), as well as

investigating the ability to enhance the biological community in the “schmutzdecke” layer with antagonistic bacteria such as *Bacillus* and *Pseudomonas* species (18).

Chemical treatments have proven to be effective against many waterborne plant pathogens. Applying chemicals to water can have detrimental effects on the environment, phytotoxic effects on plants, and health consequences on the applicator. Algaecides have been shown to be effective against *Phytophthora* in laboratory conditions, but could have environmental and plant health consequences if utilized in the field (15, 28). The most common active ingredient in commercial algaecides is CuSO_4 , which could result in copper toxicity on plants, as well as impacting non-target organisms in the aquatic ecosystem.

The most widespread water treatment strategy employed at current in the woody ornamental industry is chlorination, which was adapted from a method primarily utilized for municipal water treatment (40). Treatments can take the form of gas, liquid, or solid states of chlorine (68). Despite the form of chlorine utilized in the water treatment, when the chlorine (e.g. sodium hypochlorite) reacts with water, hypochlorous acid is formed, which actively disrupts the metabolism of microbes (68). Several site-specific biological and chemical factors can impact the efficacy of chlorination. The effectiveness of chlorine is controlled by pH, contact time, and dose. The pH of the solution is critical for how much hypochlorous acid is produced, and in turn how effective the treatment is in reducing plant pathogens in water. Chlorination is most active in reducing microbes at a pH between 5-6. When water is at or above pH 8.5, the chlorination is ineffective in controlling microbes (68). The amount of organic material in solution at the time of

chlorination also impacts efficacy, as soil and other organic particulates bind to free chlorine, and prohibits pathogen exposure to the level of chlorine needed for total mortality (i.e. 2 mg/L) (40). Filtration of water before chlorination treatment is recommended for removing soil particles and other organic particulates that would bind free chlorine (40, 68). Hong *et al.* (40) tested the effects of different chlorine concentrations on the mortality of zoospores and determined that total mortality could be achieved with 2 mg/L of free available chlorine. Phytotoxic effects on woody ornamental plants at concentrations of 2 mg/L were nil (40).

RESEARCH OBJECTIVES

The overall goal of this research was to identify the plant health risks associated with *Phytophthora* in irrigation water within a commercial ornamental nursery setting. This research was partitioned into two objectives: 1) to observe the impact of using water naturally infested with *Phytophthora* for irrigation on ornamental plant hosts, and 2) to conduct pathogenicity assays on the same hosts with *Phytophthora* taxa commonly recovered from that irrigation water in order to elucidate their potential for inciting disease.

Observing the impact of *Phytophthora*-infested water on susceptible plants.

Growers source irrigation water from a variety of water bodies including springs, rivers, lakes, retention basins, and water tanks. Although *Phytophthora* is commonly detected in irrigation water, the risk associated with using *Phytophthora*-infested water is unknown.

To demonstrate the risk associated with using *Phytophthora*-infested water, susceptible plants were placed at two different nurseries that had *Phytophthora* present in their irrigation water, and plants were irrigated over the summer growing season during 2011 and 2012. Water and plant material were then monitored and sampled over time.

As mentioned earlier, poor cultural practices also enhance the risk associated with contaminated irrigation water by creating a more conducive rhizosphere for *Phytophthora* infection. The effects associated with saturating the root zones of susceptible nursery plants irrigated with infested water were also evaluated. To demonstrate the effects of root zone saturation, susceptible plants were flooded for a period of 48 hours after normal irrigation using water naturally infested with *Phytophthora*.

Conducting pathogenicity assays with *Phytophthora* taxa commonly recovered from irrigation water. New species of *Phytophthora* recovered from aquatic environments are continuously identified through surveys, but data on their pathogenicity and host range are limited. For this very reason, research on the pathogenicity of *Phytophthora* species detected in water should be conducted to better understand the potential risks that they pose to ornamental nursery plants, as well as to plants growing in natural environments. In this study, isolates representing the most frequently recovered *Phytophthora* taxa from nursery water were screened for pathogenicity on commonly produced ornamental plants.

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Chapter 2. The risks associated with irrigating woody ornamental plants with *Phytophthora*-infested water.

ABSTRACT

Phytophthora species are water-borne plant pathogens that can be disseminated in streams and rivers, as well as in reclaimed irrigation water. *Rhododendron* and *Pieris* ‘trap’ plants at two commercial nurseries were irrigated with water naturally infested with *Phytophthora* during the 2011 and 2012 growing seasons to assess the risk of disease. *Phytophthora* was consistently recovered from water samples at every collection time, but was only detected on two of the 384 trap plants during the two growing seasons (i.e. <1% of plants). Pathogenicity assays proved that *P. hydrophatica* and *P. taxon* PgChalmydo, commonly recovered taxa in the irrigation water at both nurseries, were foliar pathogens of *Rhododendron* and *Pieris*, however neither species was able to cause root rot on these same hosts. Overall, *Phytophthora*-infested irrigation water did not act as a primary source of infection on *Rhododendron* and *Pieris* even though foliar pathogenic species of *Phytophthora* were present in irrigation water.

Additional keywords: retention basin, *Phytophthora pini*, *Phytophthora hydrophatica*, *Phytophthora taxon* PgChlamydo, waterborne pathogen

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Drought, urban competition for water sources, and legislation regarding water use are common incentives for plant producers to modify their irrigation strategies. To conserve water, preserve the surrounding ecosystems and groundwater from pesticide and nutrient runoff, and conform to stringent water regulations, growers frequently recycle or reclaim surface water for irrigation purposes. The benefits of recycling water are obvious, but the major deterrent is the potential for spreading waterborne plant pathogens and pests (29). The two most economically important genera of plant pathogens commonly found in water are *Pythium* Pringsh. and *Phytophthora* de Bary (3). Although *Phytophthora* is commonly detected in irrigation water, little is known of the risk associated with irrigating plants using water naturally infested with *Phytophthora* species. Tjosvold (42) irrigated hybrid rhododendron with stream water naturally infested with *P. ramorum* for one year, but did not detect any infected plants. Similarly, Werres and Wagner (48), found low to no infection of rhododendron by *P. ramorum* in simulated recirculation systems that had been infested with propagules of *P. ramorum*.

Concentration of *Phytophthora* propagules within an irrigation source fluctuates throughout a growing season (10); the risk of infection related to inoculum concentration of *Phytophthora* taxa present in irrigation water has not been demonstrated. A level of plant health risk is assumed when a nurseryman utilizes *Phytophthora*-infested water as a source of irrigation. Risk can be estimated based on percent infection of susceptible plants from *Phytophthora* spp. present in irrigation water when *Phytophthora*-infested water is used as the source for irrigation. To evaluate the risk associated with irrigating ornamental nursery plants with water naturally infested with species of *Phytophthora*, we

placed host ‘trap’ plants representing susceptible cultivars of *Rhododendron* sp. and *Pieris japonica* at two different commercial ornamental nurseries with a history of *Phytophthora*-infested irrigation water, and watered ‘trap’ plants over a period of 5 months in the summers of 2011 and 2012. Water and plant material were monitored and assayed for *Phytophthora* throughout the trial, including a semi-quantitative water filtration assay and direct root and foliage plating on *Phytophthora*-selective media. Finally, isolates recovered from irrigation water from these two nurseries were screened for pathogenicity on two hosts.

MATERIALS AND METHODS

Nursery selection. Two woody ornamental nurseries in Western North Carolina (WNC) were selected for this study based on the presence of *Phytophthora* in previous water surveys at these locations. Nursery I, located in Burke County, irrigates plants from a retention basin that has been reclaiming total runoff for over 50 years, while nursery II, located in Transylvania County, pumps water directly from the French Broad River, a natural water source which drains from a forested watershed through eight counties in WNC. The French Broad River watershed encompasses 7,330 km² and flowed at an average annual rate of 2,349 and 1,987 cubic feet per second (CFS) in 2010 and 2011 respectively.

Water sampling. Irrigation water was sampled monthly between June and October in 2011 and in May, June, August and October during 2012 using 500 ml sterile polypropylene bottles. At each collection time, water samples were collected at the depth

and nearest point of the intake pump from both the retention basin and river, and additionally from sprinklers that were located within the irrigation zone used to water the trap plants. Water samples were submerged in ice during transport and processed within 24 hr of collection.

Water processing and filtration. Water samples were mixed by inverting several times. Electrical conductivity and pH were measured with a solu-bridge (YSI 35, Yellow Springs, OH) and pH meter (Fisher Scientific Accumet 910, Waltham, MA) prior to filtration (*data not shown*). A filtration assay adapted from MacDonald *et al.* (31) was used to estimate the concentration of propagules of *Phytophthora* present in each sample. Aliquots of 200 ml were passed through a 1-5 μm P2 filter (Fisher Scientific, Waltham, MA) using a Büchner funnel attached to a vacuum pump. An additional 200 ml aliquot was passed through a 4-8 μm P4 filter (Fisher Scientific, Waltham, MA) with the same vacuum apparatus. Filters were removed and placed top-side down on V8-PARPH, a *Phytophthora*-selective media previously described by Jeffers and Martin (26). Plates with filters were incubated in the dark at RT (25°C) for 24 h. After initial incubation, filter papers were removed, and plates were incubated in the dark at RT for an additional 48 h. Colony forming units (CFUs) were counted after the second incubation, and converted to CFUs per liter. The CFUs per liter between filters were added together and divided by two representing the mean CFUs/liter per sample time. Isolations were made from a subset of colonies with visually distinctive morphological characters and hyphal branching typical of *Phytophthora*. Subcultures were obtained from each colony by transferring a hyphal tip to V8-PARPH for further characterization.

***In situ* plant bioassays.** During the summer of 2011 and 2012, containerized hybrid *Rhododendron* L. ‘Nova Zembla’ (2011), ‘English Roseum’ (2012) and *Pieris japonica* ‘Mountain Fire’ were used as susceptible ‘trap’ hosts and placed within the irrigation zones of both nurseries under approximately 55% shade. Plants were purchased from commercial sources and potted into a pine bark mix consisting of 1.8 kg of lime and 965 g of MicroMax (Scotts, Marysville, OH) per cubic meter of pine bark. Osmocote Plus (16-9-12) was surface applied using the recommended middle rate (52 and 21g/pot for 11.4L and 3.8L pots, respectively). Plants were potted into 11.4 liter and 3.8 liter containers in 2011 and 2012, respectively. Plants were arranged in a randomized complete block design with eight blocks of six plants each for both *Rhododendron* and *Pieris*. Blocks were spaced 1 m apart, and pots were placed adjacent to each other within each block. Plants were watered as necessary; daily water input (rain plus irrigation) was measured on site with a HOBO Data Logging Rain Gauge (Onset, Pocasset, MA) using BoxCar Pro 4.0 software.

