Phytophthora Community Structure Analyses in Oregon Nurseries Inform Systems Approaches to Disease Management

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ABSTRACT

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Nursery plants are important vectors for plant pathogens. Understanding what pathogens occur in nurseries in different production stages can be useful to the development of integrated systems approaches. Four horticultural nurseries in Oregon were sampled every 2 months for 4 years to determine the identity and community structure of *Phytophthora* spp. associated with different sources and stages in the nursery production cycle. Plants, potting media, used containers, water, greenhouse soil, and container yard substrates were systematically sampled from propagation to the field. From 674 *Phytophthora* isolates recovered, 28 different species or taxa were identified. The most commonly isolated species from plants were *Phytophthora plurivora* (33%), *P. cinnamomi* (26%), *P. syringae* (19%), and *P. citrophthora* (11%). From soil and gravel substrates, *P. plurivora* accounted for 25% of the isolates, with *P.* taxon Pgchlamydo, *P. cryptogea*, and *P. cinnamomi* accounting for 18, 17, and 15%, respectively. Five species (*P. plurivora, P. syringae*, *P.* taxon Pgchlamydo*, P. gonapodyides*, and *P. cryptogea*) were found in all nurseries. The greatest diversity of taxa occurred in irrigation water reservoirs (20 taxa), with the majority of isolates belonging to internal transcribed spacer clade 6, typically including aquatic opportunists. Nurseries differed in composition of *Phytophthora* communities across years, seasons, and source within the nursery. These findings suggest likely contamination hazards and target critical control points for management of *Phytophthora* disease using a systems approach.

Additional keywords: hazard analysis.

Greenhouse and nursery plants can be important vectors of invasive pests and pathogens that affect ornamental plants, agricultural crops, and forests (43). Recent examples include boxwood blight, citrus long-horned beetle, emerald ash-borer, light-brown apple moth, and *Ralstonia solanacearum* biovar 3 race 2. Several exotic *Phytophthora* spp. have spread from nursery plants to forests, where they cause epidemics on native vegetation. For example, *Phytophthora lateralis*, first reported from Seattle-area horticultural nurseries in the 1920s, spread to the native range of Port-Orford cedar (*Chamaecyparis lawsoniana*) around 1950, where it was widely dispersed in rivers and along roadways (23). The pathogen is now causing disease outbreaks on *Chamaecyparis* trees in the landscapes of France and Scotland, where it has been traced to the outplanting of trees from infested nurseries (5,49) Sudden oak death, caused by *P. ramorum*, was almost certainly introduced to North America on imported nursery plants and has subsequently been dispersed from the U.S. west coast to the east coast on shipments of nursery plants (17,19,20,35,36). As a consequence, west coast nurseries are subject to quarantines and inspections to restrict further spread, a costly and cumbersome process for state and federal regulatory agencies. In the United Kingdom, *P. ramorum* has spread from rhododendrons to forest plantations, where it is causing a large-scale epidemic on Japanese larch (7). *P. alni* in Europe, *P. austrocedrae* in Chile, and *P. kernoviae* in the United Kingdom are other examples of invasive forest pathogens caused by *Phytophthora* spp. likely to have been introduced by the horticultural plant trade (25).

Possible solutions to the threat posed by conveyance of pests and pathogens with the plant trade are to restrict the importation and movement of live plants (4), modernize plant import regulations to better reflect risks, improve inspection efficacy, require post-entry quarantines, and reduce contamination in nurseries through adoption of systems approaches for management of pests and pathogens (33,42).

In previous work, we developed a conceptual framework for how a systems approach could be applied to the management of pests and pathogens in nurseries (43). The first step in this process is to conduct an in-depth analysis of contamination hazards in the production cycle. In the current work, we describe the structure of *Phytophthora* communities discovered during a hazard analysis conducted in four Oregon commercial nurseries over a period of 4 years, including species richness and diversity partitioned within and among nurseries, contamination sources, and years. We examined the frequency of *Phytophthora* spp. isolated from different sources and stages of the production cycle, assigned them to ecological guilds (25), and sought to determine whether samples from water, plants, and soil substrates represent distinct *Phytophthora* communities. This work provides a foundation for implementation of systems approaches in nursery production systems by providing information on presence and abundance of *Phytophthora* spp. at select critical control points and by informing best management practices.

MATERIALS AND METHODS

Nursery sampling overview. Four Oregon wholesale nurseries were sampled every 2 months for 4 years. The nurseries (desig-

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nated as A, B, C, and D) ranged in size from 28 to 890 ha and all grew woody ornamentals in containers and in the field. Two of the nurseries (A and C) recycled and treated their irrigation water with sodium hypochlorite, whereas nurseries B and D used well water. All of the nurseries propagated their material from plants on site. In consultation with each nursery manager, a flow chart was made to show how plants were moved within the nursery during the production cycle. Plants at all stages of production, potting media, containers, irrigation water reservoirs (nurseries A and C), greenhouse soil, container yard soil and gravel substrates, and field soil were sampled on every visit. Irrigation water was sampled during months when the irrigation system was turned on, typically starting in March or April and ending in October or November.

