Soilborne *Phytophthora* and *Pythium* Diversity From Rhododendron in Propagation, Container, and Field Production Systems of the Pacific Northwest

Jerry E. Weiland,^{1,†} Carolyn F. Scagel,¹ Niklaus J. Grünwald,¹ E. Anne Davis,¹ Bryan R. Beck,¹ Zachary S. L. Foster,² and Valerie J. Fieland²

¹ Horticultural Crops Research Laboratory, U.S. Department of Agriculture Agricultural Research Service, Corvallis, OR 97330

² Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR 97331

Abstract

Rhododendron root rot is a severe disease that causes significant mortality in rhododendrons. Information is needed about the incidence and identity of soilborne *Phytophthora* and *Pythium* species causing root rot in Pacific Northwest nurseries in order to better understand the disease etiology and to optimize disease control strategies. The last survey focusing solely on soilborne oomycete pathogens in rhododendron production was conducted in 1974. Since then, advances in pathogen identification have occurred, new species may have been introduced, pathogen communities may have shifted, and little is known about *Pythium* species affecting this crop. Therefore, a survey of root-infecting *Phytophthora* and *Pythium* species was conducted at seven nurseries from 2013 to 2017 to (i) document the incidence of root rot damage at each nursery and stage of production, (ii) identify soilborne oomycetes infecting rhododendron, and (iii) determine whether there are differences in pathogen diversity among nurseries and production systems. Rhododendrons from propagation, container, and field systems were sampled and *Phytophthora* and *Pythium* species were isolated from the roots and collar region. Root rot was rarely evident in propagation systems, which were dominated by *Pythium* species. However, severe root rot was much more common in container and field systems where the genus *Phytophthora* was also more prevalent,

suggesting that *Phytophthora* species are the primary cause of severe root rot and that most contamination by these pathogens comes in after the propagation stage. In total, 20 *Pythium* species and 11 *Phytophthora* species were identified. *Pythium cryptoirregulare*, *Pythium* aff. *macrosporum*, *Phytophthora plurivora*, and *Phytophthora cinnamomi* were the most frequently isolated species and the results showed that *Phytophthora plurivora* has become much more common than in the past. *Phytophthora* diversity was also greater in field systems than in propagation or container systems. Risks for *Phytophthora* contamination were commonly observed during the survey and included placement of potting media in direct contact with field soil, the presence of dead plants that could serve as continuous sources of inoculum, and the presence of excess water as a result of poor drainage, overirrigation, or malfunctioning irrigation equipment. In the past, research on disease development and root rot disease control in rhododendron focused almost exclusively on *Phytophthora cinnamomi*. More research is needed on both of these topics for the other root-infecting species identified in this survey.

Keywords: ornamentals, oomycetes, pathogen detection, pathogen diversity, woody ornamentals, yield loss and economic impacts

Rhododendrons (*Rhododendron* species and cultivars) are an important component of the Pacific Northwest (PNW) nursery industry, with an annual value of \$11.6 million (USDA National Agricultural Statistics Service 2015). They are grown for their spring flowers and evergreen leaves. Nursery production of this crop is often severely compromised by root rot, with losses of up to 100% reported (White 1937). Several *Phytophthora* species cause root rot, including *Phytophthora cactorum* (Lebert & Cohn) J. Schröt., *Phytophthora cinnamomi* Rands, *Phytophthora citricola* Sawada or *Phytophthora*

plurivora T. Jung & T.I. Burgess, and *Phytophthora cryptogea* Pethybr. & Laff., as well as other less common species (Hoitink and Schmitthenner 1974b; Parke et al. 2014; Weiland et al. 2018). *Pythium* species, such as *Pythium cryptoirregulare* (Garzón, Yáñez, and G.W. Moorman) and *Pythium prolatum* F. F. Hendrix & W. A. Campb., may also cause root rot (Botha and Crous 1992; Hendrix and Campbell 1966; Weiland et al. 2018).

To better understand rhododendron root rot in the PNW, information is needed about the current diversity of soilborne *Phytophthora* and *Pythium* species affecting rhododendron production in this region. The last systematic survey of soilborne *Phytophthora* species that infect rhododendron was published in 1974 (Hoitink and Schmitthenner 1974b). Many advances in pathogen identification using DNA sequence analysis have been made since then and species abundances may have changed in the intervening ≥40 years. New species may also be present. Much of the more recent information about *Phytophthora* species diversity on rhododendron is based on nursery surveys of aboveground plant organs (stems, leaves) that were conducted in response to the quarantine pathogen, *Phytophthora ramorum* (Blomquist et al. 2016; Dart and Chastagner 2007; Knaus et al. 2015; Prospero et al. 2013), and may therefore not be reflective of soilborne species diversity. In addition, results from these surveys are often combined across aboveground and belowground plant samples, host species, or sample sources (e.g., plants, irrigation water, containers, and potting media or soil), making it difficult to make inferences about the diversity of soilborne oomycete pathogens that specifically affect rhododendron production (Benson et al. 1982; Bienapfl and Balci 2014; Parke et al. 2014; Schwingle et al. 2007). *Pythium* species have rarely been included in these surveys despite their potential to also cause root rot (Botha and Crous 1992; Hendrix and Campbell 1966; Weiland et al. 2018).