Root cores from one of the six plants in each block were taken with a 2.5 cm diameter soil probe each month and transported to the lab in a cooler on ice. Samples were stored at 4°C and assayed within one week. Roots were rinsed free of debris, cut into 1-cm long sections and submerged in V8-PARPH (26). Plates were incubated in the dark at RT for 24-96 h, and monitored daily for any growth. Colonies representing typical morphology of *Phytophthora* were pure cultured by hyphal-tip transfers to *Phytophthora*-selective medium (V8-PARPH).

Flooding the roots. In the summer of 2012, one plant from each block of both hosts was flooded on 25 June, 13 August, and 9 October at both nurseries. Plants were placed into 19 liter buckets and well water was filled up to the crown level of each plant. Root zones of plants were submerged for a period of 48 h, and then removed and monitored for symptoms over the rest of the growing season. On 11 October, all plants subjected to flooding from both nurseries (48 plants of each host), were destructively harvested and roots and leaves with disease symptoms were rinsed, plated on V8-PARPH, and monitored for *Phytophthora*. If *Phytophthora* was observed growing from the roots on V8-PARPH, pure cultures were obtained as previously described.

Root baiting. One plant from each block within each host type (i.e. *Pieris* and *Rhododendron*) was subject to a baiting assay to detect *Phytophthora* propagules present in the substrate and asymptomatic roots. In 2011, root balls of each cultivar were standardized by weight. Standardized root balls were placed in plastic crisper boxes (30 x 22 cm) and flooded with 1 liter of distilled water. Symptomless leaves of a native *Rhododendron maximum* plant were collected and ten, 3-mm leaf discs were floated on the water surface in each container and incubated for 48 h in the dark at RT (25°C). After incubation, six leaf discs were randomly selected from each box, submerged into V8-PARPH, incubated in the dark at RT for 48-96 h and monitored for growth of *Phytophthora*. In 2012, a method developed to detect asymptomatic infections of *P. ramorum* (45) was used to bait roots. For this method, two rhododendron leaves were wounded by cutting six, 2 cm slits evenly spaced along the edges of the entire leaf, and placed directly under the root ball within the pot of one plant from each block of both

host type at both locations, totaling 32 plants. Pots were placed in clay saucers filled with distilled water and incubated *in situ* for 48 h. Leaves were collected, rinsed free of debris and wounded areas of the leaves were excised and submerged in V8-PARPH. In both baiting assays, submerged leaf pieces were monitored daily for growth, and colonies representative of *Phytophthora* were transferred to V8-PARPH for identification.

Disease assessments and destructive sampling. Plants were monitored for foliar and root rot symptoms over the entire 5 month growing season for both 2011 and 2012. At the end of each season, the above ground portion of one plant from each block was abscised and weighed. Roots from each plant were rated for root rot symptoms on a scale of 1-5, where 1 = healthy roots, 2 = >25% root necrosis, 3 = 25-49% root necrosis, 4 = 50-75% root necrosis, and 5 = 76-100% root necrosis or dead plant. Averages of the visual root rot rating and above ground plant weight were analyzed by ANOVA (Proc GLM, SAS 9.1, SAS Institute, Cary, NC) and separated by Waller-Duncan k-ratio test.

Morphological and molecular identification. Isolates collected in this study were identified to species with standard molecular and morphological methods, including DNA sequencing of the internal transcribed spacer (ITS) region, colony morphology, and by characterization of sporangia, chlamydospores and oospores (11, 12, 19, 20, 22). Isolates were grouped based on colony morphology and hyphal branching, and a subset of these isolates (>20%) was identified based sequence analysis of the ITS region of the ribosomal DNA (rDNA). DNA was extracted from lyophilized mycelia with the Pure gene kit® (Qiagen, Valencia, CA). Amplification was performed with primers ITS4 and ITS6 (49) with thermocycling conditions of 1 cycle of 94°C for 5 min; 35 cycles of 94°C

for 1 min, 48°C for 1 min, 72°C for 1 min; and a final cycle of 72°C for 5 min, resulting in an approximately 900 bp fragment. Amplicons were cleaned with the QIAquick® PCR purification kit (Qiagen, Valencia, CA) and sequenced with ABI sequencing technology (MCLab, San Francisco, CA). Raw sequence reads were imported and aligned in Sequencher v.4.9 and visually edited accounting for heterozygous sites, if present. Edited sequences were compared with the sequences deposited in the *Phytophthora* Database (www.phytophthoradb.org) (34) and the non-redundant nucleotide database GenBank available through the National Center for Biotechnology Information (NCBI, Bethesda, MD) using the BLAST algorithm (2). Isolates were identified based on highest percent similarity within the ITS region. Isolates not formally named but with sequence depositions in the databases were given the name of the best match within GenBank to avoid ambiguity. Sequences generated in this study were deposited in GenBank (Table 2.1).

Morphological features of chlamydospores, oospores, and sporangia for at least one isolate representing each unique ITS taxon were examined by microscopy (Table 2.1). Sporangia were produced by growing each isolate in 5% V8 broth and incubating at RT (25°C) in the dark for 3-4 d. After incubation, V8 broth was removed and mycelial mats were rinsed with sterile, distilled water, flooded with 2% non-sterile soil extract (NSSE) (25) and incubated under continual fluorescent light at a luminous intensity of 181.4 candelas at RT for 2-4 days (25).

Pathogenicity assays of isolated *Phytophthora* taxa. During the summer of 2012, isolates representing four unique taxa of *Phytophthora* recovered from irrigation

water from both nurseries during 2011 were tested for pathogenicity to both roots and leaves of *Rhododendron* sp. 'English Roseum' and *Pieris japonica* 'Mountain Fire'. The four isolates were representatives of *Phytophthora pini* Leonian (*P. citricola*-complex), *P. hydropathica* Hong & Gallegly, and undescribed *Phytophthora* sp. BF4 and *Phytophthora* taxon PgChlamydo. Leaf and root pathogenicity tests were performed in Raleigh at the NC State Horticultural Field Lab (HFL) and in Mills River at the Mountain Horticultural Crops Research Station (MHCRS). Locally sourced plants were repotted into pine bark substrate amended with 2.4 kg of lime/m³ and 4.2 kg/m³ of slow release 18-6-12 fertilizer Multicote 8 (HAIFA NutriTech, Matam-Haifa, Israel). The experiments included treatments consisting of four different isolates of *Phytophthora* each representing a unique taxon and a non-inoculated control for each plant type. The treatments were arranged in randomized complete block design with four replications and two observations per host plant per treatment. Plants were placed on top of inverted plastic trays to avoid interplot interference and irrigated twice daily for 45 min/d (0.2 cm/d). Water was sourced from a manmade lake pumped with city water and chlorine-treated retention pond water at the HFL and MHCRS locations, respectively.

Root assays. Rice grain inoculum of all four isolates was prepared as described by Holmes and Benson (18). On 19 July and 26 July at MHCRS and HFL, respectively, three holes approximately 4-cm deep were made in the soilless medium of each container, and three colonized rice grains of the corresponding treatment were placed into each hole, for a total of nine colonized rice grains per plant. Non-infested rice grains were used for the negative control treatment and rice grains colonized with isolate 2386 of

Phytophthora cinnamomi served as the positive control. Plants were monitored weekly for six weeks and rated for visual above ground symptoms (*data not shown*). At the end of each experiment, one observation per host from each treatment replication was baited with wounded rhododendron leaves for a period of 48 h (45) and recovered leaves were washed in running water prior to culturing on V8-PARPH. Plants were destructively harvested on 11 and 25 September at HFL and MHCRS, respectively. Above ground weight and root rot ratings were recorded using the rating system mentioned earlier and analyzed by ANOVA (Proc GLM, SAS Institute, Cary, NC).

Leaf assays. Three different inoculation methods were used to evaluate foliar pathogenicity of the four *Phytophthora* isolates on *Rhododendron* and *Pieris*. The three leaf assays were a zoospore or mycelial suspension spray, the ‘Tjosvold’ clip method, and a detached leaf assay. All three inoculation methods were replicated over time or space, with the exception being the ‘Tjosvold’ clip inoculation method, which was only conducted at MHCRS due to a shortfall of plants.

Zoospore or mycelial spray. To produce zoospores for inoculation, sporangia of each isolate were produced as described above. To induce the release of zoospores from sporangia, cultures were incubated at 4°C for 1 h, and then placed on the bench top at RT for 1-2 h. Zoospores were quantified by taking a 1ml aliquot from the original zoospore suspension and mixing with 1ml of lactophenol cotton blue (BD, Sparks, MD) to encyst and stain zoospores for counting using a hemacytometer. Each suspension was diluted to a final concentration of 2.5×10^3 zoospores/ml if zoospores were observed (i.e. *P. pini* (*citricola*-complex) and *P. taxon* PgChlamydo). If sporangia did not form or did not

release zoospores (i.e. *P. hydropathica* and *P. sp.* BF4), mycelial mats were agitated with a sterile glass rod and the suspensions diluted with distilled water at a dilution of one part *Phytophthora* suspension to four parts deionized water for a total volume of 200 ml.

Forty *Rhododendron* and *Pieris* plants each were inoculated *in vivo* with the zoospore or hyphal inoculum on 25 May at the HFL and 1 August at the MHCRS. Inoculum was applied with 200ml spray bottles set to a stream setting. Individual plants were sprayed with the respective treatment (*Phytophthora* isolate) until inoculum ran off the leaves. The negative control treatment consisted of 2% NSSE and four parts distilled water (1:4 v/v). After application, plants were covered with white plastic bags to create a temporary humidity chamber. To avoid heat damage, treatments were applied at dusk and bags were removed at sunrise the following day (12 h post-inoculation). Plants were rated weekly after inoculation on a scale of 1-5, with 1 = completely healthy leaves; 2 = foliage with ≤ 5 foliar lesions; 3 = foliage ≥ 6 foliar lesions; 4 = plants with stem lesions or bud lesions; and 5 = dead plants. Foliar disease ratings taken on the last date, 12 July and 24 August at HFL and MHCRS, respectively, were recorded and analyzed by ANOVA (Proc GLM, SAS Institute, Cary, NC). Necrotic leaf or stem tissue collected from one plant of each host species within each treatment replication was plated on V8-PARPH to demonstrate infection.