Plant samples. Five plants from each of four *Phytophthora*-susceptible genera (*Rhododendron, Pieris, Kalmia,* and *Viburnum*) commonly grown in Oregon were collected on each sampling date for processing in the lab. Symptomatic plants were selected to maximize the likelihood that *Phytophthora* spp. would be detected. If symptomatic plants were not found, asymptomatic plants were sampled instead. To recover the greatest diversity of *Phytophthora* spp. from the nursery, the study was expanded during the final year to include additional symptomatic host plant genera found in all four nurseries. Leaves, stems, and roots were washed in tap water to remove potting media or soil, and blotted on paper towels. Leaf pieces from the margin of necrotic areas were plated onto *Phytophthora* selective medium PAR (28). Tissue pieces from stems and roots were plated onto the same medium supplemented with Terraclor (75% pentachloronitrobenzene; 66.7 mg liter^{-1}) and hymexazol (25 mg liter^{-1}) added to reduce growth of soilborne fungi and *Pythium* spp. Plates were incubated for 7 to 10 days at room temperature (18 to 20°C) and observed for colony morphology and structures consistent with *Phytophthora* spp. Isolates representing each morphological type were transferred to fresh media to obtain clean cultures for species identification.

Water samples. Reservoirs from nurseries A and C, containing recycled irrigation water that had not yet been treated with sodium hypochlorite, were baited in situ by floating mesh bags enclosing five *Rhododendron* 'Nova Zembla' leaves on the reservoir surface. Leaves were from plants grown in the Oregon State University greenhouses not treated with pesticides. Baits were retrieved after 2 days and transported to the lab, where petioles were rinsed, blotted, and plated on PAR. Except for midwinter, irrigation water was also collected from sprinkler heads that delivered water to the sampled plants. Samples were collected in 1-gal. (3.78-liter) plastic jugs after the irrigation water had been flowing for at least 20 min. Water samples were transported to the lab, and rhododendron leaf baits were placed into the containers. After 48 h at room temperature (18 to 20°C), leaves were removed, rinsed in deionized (DI) water, and blotted, and the petioles were removed and plated onto PAR. Bait tissue was observed for outgrowth of putative *Phytophthora* colonies and transferred to fresh media as described above.

Soil and potting media samples. Potting media from container plants, potting media ingredients, soil and gravel substrates from container yards, and field soil were sampled and transported to the lab. Each sample (≈500 ml) was placed in a 1-gal. Ziploc bag with 1 liter of DI water and plated with rhododendron leaves as described above.

Used container samples. Used containers stacked and ready for reuse by the nurseries were scraped to remove old potting media and residual plant debris sufficient to obtain at least 100 ml of material. In the lab, this material was mixed with 200 ml of DI water and baited as described above.

Isolate identification. Isolates for inclusion in this study were identified to species by sequencing of the internal transcribed spacer (ITS) of the ribosomal DNA and querying these sequences in *Phytophthora*-ID, as described previously (21). Primers and polymerase chain reaction (PCR) conditions were as previously reported for ITS (5,8,21). Sequences were queried against the Phytophthora-ID database (http://phytophthora-id.org/) (21) using the blastn search algorithm (2). Samples with a sequence similarity of 99.5% or higher to a type specimen's sequence, where available, or the closest match as specified in Grünwald et al. (21), were considered a positive identification. Samples with 99 to 99.5% similarity were named based on the closest match, and designated as "-like".

For some isolates, the ITS region did not amplify and percent similarity could not be determined. These included isolates in clades 6, 7 and 9 (3) and most often contained haplotypes of hybrid origin. These sequences were cloned to determine haplotype phase using the TOPO TA Cloning (Invitrogen Corporation, Carlsbad, CA) or the pGEM-T Easy Vector System (Promega Corporation, Madison, WI), as described previously (17,18). Isolates containing two diverged haplotypes with similarity to separate taxa were labeled as putative hybrids. Some haplotypes could not be matched to any parental species and were designated "-like" in reference to the closest match.

ITS sequences alone are not sufficient to distinguish *P. pini* from *P. citricola* III (27) or *P. bilorbang* from *P.* taxon Oaksoil (1). Isolates with ITS sequences matching these taxa were designated as *P. pini*/*citricola* III or *P. bilorbang*/taxon Oaksoil, respectively.

Rarefaction, diversity, and community analyses. *Phytophthora* communities were analyzed by looking at species, diversity, richness, and evenness. Rarefaction curves representing species richness were calculated to determine whether the sampling intensity was adequate to detect the majority of *Phytophthora* taxa present in each nursery. Because sample size varied among nurseries, we employed rarefaction to explore the effect of sample size on observed species richness. The function "rarecurve" from the R (46) package vegan (40) was used to subsample nurseries, generate rarefaction curves, and summarize nursery diversity. Diversity indices included the sample size, richness (number of species), Shannon-Wiener index (a diversity metric that quantifies the ability to predict taxa based on an existing sample), evenness (the Shannon-Wiener index divided by its theoretical maximum), and Simpson's index (i.e., the probability that two individuals sampled at random will be different species).

To address the hypotheses of whether diversity is evenly partitioned among nurseries, source, year, or season, we used additive diversity partitioning as implemented in the R package vegan (34) and discussed by Crist et al. (9) and Lande (30). Among-group diversity (β diversity) can be derived from within-group diversity (α diversity) subtracted from total diversity (γ diversity). This can be made hierarchical by using α diversity at the next highest level as γ diversity for that level. We used nursery, collection source (media or used containers, plant, soil or gravel, or water), year, and season as hierarchical levels, with nursery being the highest level. Some taxa were only observed in one nursery. This is expected to increase the distinctiveness of nurseries containing these unique taxa. To avoid this, we removed taxa from the analysis that were only observed in one nursery. To correct for a potentially unbalanced sample, we used diversity estimates weighted by their proportionality. To test for significance, we randomly assigned samples among the categories and derived our diversity estimates again, repeating this for a total of 999 times.