†Corresponding author: J. E. Weiland; Jerry.Weiland@usda.gov

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The last comprehensive survey of foliar and soilborne *Phytophthora* species in PNW nurseries showed that *Phytophthora cinnamomi* and *Phytophthora plurivora* were common on four plant hosts, including rhododendron (Parke et al. 2014). Parke et al. (2014) also showed that there was relatively little *Phytophthora* inoculum in the propagation stage of the production cycle, but it was unclear how species diversity might change once newly propagated plants are moved into subsequent container or field production systems. To obtain a more in-depth assessment of the diversity of soilborne oomycete pathogens that infect rhododendron and to get a better understanding of whether oomycete diversity changes among different systems (propagation, container, and field) in rhododendron production, a survey of soilborne *Phytophthora* and *Pythium* species of rhododendron in PNW nurseries was conducted. The objectives were to (i) document the incidence of root rot damage caused by soilborne pathogens at each nursery and stage of production, (ii) identify soilborne oomycetes infecting rhododendron in PNW nurseries, and (iii) determine whether there are differences in pathogen diversity among rhododendron nurseries and production system types.

Materials and Methods

Sample collection and processing. Rhododendrons were sampled from propagation, container, and field production systems at seven nurseries from 2013 to 2017 (nurseries A to G). Fifty to 100 samples (one sample per plant) were obtained from three nurseries for each type of system (Table 1). Plants (rooted cuttings) from propagation systems were 3 to 6 months old and were located inside greenhouses at nurseries C, D, and E. Plants from container systems were 1 to 3 years old and were located in an outdoor canyon (nursery B) or in both outdoor canyons and greenhouses (nurseries A and C). Plants from field systems were 1 to 5 years old and were always located in uncovered (no canopy), outdoor fields at nurseries B, F, and G. In each case, plants from multiple sites (benches, beds, or blocks) and cultivars were primarily sampled at each nursery during the cool, wet fall, winter, or spring months when temperatures were between 5 and 15°C to control for any potential seasonal effect. However, previous research from Oregon found little to no effect of either season or sample year on *Phytophthora* diversity from nursery crops, including rhododendron (Parke et al. 2014). Identification labels were missing or unavailable at some nurseries; therefore, it was not always possible to obtain cultivar data for many of the rhododendrons sampled. However, a frequency list of all symptomatic, identified cultivars from which isolates were obtained was compiled (Supplementary Table S1). The incidence of root rot damage was estimated from each site by counting the number of plants exhibiting symptoms of root rot (chlorosis, stunting, wilting, and plant death) per total number of plants for each cultivar affected.

Rhododendrons with symptoms of root rot were sampled at random from both container and field sites. Symptoms of root rot were

largely absent on plants at propagation facilities; therefore, a similar number of asymptomatic plants were sampled at random for this type of production system. In each production system, the entire plant was collected and all leaves and stems located 6 cm above the soil line were cut off and discarded. Soil or potting media was shaken off from the root system, roots were washed in running tap water, and necrotic plant tissues were excised from the roots or from lesions extending up into the root collar region. Similar tissues were sampled from plants without symptoms from the propagation facilities. All tissues were surface disinfested for 1 min in 0.5% NaOCl and for 30 s in 70% EtOH, and they were then air dried for up to 1 min in a laminar flow hood before plating on PARP (pimaricin, ampicillin, rifampicin, pentachloronitrobenzene), a semiselective medium for the isolation of Pythiaceae species (Kannwischer and Mitchell 1978).

A minimum of 50 isolates were obtained from each nursery × production system combination and 624 isolates were obtained in total. All isolates were identified on the basis of the internal transcribed spacer (ITS) sequence, compared with published type or authenticated sequences when available (Supplementary Table S2), and confirmed by morphology as described previously (Weiland et al. 2018). Isolates identified as *Phytophthora pini* were additionally confirmed by sequencing the translation elongation factor 1α and cytochrome c oxidase subunit I genes (Hong et al. 2011). Isolates identified as *Phytophthora plurivora* were confirmed using a PCR-restriction fragment length polymorphism assay (Knaus et al. 2015). *Pythium oopapillum/dissotocum* hybrids were identified by ITS sequences alone.