‘Tjosvold’ clip method. An agar plug inoculation method originally described by Tjosvold *et al.* (44) was used as the second *in vivo* inoculation method for foliar pathogenicity tests of the *Phytophthora* isolates on *Rhododendron* and *Pieris*. The Tjosvold method holds a colonized agar plug within a microfuge cap flush to the abaxial

side of a leaf with a modified hair clip. The microfuge caps were lined with cotton and then saturated with sterile distilled water prior to adding the colonized agar plug. Two leaves of all 32 plants of each host type were inoculated with each isolate [*P. pini* (*P. citricola*-complex) not tested] as described above on 31 August at the MHCRS location; with the cap assembly held in place on each leaf for 10 days as lesions developed. After 10 days, leaves with lesions were collected and placed in a flat-bed color scanner (Epson Perfection V500 Photo, Long Beach, CA). Lesions area was measured using APS Assess 2.0 software and then analyzed by ANOVA (Proc GLM, SAS Institute, Cary, NC).

Detached leaf assay. The third inoculation method evaluated pathogenicity of the *Phytophthora* isolates using detached *Rhododendron* leaves with agar plug inoculum. Detached leaves from a large *Rhododendron* sp. L. ‘Roseum Elegans’ plant were placed in plastic crisper boxes lined with three layers of moistened paper towels to create a humidity chamber. Each leaf was inoculated on two sites, which were separated by the midrib; one site was wounded by piercing the leaf with an inoculation needle, and the other site was not wounded. Seven mm-diameter agar plugs were cut from the leading edge of a 7-10 day old culture of all four isolates and placed on both the wounded and unwounded sites within each leaf. In addition to the four isolates tested, a positive control of *P. heveae* A.W. Thompson (4) and a negative control of non-colonized CMA (BD, Sparks, MD) were included. Eight inoculated leaves per isolate were incubated in the humidity chambers for 10 days at RT, and misted to restore moisture every other day. Treatments were setup in a randomized complete block design with two observations per

treatment replication; this test was performed twice starting on 13 and 21 September. Leaf lesion area was assessed as described above, and analyzed as described previously.

RESULTS

Detection of *Phytophthora* in irrigation water. An average of 20 CFUs/liter (5-38 CFUs/liter) and 16 CFUs/liter (5-33 CFUs/liter) were detected from water collected in the retention basin throughout the growing season at nursery I in 2011 and 2012, respectively. After passing through the intake and pumping system, an average of 7 CFUs/liter (0-15 CFUs/liter) and 7 CFUs/liter (0-23 CFUs/liter) were detected from water collected at the sprinkler used to irrigate the ‘trap’ plants in 2011 and 2012, respectively. For nursery II, which pumped water directly from the French Broad River for irrigation, an average of 74 CFUs/liter (30-133 CFUs/liter) and 59 CFUs/liter (38-85 CFUs/liter) were detected during the growing season in 2011 and 2012, respectively. After the river water passed through the irrigation system, an average of 25 CFUs/liter (0-70 CFUs/liter) and 15 CFUs/liter (0-33 CFUs/liter) were detected from water collected at the sprinkler used to irrigate the ‘trap’ plants in 2011 and 2012, respectively (Table 2.1).

Molecular and morphological identification of isolates. A total of 80 isolates were isolated from the water at both nurseries over both growing seasons. Based on the described molecular methods employed for culture identification, isolates recovered from the irrigation water at the two nurseries represented taxa in four clades, being *P. hydropathica* (clade 9), *P. irrigata* (clade 9) C. Hong and M. Gallegly, *P. pini* (*P. citricola*-complex) (clade 2) and uncharacterized taxa including *P. sp.* BF4 (clade 9), *P.*

sp. BFA (clade uncertain), and *P. taxon PgChlamydo* (clade 6). *Phytophthora hydrophatica* and *P. taxon PgChlamydo* were recovered in the highest frequency from nursery I and nursery II, respectively (Table 2.2). *Phytophthora* sp. BF4 and *P. hydrophatica* were the only taxa recovered from both nurseries. All isolates identified as *P. pini* (*P. citricola*-complex) based on ITS sequence analysis were between 99-100% similar with deposited sequences labeled as *P. citricola* in GenBank and the *Phytophthora* database. Although both databases have isolates of *P. citricola* with ITS sequences similar to isolates in this study, identification based on one region may not provide the resolution needed to differentiate species within this complex. Morphological characteristics of these isolates, such as irregular hyphal swellings, demonstrate a unique characteristic observed in *P. pini*, but never observed in *P. citricola* s.s (19).

Phytophthora isolates recovered from water in 2011 from both nurseries were characterized based on microscopic morphological characters (Table 2.3). *Phytophthora pini* (*P. citricola*-complex) isolates (n=6) produced ellipsoid to ovoid, semi-papillate sporangia 47.5 x 35.4 μm (range 38.1 - 56.3 μm). All isolates were homothallic and produced oospores that were mostly plerotic with large, capitate paragynous antheridia. Chlamydospores were not observed in any of the isolates examined. Isolates of *Phytophthora hydrophatica* produced ovoid to obpyriform, non-caducous non-papillate sporangia 47.5 x 35.4 μm (range = 29.6 - 77.5 x 19.1 - 48.6 μm). The most distinctive characteristic was obovate hyphal swellings that were longitudinally produced within mycelia on V8A media. Chlamydospores were terminal and most often observed as germinating propagules.

Phytophthora taxon PgChlamydo isolates (n=29) produced ellipsoid to obpyriform, non-caducous, non-papillate sporangia 51.3 x 34.8 µm (range = 29.1 - 82.4 x 18.6 – 62.7 µm). Isolates from this group produced an abundant amount of intercalary chlamydospores with a mean diameter of 34 µm. Oospores were not observed in CMA for this taxon when grown at RT (25°C) in the dark.

Phytophthora sp. BF4 (n=5) isolates produced small, ellipsoid, non-caducous, non-papillate sporangia that were on average 33.5 x 29.4 µm (ranging from 13.7-61.9 x 18.2-41.3 µm). Chlamydospores, hyphal swellings, and oospores were not observed in CMA for this taxon when grown at RT (25°C) in the dark.

Phytophthora irrigata (formerly taxon *P. sp.* 23J7) isolates (n=3) produced ellipsoid, non-papillate, non-caducous sporangia 47.1 x 27.1 µm (ranging from 33.8-59.4 x 20.5-34.4 µm). Chlamydospores and hyphal swellings were not observed. In addition, oospores were not observed in CMA when grown at RT (25°C) in the dark. (Fig.1).

Irrigating ‘trap’ plants. Foliar and root infections caused by *Phytophthora* taxa present in the irrigation water were not observed among the trap plants at either nursery during this study, except for two rare events where *Phytophthora* taxon PgChlamydo was isolated from the roots of an asymptomatic *Pieris* plant in 2011 at nursery II, and *P. hydropathica* was recovered from one symptomatic leaf of a *Rhododendron* plant at nursery I in 2012. To test for the presence of latent infections of the roots caused by species of *Phytophthora*, *Rhododendron* bait leaves were placed directly under the root ball of a subset of plants at both nurseries and processed as previously described by Vercauteren (45). *Phytophthora* was not detected with baiting methods in 2011 or 2012.

Root rot ratings and above ground weights of the *Rhododendron* and *Pieris* plants were not statistically different ($P=0.05$) at each nursery and *Phytophthora* was only detected and isolated from one symptomatic *Rhododendron* leaf and one asymptomatic *Pieris* root system.

Pathogenicity of *Phytophthora* isolates on plant roots. No visual root rot symptoms were observed on *Rhododendron* or *Pieris* test plants at either trial location eight weeks post inoculation with isolates of *P. hydropathica*, *P. pini* (*P. citricola*-complex), *P. sp.* BF4 and *P. taxon* PgChlamydo. Above ground weights and root rot ratings were not significantly different ($P=0.05$) on plants harvested from the MHCRS on 11 September (Table 2.4). At the HFL location at 8 weeks post inoculation on 26 July, no symptoms were apparent and plants were re-inoculated as described above. Six weeks following re-inoculation, all plants remained symptomless except for the positive control *Rhododendron* plants inoculated with *P. cinnamomi*. Although no root rot symptoms were observed on plants inoculated with the four *Phytophthora* isolates, above ground fresh weight of rhododendrons root-inoculated with *P. pini* (*P. citricola*-complex) and *P. hydropathica* were significantly less ($P=0.05$) than the non-inoculated control plants (Table 2.4); however average root rot ratings were statistically the same as the non-inoculated control.

Recovery of *Phytophthora* taxa from the root zone of inoculated plants.

Phytophthora cinnamomi, *P. hydropathica*, *P. pini* (*citricola*-complex), and *P. taxon* PgChlamydo were detected from rhododendron bait leaves placed in the root zone of inoculated plants or by direct isolation from roots (Table 2.5). *Phytophthora sp.* BF4 was

not detected with rhododendron bait leaves in any of the respective inoculated treatments. At the HFL location, *P. pini* was recovered from roots of 100% of the inoculated plants tested. At the MHCRS, *P. pini* was recovered from only one asymptomatic *Pieris* plant. Isolates were confirmed based on ITS sequence analysis of a subset of representative isolates from each treatment recovered from bait leaves.