To further compare the *Phytophthora* communities among sources, ordination was performed. Samples were defined by nursery, source (water; plant; or soil, gravel, media, or used containers), year, and season and were ordinated by using taxon counts as descriptors for each sample. A distance matrix was constructed using Bray-Curtis distance as implemented by the function "vegdist" (40). Samples with no observed *Phytophthora*

taxa are uninformative and were omitted from the ordination. Some pairwise comparisons resulted in values of zero in the distance matrix. Because these are problematic, they were replaced with a trivially small number (10^{-8}). Ordinations were performed with the R function "isoMDS" (46). Ordinations appeared to have problems with convergence. To explore this, 100 ordinations were performed using a random starting configuration and a maximum iteration number of 900. Plots of the resulting stress indicated a bimodal pattern of ordinations with a stress near 20.3 and 32.3. When the ordination was constrained to one iteration (its starting configuration), stress was ≈35. This suggests that the ordinations which resulted in a stress of ≈32 may have encountered a local optimum and failed to improve much from a random configuration. Ordinations with a stress ≈20 appear to minimize the stress and appear to be a better solution. The ordination with the lowest stress, 19.405, is presented.

Ecological guilds. A guild is a group of species that exploit the same resource in a similar way (53). *Phytophthora* taxa were divided into guilds (i) water, (ii) plant-associated, and (iii) soil, gravel, media, or used containers based on the habitats from which the majority of isolates for each taxon were recovered.

RESULTS

Overall summary. Of the 6,811 cultures isolated during the 4-year study, 1,269 isolates (18.6%) were submitted for molecular identification and 674 of those were identified as *Phytophthora* isolates belonging to 28 taxa (Table 1). These included 20 species, 3 recognized taxa, 1 undescribed taxon, and 4 hybrids: *P. lacustris*/taxon Pgchlamydo, *P. lacustris*/*P. riparia*, a *P. cambivora*-like hybrid, and a *P. parsiana*-like hybrid. ITS sequences representing each of the 28 taxa were submitted to GenBank. *P. cryptogea, P. gonapodyides, P. plurivora, P. syringae*, and taxon Pgchlamydo were found in all four nurseries (Table 1). Several taxa were encountered only rarely: *P. foliorum, P. inundata, P. lateralis, P. obscura, P. parsiana*, and the *P. cambivora*-like hybrid were detected only once; *P. cactorum, P. nemorosa, P.* taxon Pgchlamydo-like, and *P.* taxon Raspberry were detected twice. Overall, the most frequently encountered species were *P. plurivora* (22% of all isolates), *P. cinnamomi* (16%), *P. syringae* (11%), taxon Pgchlamydo (9%), and *P. citrophthora* (8%) (Table 1). *P. cinnamomi* and *P. citrophthora* were among the most abundant taxa but were only observed at three of the four nurseries.

Despite the fact that nurseries A and C used recycled water for irrigation, the species composition at nurseries A and B were most similar in being dominated by the plant-pathogenic species *P. cinnamomi, P. citrophthora, P. plurivora,* and *P. syringae* (Table 1). Nursery C differed from A and B by the near absence of *P. cinnamomi*. Nursery D had much less species diversity overall, with no *P. cinnamomi* or *P. citrophthora*, and with the great majority of isolates being either *P. plurivora* (66%) or *P. syringae* (23%). Taxon Pgchlamydo represented 5 to 14% of the isolates in each of the nurseries.

Rarefaction curves (Fig. 1) suggested that sampling intensity was adequate to detect a majority of the *Phytophthora* taxa present in each of the nurseries. Nurseries A and C had a higher number of samples and observed taxa relative to nurseries B and D. This was largely due to the reservoirs for recycled water in nurseries A and C, which resulted in a greater number of observed taxa (Fig. 2; Table 2). A sample size of 122 (the sample size of nursery B) was common to all nurseries. Comparison of rarefaction curves demonstrates that the observed differences in diversity among these nurseries is robust at this common sample size (Fig. 1), with nurseries A and C having comparable amounts of diversity as well as being more diverse than nurseries B and D, respectively.

Differences between nurseries, sources, years, and seasons. To better understand differences in diversity among nurseries as well as differences within nurseries, we employed additive partitioning of diversity. Additive partitioning of diversity is analogous to an analysis of variance where the independent variables are defined by hierarchical arrangements of the samples