Data analyses. Frequency data (disease incidence and isolation frequency) were analyzed using a χ^2 test of independence with Yates' correction (Mantel 1974) using Statistica (version 13; TIBCO Software Inc.). Species richness curves, computed with EstimateS version 9.1.0 (Colwell 2013), were used to evaluate whether sampling detected the majority of species at each nursery. The *Phytophthora* and *Pythium* species occurring in each nursery were counted and diversity was quantified by calculating species richness, abundance, Shannon's diversity index, and the inverse Simpson index (Morris et al. 2014). Differences in mean alpha diversity among systems were determined using analysis of variance (ANOVA) of the Shannon and inverse Simpson indexes followed by a post hoc Tukey's honestly significant difference test. The Levene test for homogeneity of variance and the Shapiro-Wilk test for normality were used to test for deviations in the data from the assumptions of ANOVA. Nonmetric multidimensional scaling of Bray-Curtis dissimilarities was used to visualize the differences in community composition between samples using the function metaMDS from the vegan R package (Oksanen et al. 2019).

Results

The incidence of root rot varied among nurseries and systems (Table 1). Propagation systems had the least amount of root rot (0

Table 1. Incidence of root rot across three production systems at seven rhododendron nurseries

Production stage	Nursery	Symptomatic plants (n)/total number of plants (%) ^y	Range ^z
Propagation	C	0/600 (0) a	0–0
	D	5/550 (1) a	0–10
	E	0/980 (0) a	0–0
Container	A	22/191 (12) c	4–25
	B	107/781 (14) c	4–50
	C	36/239 (15) c	6–36
Field	B	63/205 (31) d	7–90
	F	331/808 (41) e	5–90
	G	46/1,820 (3) b	1–25
Propagation	All	5/2,130 (<1) A	0–10
Container	All	165/1,211 (14) B	4–50
Field	All	440/2,833 (16) B	1–90

^y Number of rhododendrons with symptoms of root rot/total number of plants (%). Values within a column followed by different lowercase letters or uppercase letters are significantly different at $P < 0.050$.

^z Range of disease incidence observed across sites at each nursery or for each system type.

to 10% incidence), with only five symptomatic plants occurring at nursery D. Container systems, on the other hand, had low to moderate amounts of root rot (4 to 50%), depending on the site sampled. Field systems at nurseries B and F had several sites with a high incidence of root rot (up to 90%), resulting in a greater number of symptomatic plants overall compared with container systems. However, the field system at nursery G had the lowest incidence of root rot compared with any other container or field system surveyed.

A total of 31 oomycete taxa (11 *Phytophthora* and 20 *Pythium* species) were identified from the 624 isolates obtained during the survey (Table 2). The genus *Pythium* was isolated twice as often (67%) as the genus *Phytophthora* (33%), with *Pythium cryptoirregularis* (32%), *Pythium* aff. *macrosporum* (13%), *Pythium dissotocum* (6%), *Pythium irregulare* (4%), *Pythium attrantheridium* (3%), and *Pythium sylvaticum* (3%) being the most frequently detected *Pythium* species. *Phytophthora plurivora* (14%), *Phytophthora cinnamomi* (10%), *Phytophthora pseudocryptogea* (3%), and *Phytophthora cambivora* (2%) were the most commonly isolated *Phytophthora* species. These 10 oomycete species accounted for 90% of all isolates found. Species richness

curves approached an asymptote, indicating that few additional species would be detected if the sampling effort were increased (Fig. 1).

There were broad shifts in the richness and abundance of both pathogen genera among production systems. Overall oomycete species richness (across all *Phytophthora* and *Pythium* species) was approximately double in field systems compared with either propagation or container systems (Fig. 1). This was primarily attributable to the large increase in the number of *Phytophthora* species isolated from field systems while the number of *Pythium* species remained relatively constant across the three system types (Table 2). *Pythium* dominated propagation systems, accounting for >92% of all species and isolates obtained (Figs. 2 and 3). *Phytophthora cinnamomi* was the only *Phytophthora* species isolated from this system type (nursery D only). Although the number of *Pythium* species remained fairly constant across system types (Table 2), the genus *Pythium* was isolated less frequently from container (60% of all isolates) and field systems (47%) than from propagation systems (98%) (Figs. 2 and 3). *Phytophthora* species, on the other hand, were isolated more frequently from container (40% of all isolates) and field

Table 2. *Phytophthora* and *Pythium* species isolated from rhododendron plants in propagation, container, and field nursery production systems at seven rhododendron nurseries