Pathogenicity of *Phytophthora* isolates on inoculated foliage. Foliage of *Rhododendron* and *Pieris* plants inoculated with a zoospore or mycelial suspension of the isolates at the MHCRS and HFL developed lesions depending on taxa or location (Fig. 2). Although final foliar ratings were conducted at five and six weeks post inoculation at MHCRS and HFL respectively, lesions, if produced, developed within 14 days post inoculation for all isolates. For instance, at HFL both *Rhododendron* and *Pieris* foliage developed lesions within 5 days of inoculation with *P. hydropathica*. On *Rhododendron*, the isolate of *P. hydropathica* used for inoculation caused the most severe dieback at HFL; however, when the same isolate of *P. hydropathica* was used to inoculate *Rhododendron* at MHCRS, dieback was significant ($P=0.05$) but only moderately severe (Table 2.6). *Phytophthora pini* (*P. citricola*-complex) caused the most severe symptoms on *Rhododendron* and *Pieris* at the MHCRS, but at the HFL, foliar infection of *Pieris* plants inoculated with this taxon were not significant when compared to the non-inoculated control. *Phytophthora* sp. BF4 did not infect *Pieris* foliage at either location, but slight disease (rating scale: 1-5; 1=healthy, 5=dead) was observed on *Rhododendron* at HFL (Table 2.6). At the HFL, *Phytophthora* taxon PgChlamydo caused disease on the foliage of *Rhododendron* but not *Pieris* while neither host was infected in the experiment

at MHCRS. Average mean temperature throughout the duration of this test was 24.8 and 22.4°C at HFL and MHCRS, respectively and the average high and low temperatures were 31.0 and 28.0 °C and 18.5 and 16.8 °C at HFL and MHCRS, respectively.

In the second foliage inoculation experiment utilizing the ‘Tjsovold’ clip method, only *Rhododendron* plants inoculated with *P. hydropathica* and *P. taxon PgChlamydo* developed lesions (Table 2.7), which occurred within 10 days post inoculation. Average lesion area was not significant across any treatment on *Pieris*, but *P. hydropathica* produced lesions on two individual *Pieris* leaves of one plant with lesion areas of 1.1 and 0.9 cm². On *Rhododendron*, *Phytophthora taxon PgChlamydo* produced the largest lesion area with a mean area of 0.48 cm² (sd=0.37 cm²). *Phytophthora hydropathica* produced lesions with a mean area of 0.26 cm² (sd=0.40 cm²), which was significantly smaller than *PgChlamydo*. The non-inoculated control, which was a non-infested CMA plug, and *P. sp. BF4* did not produce lesions on *Rhododendron* or *Pieris*. *Phytophthora pini* (*P. citricola*-complex) was not tested in the ‘Tjsovold’ clip method because there were not enough plants.

In the third foliage inoculation experiment utilizing agar plugs on detached leaves, only the wounded *Rhododendron* leaves developed lesions irrespective of the *Phytophthora* taxa tested. Ten weeks post inoculation, lesions were not observed on nonwounded sites of the rhododendron leaves. However, there were significant differences in lesion areas on the wounded site of the rhododendron leaves tested. *Phytophthora hydropathica* produced the largest lesions with an average lesion area of 2.4 cm² (sd=0.97cm²). All wounded sites showed small, necrotic areas as a response to

puncturing the tissue with an inoculation needle (wound response). *Phytophthora* sp. BF4 was reisolated from small lesions, but overall, the average lesion size was not significantly different from the non-inoculated control (wound response) (Table 2.8).

DISCUSSION

Phytophthora taxa were recovered from irrigation water at both nurseries at all sampling times in 2011 and 2012. However, only one foliar infection by *P. hydropathica* on *Rhododendron* and one root infection by *P.* taxon *PgChlamydo* on *Pieris* were observed among the 384 susceptible trap plants during the study. Numerous studies have identified *Phytophthora* taxa in irrigation water (1, 3, 6, 9-11, 16, 23, 24, 27, 29, 36, 38-41, 51) and demonstrated *Phytophthora* species as causal agents of plant disease (13, 15, 17, 21, 28, 30, 33, 43, 46, 47, 50). However, the overall goal of this study was to determine whether disease would develop on plants irrigated with water naturally infested with *Phytophthora*. Similar studies have been conducted with *P. ramorum*, and yielded none to few infections on susceptible plants (42, 48). In this study, two representative nurseries were selected that had histories of *Phytophthora* taxa present in irrigation water. The irrigation system at nursery I represented a retention basin that recycled almost total runoff water from the container growing area. *Phytophthora hydropathica*, a foliar pathogen of *Rhododendron* (22) and stem pathogen of *Pieris* (21) had been previously recovered from the retention basin at Nursery I. The irrigation system at nursery II represented a natural irrigation source, the French Broad River, which drains from a forested watershed. *Phytophthora pini* (*P. citricola*-complex, had

been previously recovered from this river at this location and this species has been documented as a stem and leaf blight and root rot pathogen of *Rhododendron* (5, 9, 19) as well as many other shrubs and trees.

Isolates recovered from water represented seven unique taxa. Four of the six taxa detected were tested for their ability to cause disease on leaves and roots of *Rhododendron* and *Pieris*. The taxa chosen were the most commonly recovered species from irrigation water, which were *P. hydropathica* and *P. taxon PgChlamydo* from nursery I and II, respectively, the taxa recovered from both nurseries, *P. sp. BF4*, and *P. pini* (*P. citricola-complex*), which is known to cause dieback and root rot of *Rhododendron* (5) (Table 2.2). *Phytophthora hydropathica*, *P. pini* (*P. citricola-complex*), and *P. taxon PgChlamydo* caused foliar disease with all assay methods tested, but no isolates caused significant root rot when compared to the positive control of *P. cinnamomi*. Isolates of *P. pini* (*P. citricola-complex*) that caused *Phytophthora* dieback in *Rhododendron* also caused root rot in the same host (5). However, we found no evidence to suggest that the isolate of *P. pini* (*P. citricola-complex*) used in our study caused root rot on *Rhododendron* or *Pieris*. Host specificity may be operating with our isolates as Olson (33) showed differences in host range and aggressiveness with isolates of *P. drechsleri* on floriculture crops.

All of the inoculation methods used for foliar pathogenicity assays showed that *P. hydropathica* and *P. pini* (*P. citricola-complex*) were the most aggressive. The zoospore spray inoculation method simulated application of *Phytophthora* propagules during sprinkler irrigation at a nursery. With this inoculation method the isolate of *P. pini* (*P.*

citricola-complex) used in this test was consistently the most pathogenic across the two trials, but isolates of *P. hydrophatica* and *P. taxon PgChlamydo* also caused a significant level of foliar disease. Temperature differences at the two test locations may have impacted overall differences in disease severity between *P. hydrophatica* and *P. pini*, but differences also could have been contributed to the age of plants or some other extenuating circumstance. Plants were purchased as first year rooted liners at the HFL, while the plants utilized at MHCRS were purchased as second year potted plants.

The 'Tjsovold' clip inoculation method with an agar plug provides a favorable microenvironment for inoculum at the infection court for an extended period of time. With this method our isolate of *Phytophthora taxon PgChlamydo* produced the largest leaf lesions on *Rhododendron*, while this isolate only caused leaf infection at one trial location with the zoospore spray inoculation method. In Minnesota nurseries, Schwingle (37) isolated *Phytophthora taxon PgChlamydo* from leaf lesions on *Rhododendron* and *Taxus*, respectively. In California nurseries, Blomquist *et al.* (8) recently demonstrated pathogenicity of *P. taxon PgChlamydo* to foliage of *Buxus sempervirens*, *Camellia sasanqua*, *Laurus nobilis*, and *Arbutus unedo*, where it caused leaf lesions on all hosts tested.

In our detached leaf assay, all isolates tested produced lesions on the wounded sites on the underside of *Rhododendron* leaves, but no isolate produced lesions on the nonwounded sites of the underside of mature, detached *Rhododendron* leaves. Wound sites were made directly before inoculation. The isolate of *Phytophthora* sp. BF4 tested was not significantly different from the non-infested CMA negative control based on

lesion area. Blomquist (7) and Denman (14) previously suggested that nonwounded sites on detached rhododendron leaves yielded inconsistent results. Our observations found that wounding mature, rhododendron leaves resulted in infection in comparison to non-wounded leaves.

Although three of the four *Phytophthora* taxa tested caused foliar infections in the artificially-infected pathogenicity assays, 'trap' plants irrigated with water naturally-infested with *Phytophthora* spp. at the two nurseries yielded only two infections throughout the entire study, representing 0.1% of the 384 trap plants..

One hypothesis explaining the lack of *Phytophthora* infection *in planta* in the *in situ* assays involves the zoospore concentration threshold needed for successful infection. Mitchell *et al.* showed that *P. cryptogea* had a zoospore concentration to infection relationship, where 20% and 50% of watercress plants were infected when exposed to 50 and 276 zoospores, respectively (32). Similarly, Benson and Jones (5) demonstrated that when *P. heveae* inoculum was prepared at concentrations of 9-32 zoospores/ml (low), 337-364 zoospores/ml (moderate), 1,875-5,491 zoospores/ml (high), 0%, 40%, and 100% of the rhododendron test plants developed *Phytophthora* dieback, respectively. In this study, the average number of zoospores exiting the sprinkler and deposited into each container daily was calculated at 2 (sd=1.4) and 5 (sd=5.6) zoospores at nursery I and II, respectively, based on the surface area of the container and the average daily irrigation volume at each nursery. Even assuming a total of 18 zoospores applied per container per day based on the highest concentration of zoospores detected at one assay date at one nursery from the sprinkler, this concentration may likely not be enough, even during

favorable conditions to cause infection based on the examples with *P. heveae* on *Rhododendron* (5) and *P. cryptogea* on watercress (32). *Phytophthora hydropathica*, *P. pini* (*P. citricola-complex*), and *P. taxon PgChalmydo* all caused foliar infection of *Rhododendron* and *Pieris* when inoculated at 2500 zoospores/ml, but not at natural concentrations observed at the two nurseries.

Werres (48) observed that rhododendron plants irrigated with water containing an estimated 74 zoospores/ml of *P. ramorum* were able to infect <19% of *Rhododendron* and *Viburnum* test plants. In our study, the concentration of 74 zoospores/ml is three magnitudes greater than the highest concentrations detected at the sprinklers at any sampling date or location. Likewise, Tjosvold and Chambers (42) irrigated rhododendron plants with stream water naturally infested with *P. ramorum* and observed no disease or infections, although zoospore concentration was not determined. These data suggest the practice of using *Phytophthora*-infested irrigation water as a primary water source may not be a direct risk to susceptible plants unless high levels of inoculum exist.