TABLE 1. *Phytophthora* taxa isolated from each nursery

Phytophthora taxon	GenBank[a]	Number of isolates in each nursery					Number of nurseries
		A	B	C	D	Sum	
bilorbang/taxon Oaksoil	KJ405927	2	…	1	…	3	2
cactorum	KJ405928	…	…	1	1	2	2
cambivora	KJ405929	3	…	…	1	4	2
cambivora-like hybrid	KJ405930	0	1	0	0	1	1
cinnamomi	KJ405931	56	48	2	…	106	3
citrophthora	KJ405932	23	10	21	…	54	3
cryptogea	KJ405933	11	8	16	3	38	4
drechsleri	KJ405934	…	…	3	…	3	1
foliorum	KJ405935	1	…	…	…	1	1
gonapodyides	KJ405936	11	1	24	1	37	4
inundata	KJ405937	…	…	1	…	1	1
lacustris	KJ405938	15	…	13	…	28	2
lacustris/Pgchlamydo hybrid	KJ405939	8	…	31	…	39	2
lacustris/*riparia* hybrid	KJ405940	2	…	13	…	15	2
lateralis	KJ405941	1	…	…	…	1	1
megasperma	KJ405942	1	4	2	…	7	3
nemorosa	KJ405943	…	…	2	…	2	1
obscura	HQ917911	…	…	…	1	1	1
parsiana	KJ405944	…	…	1	…	1	1
parsiana-like hybrid	KJ405949	6	0	14	0	20	2
pini/*citricola* III	KJ405945	6	1	8	…	15	3
plurivora	KJ405946	21	12	32	86	151	4
riparia	KJ405947	…	…	3	…	3	1
syringae	KJ405948	21	18	5	30	74	4
Taxon Pgchlamydo	KJ405950	12	15	25	7	59	4
Taxon Pgchlamydo-like	KJ405951	0	2	0	0	2	1
Taxon Raspberry	KJ405952	1	0	1	0	2	2
Taxon Walnut	KJ405953	2	2	0	0	4	2
Sum	…	203	122	219	130	674	…

[a] GenBank internal transcribed spacer accession number.

(i.e., observed within nursery taxa, observed within source among nursery, and so on) and the response, or dependent variable, is a measure of diversity (e.g., Shannon-Wiener, Simpson, and so on). Additive partitioning of diversity allows us to dissect differences among nurseries into categorizations within each nursery to identify factors which contribute to these observed differences.

The diversity of *Phytophthora* communities within each nursery varied dramatically. Shannon-Wiener indices were 0.64 to 2.55, evenness was 0.48 to 0.96, and Simpson's index was 0.44 to 0.91 (Table 2). Evenness and Simpson's indices have a theoretical range from 0 to 1, such that maximal diversity is observed as these metrics approach 1 and minimal diversity is observed as the metrics approach 0. Differences in diversity among collection source for samples demonstrated differences (Table 2). Media or used containers represented the lowest diversity source in all but one nursery. Water was the most diverse sample source for the two nurseries (A and C) which used recirculated water. It is notable that the diversities for these water samples were the greatest diversities observed for any source in the sample (Table 2).

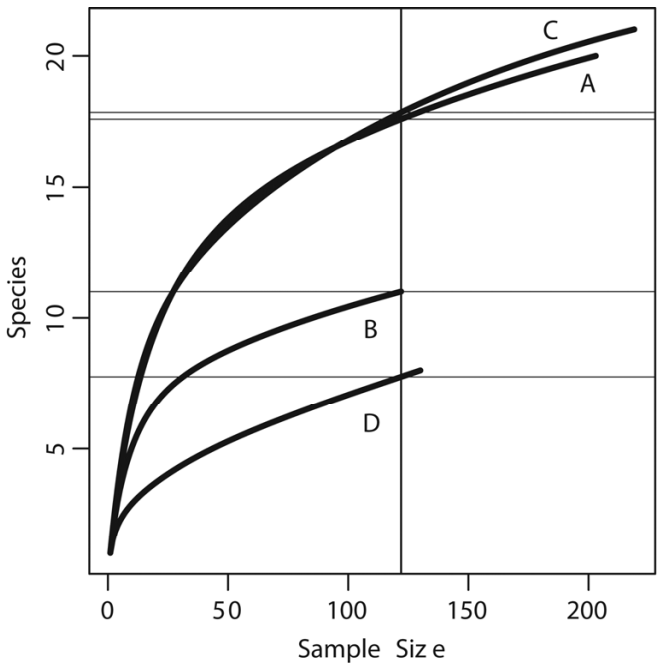

Fig. 1. Rarefaction curves for sampling *Phytophthora* isolates in nurseries A, B, C, and D.

Differences among nurseries. Among nurseries, diversities differed significantly from an even distribution of diversity (Table 3). This is due, in part, to the contribution of waterborne taxa observed in the water samples from the two nurseries using recycled water (nurseries A and C). Sources other than water appeared to contribute to the observed diversity as well. For example, plant-associated samples from nursery A demonstrated a large amount of diversity (Fig. 2), as did plant-associated samples from nurseries B and C. A final factor contributing to this deviance from an even distribution of diversity is the low diversity observed in nursery D.

Differences among sources of samples. Differences among the source of the samples (i.e., samples of symptomatic tissue or baited from media, soil, or water) within nurseries also deviated significantly from a uniform distribution of diversity (Table 3). Factors discussed above, which contributed to among-nursery differences, are similarly relevant here. Samples baited from water may have contributed to the diversity observed in the dataset more than any other category but this was driven largely by samples from nursery C (Fig. 3). Samples derived from plant tissue also contributed to the observed diversity, yet this pattern appears to have been largely driven by samples from nurseries A and B (Fig. 3). Samples derived from soil or gravel also contributed to differences among sources but at a lower magnitude than water- or plant-based samples.