Genus and species	Isolation frequency (% total)	Total isolates (n)	Isolates (n)		
			Propagation	Container	Field
<i>Phytophthora</i> ^y					
<i>Phytophthora cambivora</i>	2.4	15 b	0 A	0 A	15 bB
<i>Phytophthora chlamydospora</i>	0.6	4 a	0 A	0 A	4 aB
<i>Phytophthora cinnamomi</i>	9.5	59 c	4 A	21 bB	34 dC
<i>Phytophthora crassamura</i>	0.2	1 a	0	0	1 a
<i>Phytophthora pseudocryptogea</i>	3.4	21 b	0 A	0 A	21 bcB
<i>Phytophthora megasperma</i>	0.5	3 a	0	0	3 a
<i>Phytophthora pini</i>	0.8	5 a	0 A	0 A	5 abB
<i>Phytophthora plurivora</i>	14.4	90 d	0 A	53 cC	37 dB
<i>Phytophthora syringae</i>	0.2	1 a	0	1 a	0 a
<i>Phytophthora taxon raspberry</i>	0.3	2 a	0	0	2 a
<i>Phytophthora</i> × <i>stagnum</i>	0.3	2 a	0	0	2 a
<i>Pythium</i>					
<i>Pythium</i> aff. <i>attrantheridium</i>	1.8	11 cde	11 bcB	0 A	0 A
<i>Pythium</i> aff. <i>cederbergense</i>	0.2	1 a	1 a	0	0
<i>Pythium</i> aff. <i>macrosporum</i>	12.7	79 h	55 dB	15 bA	9 bA
<i>Pythium</i> aff. <i>rostratum</i>	0.3	2 ab	2 a	0	0
<i>Pythium</i> aff. <i>undulatum</i>	0.3	2 ab	0	2 a	0
<i>Pythium anandrum</i>	0.3	2 ab	0	0	2 ab
<i>Pythium attrantheridium</i>	2.9	18 def	0 A	0 A	18 bB
<i>Pythium cryptoirregularis</i>	31.7	198 i	82 eB	76 cB	40 cA
<i>Pythium dissotocum</i>	5.8	36 g	16 cA	12 bA	8 bA
<i>Pythium intermedium</i>	0.8	5 abc	4 ab	1 a	0
<i>Pythium irregulare</i>	4.0	25 fg	19 c	2 a	4 ab
<i>Pythium litorale</i>	1.3	8 bcd	0 A	0 A	8 bB
<i>Pythium mammilatum</i>	0.2	1 a	0	0	1 a
<i>Pythium oopapillum</i>	0.2	1 a	0	1 a	0
<i>Pythium oopapillum/dissotocum</i> hybrid	0.5	3 abc	3 ab	0	0
<i>Pythium</i> sp. ZSF0069	1.0	6 abc	5 ab	1 a	0
<i>Pythium spiculum</i>	0.2	1 a	0	1 a	0
<i>Pythium spinosum</i>	0.2	1 a	1 a	0	0
<i>Pythium sylvaticum</i>	3.2	20 ef	1 aA	0 A	19 bB
<i>Pythium undulatum</i>	0.2	1 a	0	0	1
Total species ^z		31	13	12	20
<i>Phytophthora</i>	35.5	11 a	1 aA	3 aAB	10 aB
<i>Pythium</i>	64.5	20 b	12 bA	9 bA	10 aA
Total isolates		624	204	186	234
<i>Phytophthora</i>	32.5	203 a	4 aA	75 aB	124 aC
<i>Pythium</i>	67.5	421 b	200 bA	111 bB	110 aB
Isolates of species <3% of total		21	25	26	18
<i>Phytophthora</i>	28.6	7 a	10 aA	9 aA	5 aA
<i>Pythium</i>	71.4	14 a	15 aA	17 bA	13 bA

^y Numbers of isolates for each species with lowercase letters within a column and genus and uppercase letters within a row are significantly different ($P < 0.050$).

^z Numbers of isolates for each genus with lowercase letters within a column and uppercase letters within a row are significantly different ($P < 0.050$).

systems (53%) than from propagation systems (2%), with most species of *Phytophthora* occurring only in field systems (8 of 11 or 73% of *Phytophthora* species). Diversity indices showed similar trends (Fig. 4).

There were also shifts in the presence and abundance of individual species within each genus depending on the system type (Table 2). For the genus *Pythium*, *Pythium cryptoirregulare*, *Pythium dissotocum*, *Pythium irregulare*, and *Pythium* aff. *macrosporum* were the only four species found in all three system types. However, *Pythium* aff. *macrosporum* and *Pythium irregulare* were more common in propagation systems, whereas *Pythium dissotocum* was isolated with similar frequency across all three system types. *Pythium cryptoirregulare* was isolated in approximately equal numbers from both propagation and container systems but less often from field systems. However, as a percentage of total *Pythium* isolates, *Pythium cryptoirregulare* was isolated more frequently from container systems (76 of 111 or 68%) than from either propagation (82 of 200 or 41%) or field systems (40 of 110 or 36%) ($P < 0.001$). Lastly, *Pythium* aff. *atrantheridium* was only found in propagation systems, whereas *Pythium atrantheridium*, *Pythium litorale*, and *Pythium sylvaticum* were mostly or only isolated from field systems.