Many species of *Phytophthora* that cause Phytophthora dieback, including *P. pini* and *P. ramorum*, have been shown to overwinter and persist as latent root infections until favorable conditions conducive for above ground infection occur (5, 35). Although none of the *Phytophthora* taxa tested caused root infections on *Rhododendron* and *Pieris*, three of four taxa were detected in the root zone 6 weeks post inoculation by the rhododendron leaf bait method. For instance, the *P. pini* (*P. citricola-complex*) isolate was recovered from 100% of the root zone inoculated, from asymptomatic plants of *Rhododendron* and *Pieris* baited with *Rhododendron* leaves at the HFL and from 13% of the *Pieris* plants at

the MHCRS location. In addition to *P. pini* (*P. citricola-complex*), *P. hydropathica* and *P. taxon* 'PgChalmydo' were recovered with the *Rhododendron* bait leaves 6 weeks post inoculation at the HFL location from 7% of plants inoculated, test plants. Although these three taxa did not cause root infections, survival in the root zones of asymptomatic plants could be a source of inoculum for foliar infections. Parke and Lewis (35) demonstrated that asymptomatic root infection in hybrid *Rhododendron* by *P. ramorum* present in the potting mix could result in foliar infections as the pathogen colonized asymptomatic vascular tissues in the root system to reach leaves in the foliage. In our study, the risk of irrigating susceptible nursery plants with water naturally infested with *Phytophthora* was negligible, however, our conclusion is based on the low concentration of zoospores of *Phytophthora* taxa detected in irrigation water used at the two nurseries. When zoospores of select isolates were standardized to high concentration (2500 zoospores/ml) in the laboratory and used to inoculate plants, three of the four *Phytophthora* taxa detected in the irrigation water at the two nurseries caused foliar infections in pathogenicity assays on *Rhododendron* and *Pieris*. Management decisions by woody ornamental growers to install water treatment equipment should take these parameters into consideration.

Phytophthora sp. BF4 caused negligible disease on *Rhododendron* or *Pieris* in any of the pathogenicity assays and does not appear to be a pathogen of these commonly susceptible hosts. Pathogenicity of the isolate representing *P. sp.* BF4 in our test was not observed; further tests conducted on this taxon as well as many other common non-characterized waterborne *Phytophthora* taxa would yield interesting results no doubt.

Overall, low risk was associated with irrigating susceptible *Rhododendron* and *Pieris* plants with water naturally infested with species of *Phytophthora* at two woody ornamental nurseries in Western North Carolina. Even though multiple taxa of *Phytophthora* were consistently recovered from irrigation water samples throughout the duration of the test during both years at both nurseries, the numbers of propagules that persisted from the retention basin or river water was most likely too low to infect the leaves or roots of the trap plants. These data suggest that under shaded and irrigated conditions, the primary factor that determines the level of risk associated with irrigating woody ornamental plants with *Phytophthora*-infested water is correlated with the concentration of *Phytophthora* propagules present. This preliminary finding should warrant future studies to evaluate infection threshold concentrations of different species of *Phytophthora* present in irrigation water elsewhere to determine relative risks to the woody plant nursery industry..

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Table 2.1. *Phytophthora* propagule concentrations in irrigation water at two ornamental nurseries in 2011 and 2012

Time	Sample	Nursery I ^a		Nursery II	
		CFUs/liter (\bar{x}) ^b		CFUs/liter (\bar{x})	
		2011	2012	2011	2012
May	Source	38 ± 3.5 ^a	33 ± 3.5	90 ± 7.1	85 ± 7.1
	Sprinkler	10 ± 0.0	23 ± 3.5	70 ± 21.2	5 ± 0.0
Jun	Source	3 ± 3.5	13 ± 3.5	30 ± 0.0	38 ± 3.5
	Sprinkler	0 ± 0.0	5 ± 0.0	23 ± 3.5	33 ± 14.1
Jul	Source	18 ± 3.5	-	55 ± 21.2	-
	Sprinkler	15 ± 7.1	-	23 ± 3.5	-
Aug	Source	5 ± 7.1	5 ± 0.0	60 ± 0.0	50 ± 14.1
	Sprinkler	8 ± 10.6	0 ± 0.0	0 ± 0.0	20 ± 0.0
Oct	Source	33 ± 10.6	13 ± 3.5	133 ± 24.8	60 ± 14.1
	Sprinkler	0 ± 0.0	0 ± 0.0	5 ± 0.0	0 ± 0.0

^a Water sources of nursery I and II represent a retention basin and a river, respectively.

^b Average CFUs/liter, represented by \bar{x} , and is followed by \pm the standard deviation of CFUs/liter water samples filtered through P2 and P4 filters.

Table 2.2. *Phytophthora* isolates recovered from irrigation water at two ornamental nurseries in North Carolina in 2011 and 2012

<i>Phytophthora</i> Taxa	2011		2012		Overall Total ^b
	Nursery I ^a	Nursery II	Nursery I	Nursery II	
<i>P. hydropathica</i>	8	0	6	0	14
<i>P. pini</i> (<i>P. citricola</i> -complex)	0	6	0	3	9
<i>P. irrigata</i> (formerly taxa <i>P. sp.</i> 23J7 and <i>P. sp.</i> BF3)	0	4	0	0	4
<i>P. sp.</i> BF4	4	1	0	1	6
<i>P. sp.</i> BFA	0	1	0	1	2
<i>P. taxon</i> PgChlamydo	0	29	0	16	45

^a Water sources of nursery I and II represent a retention basin and a river, respectively.

^b A total of 80 isolates were collected over the course of the two year, nursery field trial. Nursery I and II represented a retention basin and a river, respectively.

Table 2.3. Morphological characteristics of taxa recovered from irrigation water and respective GenBank accession numbers

<i>Phytophthora</i> a taxa	GenBank Accession No.	Sporangia characteristics ^b	n ^c	mean (µm)		min (µm)		max (µm)		st. dev ^a		Oospores ^d	Chlamydo­spores ^d
				L1	L2	L1	L2	L1	L2	L1	L2		
<i>P. hydropathica</i>	JX996049	range from ovoid to obpyriform, non-caduceus, non-papillate	33.0	47.5	35.4	29.7	19.1	77.5	48.6	11.7	7.1	absent	present, terminal: often germinated upon observation
<i>P. pini</i> (<i>P. citricola</i> -complex)	KC122237	range from ellipsoid to ovoid, non-caduceus, semi-papillate	47.0	63.2	37.8	38.2	23.8	92.7	56.3	10.9	6.6	present, paragnous antheridia, mostly plerotic, and thick cell walls	absent
<i>P. irrigata</i> (formerly <i>P. sp.</i> 23J7)	JX996046	ellipsoid, non-caduceus, non-papillate small, non-papillate, ellipsoid to obpyriform, non-caduceus	10.0	47.1	27.1	33.8	20.5	59.4	34.4	8.5	4.8	absent	absent
<i>P. sp.</i> BF4	JX996050	papillate, ellipsoid to obpyriform, non-caduceus	21.0	33.5	29.4	13.7	18.2	61.9	41.3	15.8	5.5	absent	absent
<i>P. taxon</i> 'PgChlamydo'	JX996047	ellipsoid to obpyriform, non-caduceus	79.0	51.3	34.9	29.1	18.6	82.4	62.7	9.9	8.6	absent	present, large, intercalary

^a Standard deviation represents the standard deviation between sporangia lengths within a species.

^b Sporangia were measured from apex to place of attachment to pedicel, as well as across horizontally.

^c Number of sporangia measured within a particular taxa.

^d Oospores were screened on cornmeal agar, and Chlamydo­spores were observed in V8-A, if present.

Table 2.4. Assessment of four isolates representing unique taxa of *Phytophthora* recovered from irrigation water to cause root rot of *Rhododendron*

<i>Phytophthora</i> Taxa	HFL ^y		MHCRS	
	Root Rot Rate	Weight (g)	Root Rot Rate	Weight (g)
<i>P. sp.</i> BF4	1.0 b ^z	42.0 ab	1.0	35.0
<i>P. pini</i> (<i>P. citricola</i> -complex)	1.0 b	33.0 bc	1.0	29.0
<i>P. hydrophatica</i>	1.0 b	34.0 bc	1.0	28.0
<i>P. taxon</i> PgChlamydo	1.0 b	48.0 ab	2.0	28.0
Non-inoculated ctrl	1.0 b	54.0 a	2.0 ns	33.0 ns
<i>P. cinnamomi</i> (Pos. Ctrl)	4.0 a	16.0 c	NT	NT

^y HFL and MHCRS represent the Horticultural Field Lab and Mountain Horticultural Crop Research and Extension Center, respectively. These were the two locations where these experiments were conducted.

^z Values followed by different letters in each column are statistically different from one another according to Waller-Duncan k-ratio means separation, $P=0.05$.

Table 2.5. Recovery of *Phytophthora* isolates with leaf baits and direct root plating from roots of inoculated, asymptomatic plants.

<i>Phytophthora</i> Taxa ^c	Rhododendron				Pieris			
	HFL ^a		MHCRS ^b		HFL		MHCRS	
	bait leaves	direct root	bait leaves	direct root	bait leaves	direct root	bait leaves	direct root
<i>P. sp.</i> BF4	-	-	-	-	-	-	-	-
<i>P. pini</i> (<i>P. citricola</i> -complex)	++++	+	-	-	++++	+	+	-
<i>P. hydropathica</i>	+	-	-	-	-	-	-	-
<i>P. taxon</i> PgChlamydo	+	-	-	-	-	-	-	-
<i>P. cinnamomi</i> (Pos. Ctrl)	++	++ ^d	NT ^e	NT	NT	NT	NT	NT

^a Plants were inoculated six weeks prior to baiting and direct root plating at HFL.

^b Plants were inoculated eight weeks prior to baiting and direct root plating at MHCRS.

^c Nine infested rice grains of each taxa were used to inoculate individual root zones.

^d Four plants were assayed at both locations. Each + represents recovery of *Phytophthora* taxon from one plant, while – represents not recovered.

^e NT means “not tested”.

Table 2.6. Assessment of four isolates representing unique taxa of *Phytophthora* recovered from irrigation water to cause foliar disease of *Rhododendron* and *Pieris*.