Differences among years. Differences in diversity within nurseries and within sources but barely among year were also significantly different from a uniform distribution of diversity (Table 3). Heterogeneity in diversities and abundances appears common throughout the sample (Fig. 2). For example, much of the diversity observed in nursery C can be attributed to the year 2008. A similar amount of diversity was also observed in nursery C during 2009 but at much lower abundances. A similar pattern is

TABLE 2. Abundance and diversity summary for four Oregon nurseries

Nursery	Source of isolates	*n*	Richness[a]	Shannon[b]	Evenness[c]	Simpson[d]
A	Total	203	19	2.39	0.81	0.87
	Media or used containers	3	2	0.64	0.92	0.44
	Plant	120	11	1.74	0.73	0.75
	Soil or gravel	24	11	2.16	0.90	0.86
	Water	56	13	2.23	0.87	0.86
B	Total	122	12	1.88	0.81	0.79
	Media or used containers	6	4	1.33	0.92	0.72
	Plant	75	7	1.44	0.73	0.70
	Soil or gravel	41	8	1.78	0.90	0.80
	Water	0	0	0.00	0.87	1.00
C	Total	219	21	2.55	0.84	0.91
	Media or used containers	5	3	1.06	0.96	0.64
	Plant	56	10	1.74	0.75	0.75
	Soil or gravel	16	7	1.73	0.89	0.80
	Water	142	15	2.26	0.83	0.87
D	Total	130	8	1.01	0.48	0.51
	Media or used containers	4	3	1.04	0.95	0.35
	Plant	94	5	0.77	0.48	0.45
	Soil or gravel	32	6	1.20	0.67	0.57
	Water	0	0	0.00	0.00	1.00

[a] Richness: number of species.
[b] Shannon-Wiener index: index of species diversity.
[c] Evenness: Shannon-Wiener index divided by the natural log of the mean of Shannon-Wiener index.
[d] Simpson's index: probability that two individuals sampled at random will be different species.

observed in nursery A, where much of its diversity occurred during 2008 and 2009; however, in this nursery, abundances were similar for both years.

Comparison of *Phytophthora* communities from different sources. Nonmetric multidimensional scaling resulted in an ordination with a final stress of 19.4 (Fig. 3). Samples from water; plant; or soil, gravel, media, or used containers were not differentiated along the first axis. However, samples derived from water were differentiated from the other sources along axis 2 (Fig. 3). This indicates that samples derived from water consisted of communities that differed in taxonomic composition from samples derived from other sources.

Species diversity at different stages of production and sample types. *Phytophthora* spp. were rarely recovered from the early propagation phase or from materials used in the initial planting of cuttings or tissue culture plantlets (Table 4). Only 3.5% of the total isolates representing seven taxa were associated with media ingredients, used containers, or irrigation water (Fig. 4). Nearly 17% of the isolates representing 15 taxa were associated with soil or gravel substrates in greenhouses, can yards, and field soils. Over 41% of the isolates (16 taxa) were associated with plant leaves, stems, roots, or the container media surrounding the roots. *P. syringae* and *P. plurivora* were the dominant species recovered from leaves; *P. cinnamomi, P. plurivora,* and *P. citrophthora* were the dominant species recovered from roots; and stems represented a combination of the two populations. The greatest diversity of *Phytophthora* isolates was found in the water reservoirs (nurseries A and C only), with 17 taxa representing 28.8% of the total isolates. Of the water isolates, the majority (49%) are characterized in the literature as aquatic opportunists (*P. gonapodyides, P. lacustris*, taxon Pgchlamydo, *P. riparia*, *P. bilorbang*/taxon Oaksoil, *P.* taxon Raspberry, and *P.* taxon Walnut), five taxa (37% of isolates) are newly reported and pathogenicity is unknown (the *P. lacustris*-Pgchlamydo hybrid, *P. lacustris-P. riparia* hybrid, *P. parsiana*-like hybrid, the *P. cambivora*-like hybrid, and *P.* taxon Pgchlamydo-like), and only seven taxa (14%) are recognized as plant-pathogenic species (*P. citrophthora, P. cryptogea, P. drechsleri, P. parsiana, P. pini/citricola* III*, P. plurivora,* and *P. syringae*). The aquatic population was dominated by *P. lacustris* (formerly taxon Salixsoil) (39), *P. lacustris*-Pgchlamydo hybrids, taxon Pgchlamydo, and *P. gonapodyides*. The dominant plant-associated species were poorly represented in the water samples; *P. plurivora, P. syringae*, and *P. citrophthora* were rarely detected, and *P. cinnamomi* was never recovered from water.

Ecological guilds. The proportion of *Phytophthora* isolates for each taxon recovered from various habitats is shown in Figure 5. There were 10 plant-associated taxa; 12 water-associated taxa; and 6 taxa mainly found in soil, gravel, media, or used containers. Few of the predominantly plant-associated taxa were also detected in water, and few of the predominantly water-associated isolates were found in association with plants; these appear to be largely separate communities. Taxon Raspberry was recovered at

TABLE 3. Hierarchical partitioning of Shannon-Wiener diversity (weighted by sample abundances)[a]

Hierarchical level	Statistic	*Z* value	Mean	*P* value
Total diversity	2.484	0.000	2.484	1.000
Among nursery	0.401	45.902	0.064	0.001
Among source	0.541	19.403	0.245	0.001
Among year	0.368	–2.024	0.402	0.047
Among season	0.171	1.746	0.153	0.093
Within nursery	2.083	–45.902	2.42	0.001
Within source	1.542	–38.335	2.175	0.001
Within year	1.174	–31.397	1.773	0.001
Within season	1.002	–33.366	1.62	0.001

[a] Hierarchical levels are nursery, source, year, and season (from highest level to lowest). Taxa that were only observed in one nursery were omitted from analysis. Sources include media or used containers, plant-associated, sand or gravel, and water.