For the genus *Phytophthora*, *Phytophthora cinnamomi* was the only species present in all three system types (Fig. 3; Table 2). It was rarely detected in propagation systems (only four plants from nursery D) and was isolated in greater numbers from field systems than container systems. However, as a percentage of total *Phytophthora* isolates, *Phytophthora cinnamomi* was isolated with equal frequency from both systems (container = 21 of 75 or 28%, field = 34 of 124 or 27%) ($P = 0.929$). *Phytophthora plurivora* was the most common *Phytophthora* species encountered in the survey ($P < 0.050$) but was never isolated from propagation systems. Based on both isolate counts and as a

percentage of total *Phytophthora* isolates, *Phytophthora plurivora* was isolated much more frequently from container systems (71%) than from field systems (30%) ($P < 0.001$), although much of this was attributable to the large number of *Phytophthora plurivora* isolates detected at nursery A (Table 3). The remaining nine *Phytophthora* species were only isolated from a single system (container or field), with the majority coming from field plants. Results were generally similar as described for shifts in *Pythium* and *Phytophthora* species abundances between the propagation system and container system at nursery C and between the container system and field system at nursery B (Table 3).

Species communities varied among nurseries but to a lesser degree than by system (Fig. 5; Table 3). *Pythium cryptoirregulare* was the only oomycete species found in all seven nurseries, whereas *Pythium dissotocum*, *Pythium* aff. *macrosporum*, *Phytophthora cinnamomi*, and *Phytophthora plurivora* were the next most frequently encountered species found at five or six nurseries each. Nursery A had the least oomycete richness of any of those surveyed, with only two *Phytophthora* species (*Phytophthora cinnamomi* and *Phytophthora plurivora*) and a single *Pythium* species (*Pythium cryptoirregulare*). Nursery F, on the other hand, had the greatest oomycete richness, with eight *Phytophthora* and nine *Pythium* species. Nursery F was also the only container or field nursery where *Phytophthora cinnamomi* was not isolated from any plants and had the least number of *Phytophthora plurivora* isolates of any nursery where this pathogen was present.

Discussion

This study represents the first survey focused on soilborne, root-infecting *Phytophthora* species of rhododendron since 1974 (Hoitink and Schmitthenner 1974b). Although many other studies since then

Fig. 1. Species richness curves A, across all pathogens, nurseries, and production systems; B, by pathogen genus; C, by production system; and D, by nursery. S(est) = estimated number of species and CI = confidence interval.

have indirectly reported on the diversity of soilborne *Phytophthora* species that infect rhododendron, results from those studies were often combined across hosts and sample types and this makes it difficult to make inferences about the community of root-infecting oomycetes that are specific to rhododendron production. Our results confirm that *Phytophthora cinnamomi* and *Phytophthora plurivora* are the most common *Phytophthora* species causing root rot of rhododendron (Weiland et al. 2018). This finding matches observations by Parke et al. (2014), who found that both species are common root pathogens of *Kalmia*, *Pieris*, *Rhododendron*, and *Viburnum* species in Oregon nurseries. Our finding also matches observations by Hoitink and Schmitthenner (1974b), who commonly isolated both

Phytophthora cinnamomi and *Phytophthora citricola* sensu lato. Their survey was conducted before *Phytophthora citricola* sensu lato was split into several other species, including *Phytophthora plurivora*, *Phytophthora pini*, and *Phytophthora citricola* sensu stricto (Hong et al. 2011; Jung and Burgess 2009), so it is impossible to know whether they might also have isolated *Phytophthora plurivora* specifically. In addition, Hoitink and Schmitthenner (1974b) isolated *Phytophthora cinnamomi* much more frequently during their survey than any other *Phytophthora* species and concluded that this pathogen was the most important species causing rhododendron root rot. This is in contrast to our observation that *Phytophthora plurivora* was more common than *Phytophthora cinnamomi*, which indicates

Fig. 2. Percentage of isolates and species of *Phytophthora* and *Pythium* from rhododendron plants in propagation, container, and field nursery production systems. Numbers in parentheses above columns are the total numbers for each group. **A and B.** Data for each genus across all nurseries in each production system. Significant ($P < 0.050$) differences between genera within a production system are denoted by lowercase letters and differences among production systems within a genus are denoted by uppercase letters. **C and D.** Data for each genus from each nursery. Significant ($P < 0.050$) differences between genera within a nursery denoted by lowercase letters and differences among nurseries within a production system and genus are denoted by uppercase letters.

that a shift in the diversity of rhododendron *Phytophthora* root pathogens has occurred since 1974. Our results were also different from surveys of *Phytophthora* species from rhododendron foliage and stems, which tended to more frequently find *Phytophthora citrophthora*, *Phytophthora syringae*, or *Phytophthora cambivora*, as well as *Phytophthora plurivora* (or *Phytophthora citricola* sensu lato) (Bienapfl and Balci 2014; Knaus et al. 2015; Parke et al. 2014; Prospero et al. 2013; Warfield et al. 2008). This highlights the ability of *Phytophthora plurivora* to infect both aboveground and belowground plant tissues (Jung and Burgess 2009; Weiland et al. 2010, 2018), a feature that may have contributed to this species becoming more common on rhododendron over time in comparison with *Phytophthora cinnamomi*, a primarily soilborne species. Analyses of the population structure of *Phytophthora plurivora* isolates from nurseries A, B, and C have further shown that most isolates are representative of a single clonal lineage (Carleson et al. 2019), providing additional support for the relatively recent spread of this species in the U.S. nursery industry (Beaulieu et al. 2017; Schoebel et al. 2014).