<i>Phytophthora</i> Taxa	<i>Rhododendron</i> Avg. Ratings ^y		<i>Pieris</i> Avg. Ratings	
	HFL ^w	MHCRS	HFL	MHCRS
<i>P. sp.</i> BF4	2.0 bc ^z	1.0 c (n=7)	1.0	1.0 b
<i>P. pini</i> (<i>P. citricola</i> -complex)	3.0 b	4.0 a (n=7)	2.0	3.0 a
<i>P. hydropathica</i>	5.0 a	2.0 b (n=7)	2.0	2.0 b
<i>P. taxon</i> PgChlamydo	3.0 b	1.0 c (n=8)	2.0	1.0 b
Non-inoculated ctrl ^x	1.0 c	1.0 c (n=5)	2.0 ns	1.0 b

^w HFL and MHCRS are the two locations that the experiments were conducted at in the Fall of 2012.

^x The non-inoculated control was a diluted non-sterile soil extract at a proportion of 1:4 with a total volume of 200 ml.

^y Avg rating mean ratings represent the averages of each treatment on a visual disease rating scale of 1-5, with each treatment replication being equal to N=8, unless otherwise stated; Six rhododendron individuals at MHCRS were thrown out because plants were infected prior to inoculation.

^z Values in each column followed by different letters are statistically different from one another according to the Waller-Duncan k-ratio means separations, $P=0.05$.

Table 2.7. Average lesion area of plants inoculated with three unique isolates of *Phytophthora* using the Tjosvold foliar clip inoculation method

Taxa	N ^z	Rhododendron	Pieris
		Lesion Area (cm ²)	Lesion Area (cm ²)
<i>P. sp.</i> BF4	14	0.00 c ^y	0.00
<i>P. hydropathica</i>	15	0.26 b	0.12
<i>P. taxon</i> PgChlamydo	16	0.48 a	0.00
Non-inoculated ctrl ^x	10	0.00 c	0.00 ns ^w

^w ns means “not significant”

^x The non-inoculated control was an a noninfested CMA plug clipped on the plant with a modified hair clip.

^y Values in each column followed by a different letter are significantly different according to the Waller-Duncan k-ratio means separation, $P=0.05$ in SAS 9.3.

^z Nine rhododendron plants were removed as samples because they were infected prior to inoculation; *Pieris* were healthy at time of inoculation (N=16).

Table 2.8. Average lesion area of wounded sites of *Rhododendron* leaves in the detached leaf assay.

Taxa	Test I ^w	Test II
	Lesion Area (cm ²) ^y	Lesion Area (cm ²)
<i>P. sp.</i> BF4	0.50 cd ^z	0.82 bc
<i>P. pini</i> (<i>P. citricola</i> -complex)	1.60 b	1.95 a
<i>P. hydropathica</i>	2.60 a	2.23 a
<i>P. taxon</i> 'PgChlamydo'	1.05 bc	2.11 a
Non-inoculated ctrl ^x	0.04 d	0.07 c
<i>P. heveae</i> (pos. ctrl)	1.65 b	1.60 ab

^w Test I and Test II were analyzed separately in SAS 9.3.

^x The non-inoculated control was a non-infested CMA plug (3 mm).

^y Lesion areas represent the lesion area means across replications.

^z Values in each column followed by different letters are statistically different from one another according to the Waller-Duncan k-ration means separation, $P=0.05$.

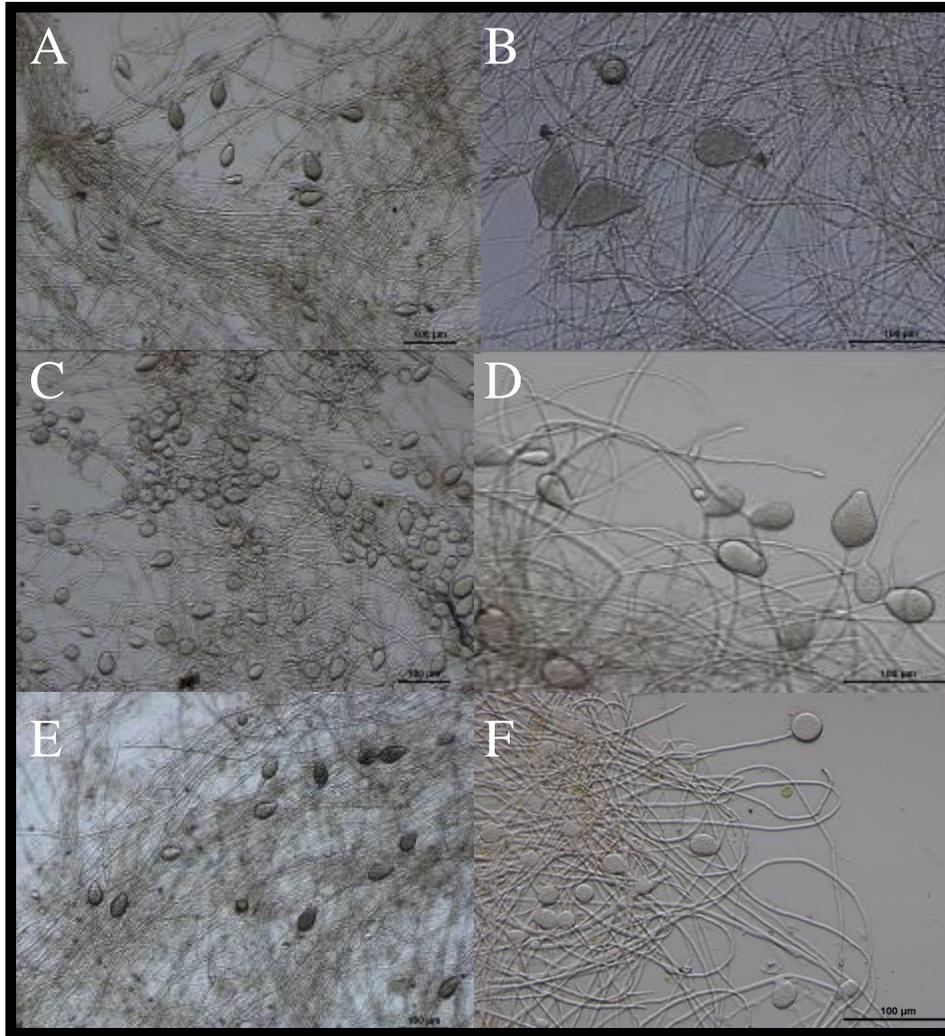


Fig. 1. A) Ovoid, non-papillate, non-caducous sporangia of *Phytophthora irrigata* (Formerly *P. sp. 23J7*); B) Irregular and obpyriform, semi-papillate and double semi-papillate sporangia of *P. pini*. Plerotic oospore with a single paragynous antheridia also observed; C) Ovoid-obpyriform, non-papillate, non-caducous sporangia of *P. taxon PgChlamydo*, as well as large, intercalary produced chlamydospores; D) Obpyriform, non-caducous, non-papillate sporangium and longitudinal, obovate hyphal swellings of *P. hydropathica*; E) Ovoid, non-caducous, non-papillate sporangia of *P. sp. BF4*; F) terminally-produced chlamydospore and hyphal swellings of *P. hydropathica*.

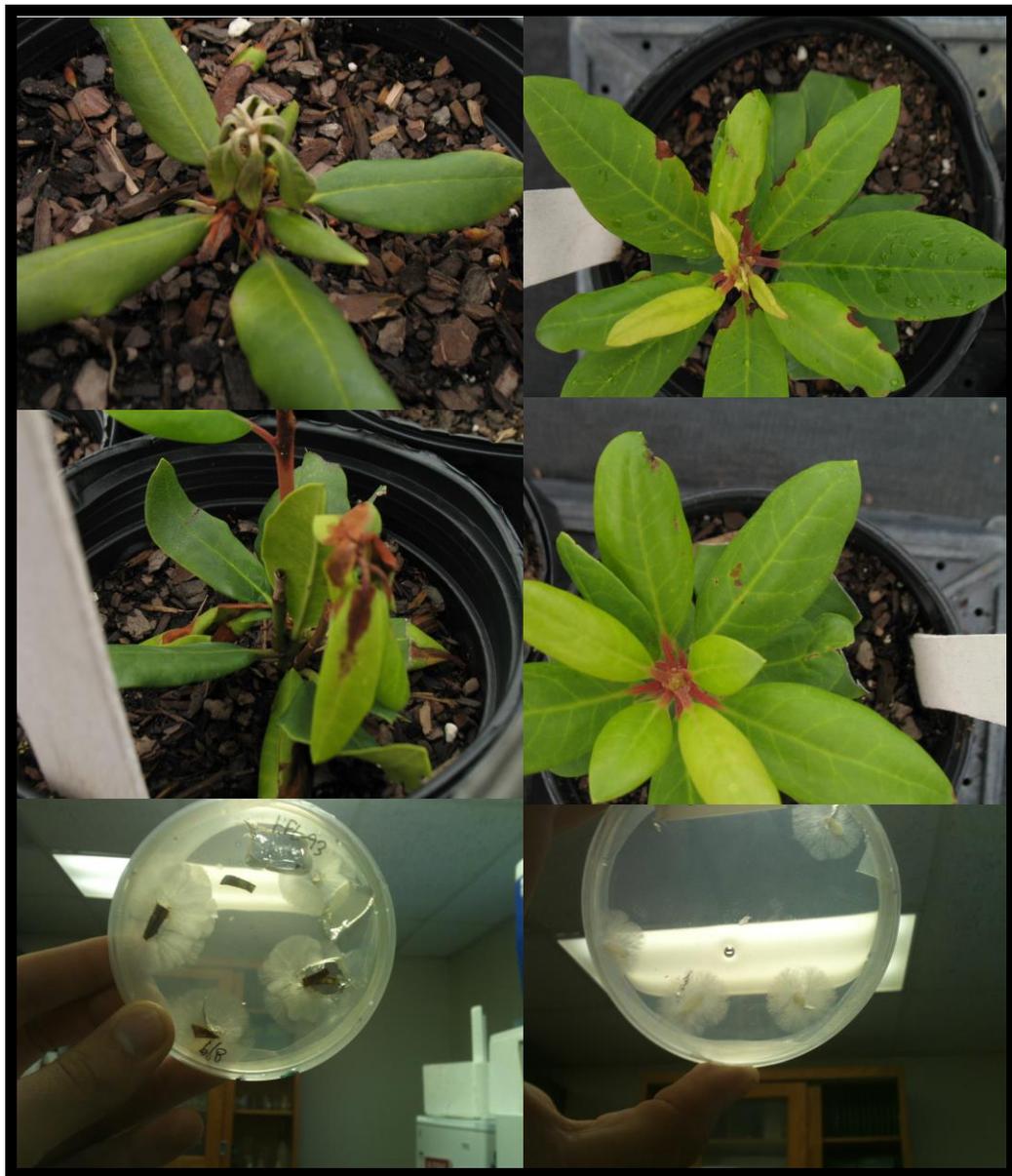


Fig. 2. Typical foliar disease symptoms of *Phytophthora hydropathica* (A), *P. taxon* PgChlamydo (B), *P. pini* (*citricola*-complex) (C), and *P. sp.* BF4 (D) on *Rhododendron* at HFL; Reisolation of *P. hydropathica* from leaf and stem lesions on CMA (E); Assessment of viability of rice grain inoculum of *P. sp.* BF4 used in root rot assay.