TABLE 4. Number of *Phytophthora* isolates associated with each sample type

	Media ingredients, containers, water			Soil or gravel (S/G) substrate				Plant					
Phytophthora taxa	Media mix	Used containers	Irrigation water	S/G greenhouse	S/G can yard	Field soil	Staging area	Media in containers	Roots	Stems	Leaves	Water reservoir	Total
bilorbang/taxon Oaksoil	…	…	1	…	…	…	…	…	…	…	…	2	3
cactorum	…	…	…	…	…	…	…	2	…	…	…	…	2
cambivora	…	…	…	…	…	1	…	2	1	…	…	…	4
cambivora-like hybrid	…	…	…	…	…	…	…	…	1	…	…	…	1
cinnamomi	2	…	…	…	…	17	…	40	32	15	…	…	106
citrophthora	2	3	…	…	2	3	…	22	9	8	…	5	54
cryptogea	…	1	2	8	3	7	1	4	3	…	1	8	38
drechsleri	…	…	…	…	…	…	…	…	…	…	…	3	3
foliorum	…	…	…	1	…	…	…	…	…	…	…	…	1
gonapodyides	1	…	1	…	2	…	…	2	…	…	…	31	37
inundata	…	…	…	…	…	1	…	…	…	…	…	…	1
lacustris	…	…	…	…	…	…	…	…	…	…	…	28	28
lacustris/Pgchlamydo hybrid	…	…	…	…	1	…	…	2	…	…	…	36	39
lacustris/*riparia* hybrid	…	…	…	…	…	…	…	…	…	…	…	15	15
lateralis	…	…	…	…	…	…	…	…	…	1	…	…	1
megasperma	…	…	…	…	…	5	…	…	2	…	…	…	7
nemorosa	…	…	…	…	…	…	…	…	2	…	…	…	2
obscura	…	…	…	1	…	…	…	…	…	…	…	…	1
parsiana	…	…	…	…	…	…	…	…	…	…	…	1	1
parsiana-like hybrid	…	…	…	…	…	…	…	…	…	…	…	20	20
pini/*citricola* III	…	…	…	1	1	2	…	3	1	2	…	5	15
plurivora	…	4	…	15	13	…	…	27	30	46	11	5	151
riparia	…	…	…	…	…	…	…	…	…	…	…	3	3
syringae	…	2	…	3	2	1	…	…	…	10	55	1	74
Taxon Pgchlamydo	3	2	…	6	6	6	…	7	…	1	…	28	59
Taxon Pgchlamydo-like	…	…	…	1	…	1	…	…	…	…	…	…	2
Taxon Raspberry	…	…	…	…	…	…	…	1	…	…	…	1	2
Taxon Walnut	…	…	…	…	…	…	2	…	…	…	…	2	4
Total	8	12	4	36	30	44	3	112	81	83	67	194	674