This study was the first to assess *Pythium* species diversity in rhododendron production and to show that *Pythium cryptoirregularis* and *Pythium* aff. *macrosporum* are the most common *Pythium*

species isolated from rhododendron roots. Isolates of *Pythium cryptoirregularis* caused mild damage to two rhododendron cultivars in an earlier pathogenicity experiment (Weiland et al. 2018). *Pythium* aff. *macrosporum*, likely a new, undescribed *Pythium* species, was also detected in the soil of PNW forest nurseries and is capable of causing significant damping-off of Douglas-fir (*Pseudotsuga menziesii*) seedlings (Weiland 2011; Weiland et al. 2013). However, the ability of *Pythium* aff. *macrosporum* and other *Pythium* species identified from this survey to cause disease on rhododendron has not yet been tested. Root damage by these pathogens is expected to be minor given the lack of severe root rot in propagation houses where these pathogens were frequently isolated. Most *Pythium* species are considered mild pathogens that primarily cause root rot or damping-off in juvenile plant material, including cuttings (Moorman 2003; Weiland et al. 2018). Although *Pythium dimorphum* was identified as a cause of severe root rot of rhododendron in the past (Ho 1986), this pathogen has been rarely isolated since the initial report and was not found during our survey. Given the high frequency of *Pythium* detection from young rhododendrons in propagation houses, further research is needed to determine whether the presence of these pathogens may reduce the ability for cuttings to root.

Fig. 3. Frequency of *Phytophthora* and *Pythium* species from rhododendron plants in propagation, container, and field nursery production systems. **A and B.** Data for each genus across all nurseries for each production system. Numbers in parentheses above columns are the total numbers for each species. Other species are those accounting for <3% of the total number of isolates for each genus. Within a species, significant ($P < 0.050$) differences among systems are denoted by lowercase letters within columns. **C.** Species data for each production system across all nurseries.

Fig. 4. Species diversity indices of *Phytophthora* and *Pythium* from rhododendron plants in propagation, container, and field production systems. **A, B, and C,** Shannon index for both genera *Phytophthora* and *Pythium*, genus *Phytophthora* only, and genus *Pythium* only, respectively. **D, E, and F,** Inverse Simpson index for both genera *Phytophthora* and *Pythium*, genus *Phytophthora* only, and genus *Pythium* only, respectively. Significant ($P < 0.050$) differences between production systems, determined by analysis of variance followed by the Tukey's honestly significant difference test, are denoted by lowercase letters within each panel.

Table 3. Five most common *Phytophthora* and *Pythium* species isolated from rhododendron plants in three propagation (C, D, and E), container (A, B, and C), and field (B, F, and G) nurseries

Genus and species	Isolates (n)								
	Propagation			Container			Field		
	C	D	E	A	B	C	B	F	G
<i>Phytophthora</i> ^y									
<i>Phytophthora cambivora</i>	—	—	—	—	—	—	1 aA	14 bB	0 aA
<i>Phytophthora cinnamomi</i>	0	4	0	1 aA	1 aA	19 cB	17 bB	0 aA	17 bB
<i>Phytophthora pseudocryptogea</i>	—	—	—	—	—	—	5 aB	16 bC	0 aA
<i>Phytophthora pini</i>	—	—	—	—	—	—	0 aA	0 aA	5 aA
<i>Phytophthora plurivora</i>	—	—	—	36 bB	10 bA	7 bA	16 bB	2 aA	19 bB
<i>Pythium</i>									
<i>Pythium aff. macrosporum</i>	1 aA	2 aA	52 cB	0 aA	15 cB	0 aA	1 aA	8 bB	0 aA
<i>Pythium attrantheridium</i>	—	—	—	—	—	—	17 bB	1 aA	0 aA
<i>Pythium cryptoirregulare</i>	34 cB	32 bB	16 bA	13 aA	35 dB	28 bB	16 bB	2 abA	22 cB
<i>Pythium dissotocum</i>	5 aAB	0 A	11 bB	0 aA	8 bcB	4 aAB	3 aA	4 abA	1 aA
<i>Pythium irregulare</i>	19 bB	0 aA	0 aA	0 aA	1 aA	1 aA	0 aA	3 abA	1 aA
Total species ^z	5	6	8	3	11	6	11	17	9
<i>Phytophthora</i>	0 a	1 a	0 a	2 aA	3 aA	2 aA	6 aA	8 aA	4 aA
<i>Pythium</i>	5 bA	5 aA	8 bA	1 aA	8 aA	4 aA	5 aA	9 aA	5 aA
Total isolates	60	50	94	50	76	60	83	74	76
<i>Phytophthora</i>	0 aA	4 aA	0 aA	37 bB	12 aA	26 aB	42 aA	40 aA	42 aA
<i>Pythium</i>	60 bA	46 bA	94 bA	13 aA	64 bC	34 aB	41 aA	34 aA	34 aA
Isolates of species <3% of total	9	8	6	2	4	4	7	7	5
<i>Phytophthora</i>	0 a	0 a	0 a	1 aA	3 aA	2 aA	4 aA	2 aA	3 aA
<i>Pythium</i>	9 bA	8 bA	6 bA	1 aA	4 aA	2 aA	4 aA	7 aA	2 aA