APPENDICES

Appendix A

>LoydOrnD 2011 ITS Sequences (using ITS4 and ITS6 Primers) of isolates recovered from irrigation water

>Phytophthora_hydropathica

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>Phytophthora_pini (citricola-complex)

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>LoydOrnD 2012 ITS Sequences (using ITS 4 and IT S6 Primers) of isolates recovered from irrigation water

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>GenBank deposit accession number and isolate annotation

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AGAAACAACGTCGCCGACTTTTCAGCCGCACATTCGTCGCCGTGTTTGATAGGTCTCAACCCGCGCCGGTCAACATATT
GCTACATCGACCAACAACAGGGAGTCCCAACTAATTTGGTAAATACGGTTCACGTGGAAAGT TTTTTAGGTGTGGT AATGA
TCTTCCGCAAGTTCACCTACGGAAAC

>gb|JX996046|Phytophthora_irrigata (formerly P. sp._23J7) [organism=Phytophthora irrigata] 2Pax1_P4_3 recovered from a river in
Western North Carolina
GATATGCTTAAGTTCAGCGGGTAAATCTTGCCTGATATCAGGTCCAATTGAGATGCA GCCAAAAGCCGCACAATTTGCCAAA
TAGGTGCTCCTCTCGGCACAAGCCACCATACTTCGCGCAACACCTGCGCGGTTCAAAAGCCAAGCCGCCATGACTACGGTGC
ACCGACCCCGCCACGCCAAAAACAGCAGAAAACAGACCCAT AAGCAGAAGTTTCAGCCGAAGCCAAACCATACCGCGAATCGA
CCTCCTCTCCACACGCTCAGCAGCAAAATCAGTTCGCCGACTGGCCACAACGGCAGCCTCCACAACCCACACAACGCACGCTTT
TCCAGCAAAGAGAAGAAGCAGTATCTTACATTTCAAAGGACTCGCCTGCCATCTTTACAAAATGACCAGCAAGACACTTCAC
GTCTGCGCGTCCCGCCACCGACTACACGGAAAGGAAGGACAAAGTTCATGTACGGACACTGATACAGGCATACTCCA
GGCATAACCCGGAAGTGCAATATGCGTTCAAAATTTTCGATGACTCACTGAATCCTGCAATTCGCATTACGTATCGCAGTTCG
CAGCGTCTTTCATCGATGTGCGAGCCTAGACATCCACTGCTGAAAGTTGCTATCTAGTTCAAAGCAGAGACTTTCGTCGCCAC
AGTATCATCAGAAAATATTA AAAAGGGTTACAAAAAGTAACTACTACCTCGACTTTGCGAGCCAGAGCCTCGTTGATG
GAGCCGACCAAGCGGCCCCCAACATCACAGACAGTTCACGTGGAAAGT TTTTAGGTGTGGT AATGATCCTTCCGCAAGTTCAC
CCTACGGAAACCTTGT A

>gb|JX996047|Phytophthora_taxon_PgChlamydo [organism=Phytophthora taxon PgChlamydo] 1Pax1_P4_3 recovered from a river in
Western North Carolina
AGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAACTTTCCACGTGAACCGTATCAACCCCTTAAATTT
TGGGGCTTCTGCTCGCGCGGTGCTGCTGGCCTGTAAATGGTTCGGCGTCTGCTGCTGGCAGGCTCTATCATGGGCGAGCG
TTTGGGCTTCCGCTCGAAGTATGCTATCAATTTAAACCTTTCTTAAATACTGAACATACTGTGGGACGAAAAGTCTCT
GCTTTTAACTAGATAGCAACTTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTA
TGCGAATTGCAAGATTGAGTATCATCGAAATTTGAACGCATATTGCACTTCCGGGTAGTCTCGGGAGTATGCCTGTATC
AGTGTCCGTACATCAACCTTGGT TTTCTTCTTCCGTGTAGTTCGGTGGAGGATATGCCAGACGTGAAGTGTCTTGCTGGCGT
CTTTCGAGTCTGCCGTGAGTCTTTGAAATGTACTGAACTGTACTCTCTTTCGTCGAAAAGCGTGGCTGTCTGCTGTTG
GAGGCTGCCGCTGTGGCAGTCCGGACCGGTTTGTAGTGTGGCTTTAAATGGAGGAGTGTTCGATTCCGGTATGGTGTG
GCTTCCGCTGAACAATCTGCTTATTGGGTGCTTTTCTGTCTTGGCGGTACGAACTGGTGAACCGTACGTGTGTGGTCTTGT

GCTTTTGAACCGGCTTTGCTTTGCGAAGT AGGGTGGCGGCTT CGGCTGT CGAGGGGT CGATCCATTTTGGGAACTTTGTGTG
T GCGGCTT CGT GCT GCGCGCAT CTCAATTGGACCTGAT ATCAGGCAAGATTACCCGCTGAACCTAAGC

>gb|JX996048|Phytophthora_heveae [organism=Phytophthora heveae]4Pax_P6_2_1 recovered from a river in Western North Carolina

GTTAAGTTCAGCGGGT AATCTTGCCT GATATCAGGT CCAATTGAGAT GCACGCCAATAAAGGCACACACAAAATTTCCCAAAT
GGATCGACCCTCGACAGCCGAAGCCGCCACTCTACTCACAACAGCAAAGCCGATTCAAAAGCCAAGCACCACATAGCTAC
GGTACACCAGCATT ACCGCCACAACAGGAAACACATCCAATAAGCGCATTGTTCAGCCGAAGCCAACCATACCGCGAATC
GAACACGCCTCCATT AACGCTGCAGCAGACAGACCGGT CGCCGACATGCCACACAGGCAGCCTCCACAACAGCAACGCCA
TGCTTTTCGAGCAAAGAGAAGTACAGTTCA GTACATTTCAAAGGACTCGCAGCCAGTTCGAGAACCAGCCGCAAGACTTC
ACATCTGGCATCTCCTCCACCGACTACACGGAAGGAAGAAAGCCAAGT TTGTTGTACGGACTGATACAGGCATACTCCCA
GGACTAACCCGGAAGT GCAAT ATGCGTTCAAAATTTGATGACTCACTGAATCCTGCAATTCGATTACGTATCGCAGTTCG
CAGCGTCTCTCATCGATGTGCGAGCCTAGACATCCACTGCTGAAAAGTTGCTATCTAGTTAAAAGCAGAGACTTTCGTCCCCAC
AGTAT AATCAGTAATTAGGAATGGGT TAAAAAGAGGGCTACTAGTTCAGACCCGAAGT CCAAACGCTCGCCATGATAGAGC
TCGTCCAGCAGCAGCAGCCGCAAACCTCGCCCCAACTATTGGT TTGTTACGGTTCACGTGGAAAAGT TTTTAGGTGTGGTAA
TGATCCTTCCGACGGTTCACCTACGGAACCTTGT

>gb|JX996049|Phytophthora_hydropathica [organism=Phytophthora hydropathica] 1CH1_P4_1 recovered from a retention basin in Western North Carolina

TAAATCTGATATCAGGTCCAATTGAGATGCGACGACGAAGCCGCAACAATCGTCCCAAATAGGTCTCTCGGCCGAAGCCAC
CATACTTCGCGCACTAGYCGCGGGTCAAAGCCAAGCCACYATGACTACGGTTCACYRACCCCGCCACGCCRAAACAGC
AGAAAAACAGCCCAT AAGCAGAAGT KCAGCCGAAGCCAACCATACCGCGAATCGAGCTCCTCTCCACACGCCTCAGCAGC
AAATCAGT CGCCGACTGGCCAT AACGGCAGCCTCCAACAACACACAACGCAAGCTTTTCCAGCAAAGAGAAGAACAGTATC
TTACATTTCAAAGGACTCGCCGGCKAACGGCCAGCAAGACTTCACTGTCTGCGGTCCCGCCACCGACTACACGGAAGG
AAGGRAGCCAAGGTGATGTACGGACTGATACAGGCATACTCCCAGGCATAACCCGGAAGT GCAATATGCGTTCAAAAT
TTGATGACTCACTGAATCCTGCAATTCGATTACGTATCGCAGTTCGACAGCTTCTTCATCGATGTGCGAGCCTAGACATCC
ACTGCTGAAAAGTTGCTATCTAGTTAAAAGCAGAGACTTTCGTCCCCACAGTATGATCAGAAAATATAGGAAGGGTTTACAAA
AAGTAACACTACCCTCGACTTTGACGCCAGACCTCGTTGATGGAGCCCGCCCAACGGGACCCGCAACGCCCAACATC
ACAGACAGTTCACGTGGAAAAGTTTAGGTGTGGTAAATGTCTTCCGAGTTCGTTACAGCTAC

>gb|JX996050|Phytophthora_sp._BF4 [organism=Phytophthora sp. BF4]
0CH1_P2_1 recovered from a retention basin in Western North Carolina