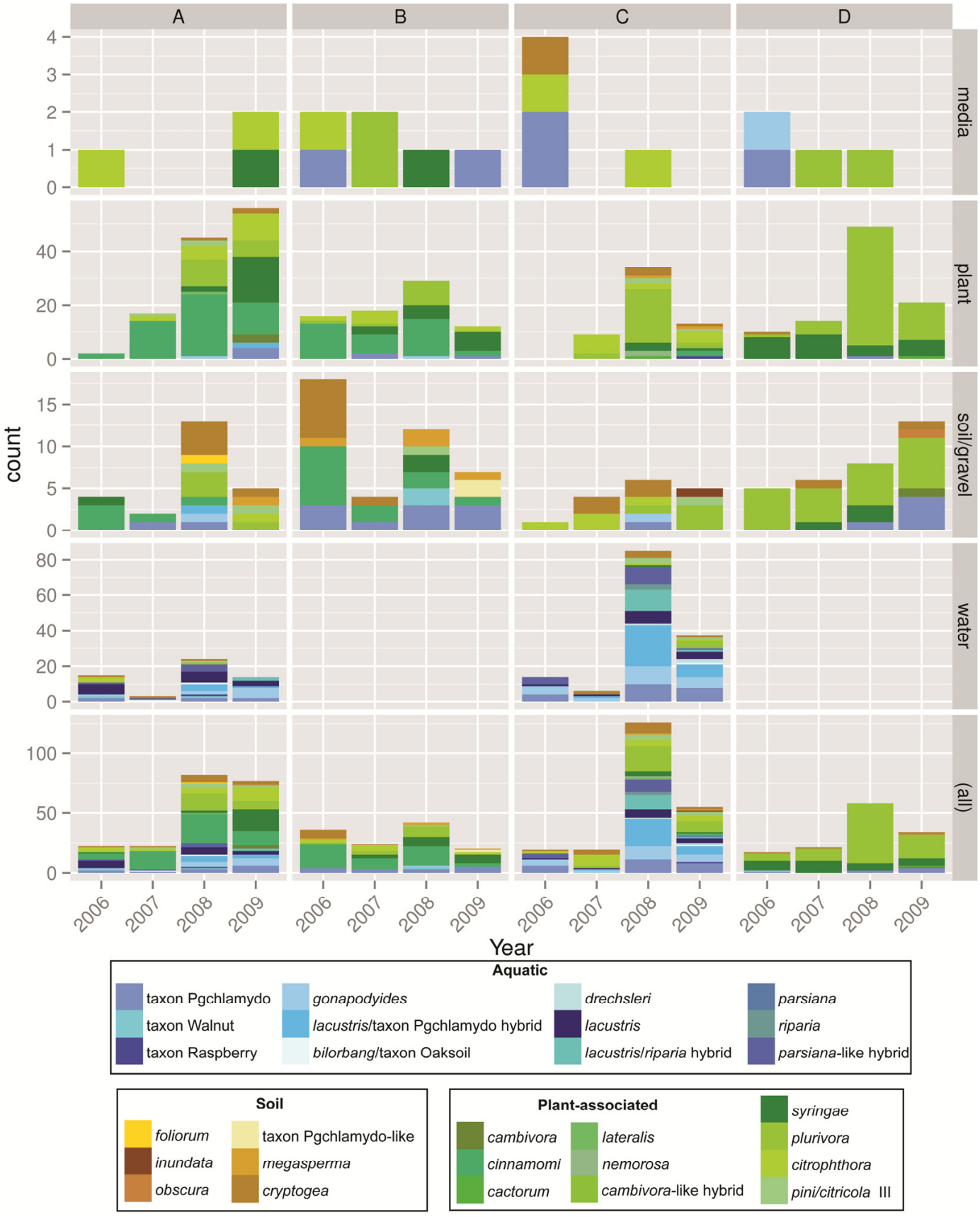

Fig. 2. Bar plot of *Phytophthora* incidence indicating the relative contribution of each nursery (A to D, in columns), source of sample within nursery (rows), and year (subcolumns) to the total number and diversity of isolates. *Phytophthora* spp. are color coded according to the primary source of isolates: aquatic (blue); soil, media, or used containers (brown); or plant-associated (green).

the same frequency from water and plants but this represents just one isolate from each habitat.

DISCUSSION

Horticultural nurseries can harbor a large diversity of *Phytophthora* spp. This diversity represents the full ecological spectrum, including well-characterized plant pathogens as well as soil-associated or aquatic facultative pathogens and opportunists. The concept of ecological guilds, based on the habitat from which species were isolated, appears to generally apply to nurseries as well as to forest ecosystems. Although Hansen et al. (25) grouped southwest Oregon forest *Phytophthora* spp. as foliar pathogens, soilborne fine-root and canker pathogens, or aquatic opportunists, nursery species were grouped according to plant-associated, soil-dwelling, or aquatic. Many species were found to occupy similar habitats, with *P. gonapodyides, P.* taxon Pgchlamydo, and *P. lacustris* (= taxon Salixsoil) dominating the aquatic samples; and *P. cinnamomi, P. cactorum, P. plurivora* (likely *P. citricola* sensu lato) isolated from the soil and root samples in both studies. Differences in the species composition between forests and nurseries were most apparent among the foliar *Phytophthora* communities, where *P. ramorum, P. nemorosa,* and *P. pseudosyringae* dominated in the forest, whereas nursery foliage yielded primarily *P. syringae* and *P. plurivora*. It is not surprising that aquatic species, including both saprophytes and opportunists, would be less affected by the presence of particular plant species than would plant-pathogenic species. *P. syringae*, for example, is particularly common on leaves of nursery hosts *Kalmia* and *Rhododendron*.

Compared with determining community structure in natural environments, assessing *Phytophthora* community structure in nurseries is challenging. Nurseries are dynamic entities, with new propagative material constantly moving through the production cycle. A single plant is likely to be moved several times within the nursery as it is successively repotted. The physical shifting of plant host material, and the variable environmental conditions due to seasonal changes, warranted repeated sampling to detect the greatest diversity of *Phytophthora* spp. However, our culture-based sampling methods may not have detected all *Phytophthora* spp. Not all *Phytophthora* spp. are baited from water (e.g., *P. cinnamomi* in this study), and no single type of bait or set of culture conditions is likely to detect all *Phytophthora* spp. present in soil or water. Ideally, a non-culture-based, metagenomic approach should be combined with traditional culture-based approaches to overcome this shortcoming. The sampling of plants in this study also targeted symptomatic plants of four *Phytophthora*-susceptible genera rather than sampling all plants. Sampling nonsymptomatic plants would have resulted in extremely low recovery of *Phytophthora* isolates, which would have made this study not feasible. We chose instead to apply a consistent sampling strategy and to limit our comparisons to within each sample source. Thus, our results must be interpreted in light of these limitations.

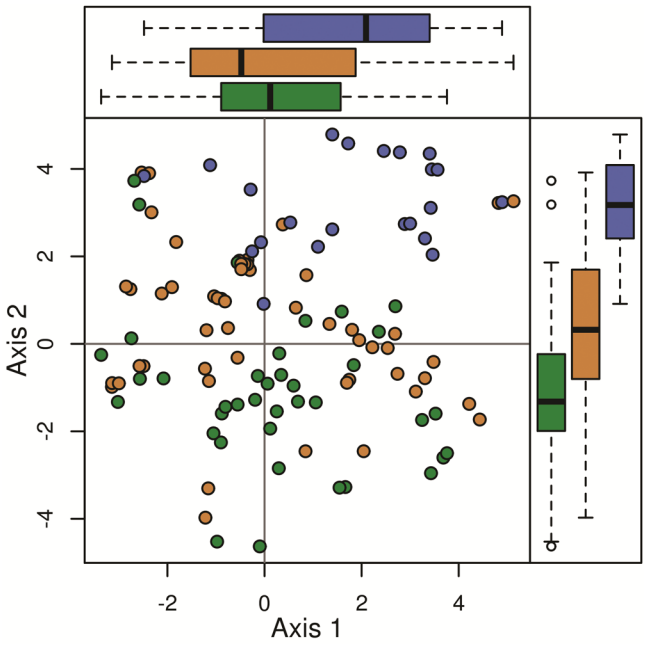

Fig. 3. Nonmetric multidimensional scaling of samples (see text) based on a Bray-Curtis distance computed from taxon counts. Samples are from water (blue); soil, gravel, media, or used containers (light brown); or plants (green). Marginal boxplots are constructed from the first and third quantiles (i.e., 50% of the data is contained), with the median indicated by a line. Whiskers include data that is 1.5 times the box length away from the box. Data points beyond the whiskers are indicated with open circles.