^y Numbers of isolates for each species with lowercase letters within a column and genus and uppercase letters within a row and production system are significantly different ($P < 0.050$).

^z Numbers of isolates for each genus with lowercase letters within a column and uppercase letters within a row and production system are significantly different ($P < 0.050$).

Since the first discovery of *Phytophthora* species as a cause of rhododendron root rot in 1930, research on disease development and control has focused almost exclusively on *Phytophthora cinnamomi* as the causal agent (Benson 1986, 1990; Benson and Parker 2005; Blaker and MacDonald 1981; Englander et al. 1980; Hoitink and Schmitthenner 1974a, b; Krebs 2013; Manning and Crossan 1966; McGuire and Jackson 1973; Ownley and Benson 1991; White 1930). However, our results indicate that *Phytophthora plurivora* is now at least as common as *Phytophthora cinnamomi* in both container and field systems and is also capable of causing severe root rot (Weiland et al. 2018). Other *Phytophthora* species are present and may likewise be important in causing disease, particularly at nurseries where they are more abundant. Nursery F, for example, had a very different community profile with more *Phytophthora* species than any other nursery (Table 3). This may have been attributable in part to the fact that this was the only surveyed container or field nursery that did not use fungicides to control root rot. Regardless, research on the ability of these other *Phytophthora* species to cause disease either alone or in combination with other species is needed because there are likely differences in biology, environmental responses, infection, and disease development that remain largely unexplored (Hoitink and Schmitthenner 1974b; Weiland et al. 2018). In addition, research is needed to assess whether these other *Phytophthora* species respond in a similar manner to disease control measures that were developed for *Phytophthora cinnamomi*. For example, isolates of *Phytophthora citricola* sensu lato from various hosts, including rhododendron, were generally less sensitive to metalaxyl than those of *Phytophthora cinnamomi* (Coffey et al. 1984). The active component of this fungicide, mefenoxam, is still in use for *Phytophthora* root rot control; if similar differences are observed among rhododendron isolates of *Phytophthora pini* and *Phytophthora plurivora* compared with those of *Phytophthora cinnamomi*, this could lead to a shift in soilborne *Phytophthora* communities toward more fungicide-resistant species and disease control failure. Whether other *Phytophthora* species respond in a similar manner to the host, fungicides, temperatures, or moisture regimes as *Phytophthora cinnamomi* is generally not known, and any differences may partially explain why practices developed for *Phytophthora cinnamomi* sometimes fail to adequately control root rot caused by other *Phytophthora* species in PNW nurseries (J. E. Weiland, *personal observation*).

Our disease survey results show that rhododendron root rot remains a serious problem for the rhododendron industry. For example, nursery F lost 90% of the crop from a 0.2-ha field consisting of approximately 2,000 rhododendrons aged 2 and 3 years to root rot at a cost of almost \$25,000. This nursery had the highest incidence of root rot of any nursery surveyed (41%) and was the only nursery that did not use fungicides to manage root rot. This might explain why nursery F also had the greatest number of *Phytophthora* and *Pythium* species of any nursery surveyed. In comparison, the nursery with the fewest number of detected oomycete pathogens, nursery A, uses two fungicides (mefenoxam and phosphorous acid) and a proprietary biocontrol blend to manage disease. Despite this, nursery A had the same incidence of root rot (12%) as the other two container nurseries (B and C at 14 to 15%) that use mefenoxam and phosphorous acid without biocontrol, and nursery A had more root rot than field nursery G (3%), which used both fungicides combined with frequent culling of unhealthy plants. These observations suggest that fungicides are at least somewhat effective for reducing root rot incidence and oomycete pathogen diversity, but there is still some residual oomycete pathogen activity capable of causing disease. It is therefore worth exploring how fungicides may change oomycete pathogen communities toward more fungicide-tolerant isolates in the nursery industry. Ultimately, combining fungicides with cultural and other disease control measures may provide the best control.