GTAAACAAGGT TTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCTAAAAACTTTCCACGTGAAGTGTCTGTGATGTTG
GGGGCTGCCCTTGGTGGTGGCTCCATCAAACGAGGCCCTGGGCTGCAAAGT CGAGGGT AGT AGTTACTTGTGTAAACCTT
TTGTAATTTTCTGATCATACTGTGGGACGAAAGTCTCTGCTTTTAACTAGTAGCAACTTTCAGCAGTGGATGTCTAGGCT
CGCACATCGATGAAGAACGCTGCGAATCGATACGTAATCGAATTGCAAGATTCAAGTTCAGTTCAGTTCGAAAATTTGAACGC
ATATTGCACTTCGGGT TATGCCTGGGAGTATGCCTGTATCAGTGTCCGTACATCAACCTTGGCTTCTTCTTCCGTGTAGTC
GGTGGCGGAACCGCAGACGTGAAGTGTCTTGTGGCGT GAGT CCGGCGAGTCTT T GAAATGT AAGATACTGTTCTTCT
CTTTGCTGGAAAAGCGT CCGT TGTGTGGT TGTGGAGGCTGCCGT TGTGGCAGT CCGCGACTGAT TTCGTGCTGAGGCGTGT
GGGAGGAGCTCGAT TCGCGGTATGGT TGGCT TCGGT GAACTTCTGCTTATGGGGCTGT TTTCTGCTGTTTCGCGGTGCG
GGGTGGTGAACCGT AGTCATGGTGGCTTTTGAACCGCGCGGCTGT TGGCGAAGTATGGTGGCTTCGCGCGAGAGG
ACGACCTATTTGGGACGATGTGCGGCTTCGTGCTGCATCTCAATTGGACCTGATATCAGGCAAGATTACCCGCTGAACCTA
AGCATATCATA

>gb|JX996051|Phytophthora_bisheria [organism=Phytophthora bisheria] Pax_1_46 recovered from rhododendron plant

ATCAGGTCCAATTGAGATGCGCACCCGAAGTACACACAACGTTTCCCAAATGGATCGACCCCTCGGCAGCCGAAGCTGCCA
CTCTACTTCGCAATAGTATCACCGGT TCAAAAAGCCAAGCCTACATAGCTACGGTTCACCAGCTCATCACGCCACAGCAGGAA
AAAAAAAAGCGTCCAATAAGCGCCTGTTACGCGAAGCCAACCATACCGCGAATCGAACACTCCTCCAGTAAAAACTGCA
GCARACAAACCGGT CGCCGACTGGCCACCGCGGACGCTCCAACAACGCAACCGCTCACTTTTCGAGCAAAGARAAGTAC
AGTT CAGTACATTTCAAAGGACTCGCAGCCGACCCGAAAAGCCAGCCGAARACACTTCAATCTGGCATATCCTCCACCGA
CTACACGGAAGGAARAAAACCAAGTTGATGTACGGACTGATACAGGCATACTCCCAGGACTAACCCGGAAGT GCAATA
TGCGTTCAAATTTGATGACTCACTGAATCCTGCAATTCGATTACGTATCGCAGTTCGACGCTTCTTCATCGATGTGCGA
GCCTAGACATCCACTGCTGAAAGTTGCTATCTAGTTAAAAGCAGAGACTTTCGTCCCCACAGTATAATCAGTATTAAGAAAT
GGTTTTAAAAAAAAGGCTACTTGT CAGACCGAAGCCAAACGTTTGCCATGATAGGACTCGCCAGCACGCAGCCTG
CCAGCCAATAAAGGCCAGCTAGCCGACACCCAGCAAATCCCCAATTAAGGGTTGATACGGTTCACGTGGAAAAGTTTTT
TTAGGTGTGGTAATGGAT

>gb|KC122237|Phytophthora_pini (citricola-complex) recovered from French Broad River 0Pax2 P4_1

ACCACACTAAAAAACTTTTACAGTGAACCGTATCAACCCTTTAGTTGGGGTCTTGCTTTTTCGAGCCCTATCATGGC
GAATGTTGGACTTCGGTCTGGGCTAGTACGCTTTTGTTTAAACCCATCTACAATACTGATTATACGTGGGGACGAAAGT
CTCTGCTTTAACTAGATAGCAACTTTCAGCAGTGGATGTTCTAGGCTCGCACATCGATGAAGAACGCTCGCAACTGCGATAC
GTAAATGCGAATTGCAAGTTCAGTGTGATCATGAAAATTTGAAACGCATATTGCACTTCGGGT TAGTCTGGGAGTATGCCT
GTATCAGTGTCCGTACATCAAACCTTGGCTTCTTCTTCCGTGTAGTGGTGGAGGATGTGCCAGATGTGAAGTGTCTTGGC

GTGT CCTTCGGGTCGTCTGCGAGT CCTTTGAAATGTACTGAACTGTACTTCTCTTTGCTCGAAAAGCGTGGTGTGCTGGTTG
TGRAGGCTGCCTGCGTGGCCAGTGGCGACCGTTTGTCTGCTGCGGCGTTAATGGAGGAGTGTTCGATTCGCGGTATGGT
TGGCTTCGGCTGAACAGGCGCTTATTGTATGCTTTTCCTGCTGTGGCGTGATGGGCTGGTGAACCGTAGCTGTGTGTGGCTTG
GCTTTTGAATCGGCTTTGCTGTTGCGAAGTAGAGTGACGGCTTCGGCTGTGAGGGTCGATCCATTTGGGAACTTTGTGTGCA
CCTCGGTGCGCATCTCAA

Appendix B

Table B1. Theoretical amount of zoospores deposited on individual plant per day.

year	Water applied to plants (cm/d)		Zoospores/ml		Daily Zoospores deposits		Average zoospores/day	
	Nursery I	Nursery II	Nursery I	Nursery II	Nursery I	Nursery II	Nursery I	Nursery II
	2011	0.885	1.08	0.01	0.07	3	18	2
2011	0.755	0.776	0	0.023	0	5		
2011	0.652	0.869	0.015	0.023	3	5	Standard deviations	
2011	0.749	0.908	0.008	0	2	0	2	6
2011	0.76	0.908	0	0.005	0	2		
2012	0.551	0.401	0.023	0.005	5	1		
2012	0.604	0.765	0.005	0.033	1	9		
2012	0.409	0.583	0	0.02	0	4		
2012	0.521	0.583	0	0	0	0		

Table B2. Quantities, pH, EC and Average CFU for Nursery I: retention basin in 2011.

Time	Sample	Water Inputs/day (cm/d)	pH	EC (μMHO)	Average CFU/L
18-Jun	Source	0.885	5.53	56	38
	Sprinkler		5.63	56	10
20-Jul	Source	0.755	5.82	68	3
	Sprinkler		5.85	80	0
14-Aug	Source	0.652	5.59	62	18
	Sprinkler		5.93	63	15
23-Sep	Source	0.749	5.69	54	5
	Sprinkler		5.73	65	8
15-Oct	Source	0.76	5.75	54	33
	Sprinkler		5.95	80	0

Table B3. Quantities, pH, EC and Average CFU for Nursery II: French Broad River in 2011.

Time	Sample	Water Inputs/day (cm/d)	pH	EC (μMHO)	Average CFU/L
18-Jun	Source	1.08	5.45	14	90
	Sprinkler		5.1	14	70
20-Jul	Source	0.776	5.14	13	30
	Sprinkler		5.25	13	23
14-Aug	Source	0.869	5.63	18	55
	Sprinkler		5.7	18	23
23-Sep	Source	0.908	5.35	20	60
	Sprinkler		5.4	20	0
15-Oct	Source	0.908	5.54	16	133
	Sprinkler		5.48	16	5

Table B4. Quantities, pH, EC and Average CFU from Nursery I: retention basin in 2012.

Time	Sample	Water Inputs/day		EC (μ MHO)	Average CFU/L
		(cm/d)	pH		
1-Jun	Source		5.15	28.5	33
	Sprinkler	0.551	5.55	34.2	23
27-Jun	Source		5.07	47	13
	Sprinkler	0.604	5.58	52.4	5
24-Aug	Source		5.77	56.7	5
	Sprinkler	0.409	5.9	-	0
11-Oct	Source		6.01	68.1	13
	Sprinkler	0.521	6.05	67.6	0

Table B5. Quantities, pH, EC and Average CFU for Nursery II: French Broad River in 2012.

Time	Sample	Water Inputs/day		EC (μ MHO)	Average CFU/L
		(cm/d)	pH		
31-May	Source	0.401	5.18	11.6	85
	Sprinkler		5.64	39.8	5
27-Jun	Source	0.765	5.7	13.1	38
	Sprinkler		5.8	19.3	33
24-Aug	Source	0.583	5.31	14.9	50
	Sprinkler		5.66	12	20
11-Oct	Source	0.583	5.26	16.1	60
	Sprinkler		5.4	16.8	0

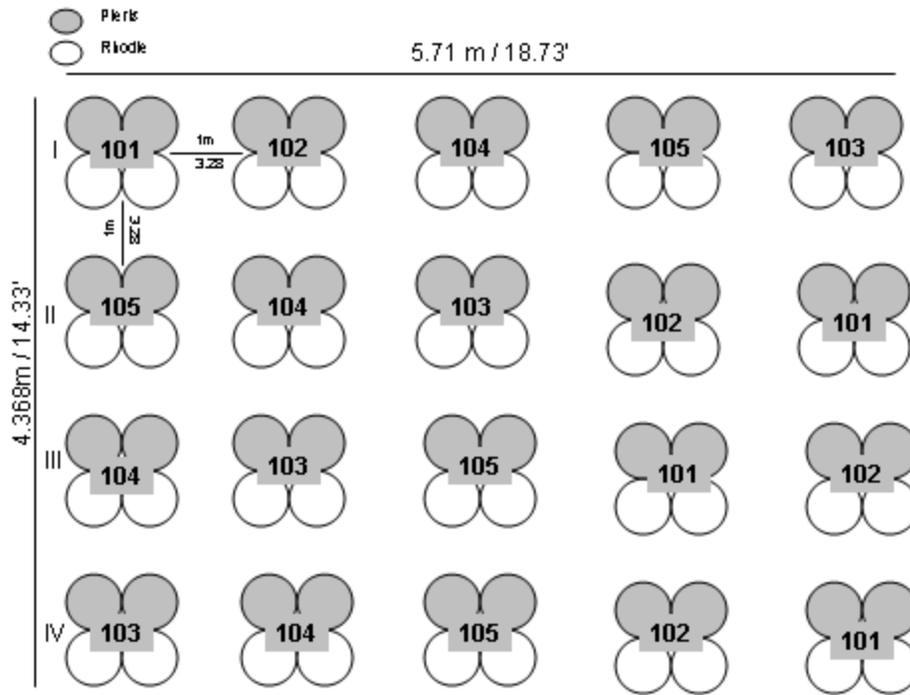


Figure B1. Experimental design for pathogenicity assays. Treatment replications were placed atop black, plastic trays to avoid interplot interference



Figure B2. Foliar clip inoculation on *Rhododendron* plant.