Symptoms of root rot were uncommon and *Phytophthora* species were rarely isolated from the rhododendron plants sampled from propagation systems in our survey. This matches observations by Parke et al. (2014), who seldom detected *Phytophthora* species from plants, media, or containers during the early stages of production. Rhododendron plants in the nurseries we surveyed were propagated only by stem cuttings and therefore posed a very low risk for moving soilborne *Phytophthora* species into propagation systems. Sanitation was also generally greater at this stage compared with container and field production stages. Cuttings were monitored frequently and any dying cuttings were usually promptly removed. In addition, most nurseries used new flats and new soilless media for each new batch of cuttings. The one exception occurred at nursery D, which was the only nursery where the genus *Phytophthora* (*Phytophthora cinnamomi*) was detected from the propagation stage. This nursery used raised wooden benches to root cuttings and the soilless rooting medium in the benches had been reused for a number of years. This likely allowed *Phytophthora cinnamomi* to survive, proliferate, and to cause root rot once it had been accidentally introduced into the system. Unfortunately, a *Phytophthora* contamination issue also developed later at nursery E (after the survey) where disease scouting and sanitation had become less stringent since the survey was originally conducted. These findings further illustrate the importance of sanitation for managing root rot, especially during early stages of production.

The sharp increase in the incidence of root rot and in the isolation of the genus *Phytophthora* from container and field systems suggests that the greatest risk for contamination comes in after the propagation stage. During the survey, we commonly observed several practices that likely introduced and spread or maintained *Phytophthora* inoculum in the production stream. Materials used to produce soilless media (sawdust, bark, perlite, pumice, or sand) were sometimes stored on open field soil next to plant production blocks where root rot was present. Ideally, these materials would be kept on concrete pads to reduce contamination by any inoculum residing in field soil. In some cases, pots were reused without proper cleaning, which could potentially transfer inoculum to the next generation of plants (Parke et al. 2014). Poor drainage, overirrigation, and leaking or malfunctioning irrigation equipment was also common, leading to frequent flooding in field soils or to puddling of water in container beds. These conditions promote the production and movement of zoospores, and they increase the likelihood of infection and spread of *Phytophthora* species through nursery beds. Similar situations have been observed in other nursery studies (Parke and Grünwald 2012).

Fig. 5. Nonmetric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities between nursery communities. Letters indicate the nursery and colors indicate the production system. Points closer together have more similar communities than points further apart.

The presence of dead or dying plants was a consistent problem in most of the surveyed nurseries that could function as a continuous reservoir of *Phytophthora* inoculum for contaminating nearby healthy plants. With the exception of nursery G, which has a rogueing program to remove unhealthy plants as soon as they are discovered, plants with symptoms of root rot were common in many of the container and field sites at the other nurseries. Although these symptomatic plants are not sold, nearby healthy plants might be contaminated with *Phytophthora* species as a result of their proximity to the infected plants (Fig. 6) and subsequently sold or possibly exported out of state. Dead plants may also have served as a direct source of inoculum at nursery A, which periodically composts dead plant material from the production stream and then uses the resulting compost as an amendment when potting new plants. If critical killing temperatures were not reached and composting was incomplete, *Phytophthora* inoculum could potentially survive and be spread to new plants. Multiple studies have shown that *Phytophthora* and *Pythium* species are frequently moved across state and national borders on infected nursery stock (Bienapfl and Balci 2014; Jung et al. 2016; Miglioni et al. 2015). Contaminated or infected plants with few or no symptoms of disease pose a significant risk for spreading soilborne pathogens throughout the nursery industry. Studies to evaluate low levels of inoculum (Weiland et al. 2018) and the potential for asymptomatic, but contaminated plants to spread soilborne pathogens and develop disease later are needed to better evaluate this risk.

In summary, our survey shows that root rot is still a significant problem in the PNW rhododendron industry. We now have a better understanding of the diversity of soilborne *Phytophthora* and *Pythium* species infecting rhododendron and have evidence for a shift in species abundance over time from *Phytophthora cinnamomi* toward *Phytophthora plurivora*. This knowledge has allowed us to compare the pathogenicity of the three most common species (*Phytophthora cinnamomi*, *Phytophthora plurivora*, and *Pythium cryptotirregulare*) using PNW isolates (Weiland et al. 2018) and enables future disease control research to focus on the root-infecting oomycete species that are the most prevalent in the industry and cause the

most damage. Disease control methods that were developed for *Phytophthora cinnamomi* may not be as effective for other *Phytophthora* species and more research is needed to explore whether there are significant differences in biology among species that affect disease development and control. Our survey also showed that most root rot damage is occurring after the propagation stage, in the container and field systems. This damage coincides with an increased presence and diversity of *Phytophthora* species and suggests that most disease control efforts should be targeted toward these production stages.

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Fig. 6. Rhododendron plants with symptoms of root rot occurring next to asymptomatic plants in **A**, a field site at nursery F and **B**, a container site at nursery B.

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