Differences in species diversity among nurseries are intriguing yet difficult to interpret. It is not clear why *P. cinnamomi* is so prevalent in nurseries A and B, for example, yet was isolated only twice from nursery C and never from nursery D. It seems likely that *P. cinnamomi*, an invasive soilborne species that has become widely established in many parts of the world, could have been introduced to all nurseries. Differing practices over a period of decades, including sanitation, pesticide use, inspection and quarantine of incoming stock, water management, crop history, and many other factors may have contributed to the observed differences in *Phytophthora* communities among the nurseries. Differences in *Phytophthora* spp. diversity among the nurseries may also be related to host plant diversity or stochastic effects such as occasional but rare introductions. Nursery D had the lowest diversity of *Phytophthora* spp. (a Shannon-Wiener index of 1.01); 89% of isolates were *P. syringae* or *P. plurivora*. Unlike the other three nurseries that each grew >40 genera of plants, this nursery grew only 10 plant genera, and >90% of the container stock consisted of *Kalmia, Rhododendron,* or *Pieris* plants. Nursery D grew successive crops that were all highly susceptible to the foliar pathogens *P. syringae* and *P. plurivora,* without crop rotation to nonhosts. Insufficient sanitation to remove infested leafy debris may have also contributed to the consistent recovery of these species every year.

From a crop loss standpoint, the main *Phytophthora* spp. of concern for nursery growers would be widespread, highly virulent plant-pathogenic species such as *P. plurivora*, *P. cinnamomi*, and *P. syringae* or pathogens of zero tolerance such as *P. ramorum* subject to federal quarantine. In the broader context of nursery crops as potential vectors of forest and agricultural pathogens, *Phytophthora* taxa of greatest concern may be those that are new or rare and potentially invasive. Targets would include recently introduced exotic species and recent hybrids with unknown pathogenicity. These rare taxa may escape detection, yet their presence in a nursery would allow their encounter with numerous potential hosts and an expanded geographical range when these hosts are shipped.

P. parsiana is an example of a rare and possibly exotic species isolated once in this study from water reservoirs. This high-temperature tolerant species, originally isolated from the rotted crown of fig (*Ficus carica*), pistachio (*Pistacia vera*), and almond (*Prunus dulcis*) in Iran and Greece (38), has never been reported from North America. Nurseries are complex assemblages containing hundreds of potential host species (including several *Prunus* spp.) and spanning several microenvironments. If the recycled water is untreated, it is conceivable that this potentially invasive species could be distributed with irrigation water, brought into contact with a suitable host, and shipped to a warm climate such as the southeastern United States, where it could spread to native vegetation. Another rare species, *Phytophthora obscura,* recently described from nursery D in this study, was

determined to be identical to German isolates collected beneath diseased horse chestnut (*Aesculus hippocastanum*) trees (22). Pathogenicity tests demonstrated that it was potentially pathogenic to *Aesculus, Kalmia, Rhododendron,* and *Pieris* spp. The azalea leaf blight pathogen *P. foliorum*, originally reported from California and Tennessee (11), was also detected in this nursery study, although it was recovered from soil and gravel, not from plants. Several unrecognized taxa were found in this study: taxon Pgchlamydo-like and several hybrid taxa, *P. cambivora*-like hybrid, *P. parsiana*-like hybrid, *P. lacustris*/Pgchlamydo hybrid, and *P. lacustris*/*riparia* hybrid. It is possible that any one of these three rarely encountered species or one of the newly detected taxa might find a suitable host and favorable environment within the nursery or among the eventual outplanting sites to which the nursery plants are shipped, with unknown consequences. As with most species introductions, few would be expected to establish a large enough population to initiate a disease epidemic, and yet this has occurred repeatedly within the genus *Phytophthora*, including *P. ramorum* and *P. kernoviae* (4,20), presumably because of the increased globalization of the live plant trade (33).

We detected several putative hybrids, including *P. lacustris*/Pgchlamydo, *P. lacustris*/*riparia, P. cambivora*-like hybrid, and *P. parsiana*-like hybrid. To determine whether these were indeed hybrids, we cloned and sequenced the ITS region to determine whether two distinctly different ITS sequences could be cloned (N. J. Grünwald and V. J. Fieland, *unpublished data*). In all cases, we were able to confirm the hybrid nature of these cultures although, in the case of *P. cambivora*-like hybrid and the *P. parsiana*-like hybrid, we could not get matches to the parental species and called them "-like". The hybrids we observed in our surveys belonged to clade 6 (*P. lacustris*/Pgchlamydo and *P. lacustris*/*riparia*) that is mostly associated with aquatic environments, but also clade 7 (*P. cambivora*-like hybrid) and clade 9 (*P. parsiana*-like hybrid). Hybrids have been detected in previous nursery surveys. For example, Leonberger et al. (27) found hybrids between *P. cactorum* × *hedraiandra* and *P. nicotianae* × *cactorum* from surveys conducted in Iowa, Michigan, and Ohio. However, these taxa belong to clade 1, reminiscent of other clade 1 hybrids (18,34). Hybrid taxa pose special concern because they may provide greater virulence or an expanded host range, albeit rarely, given the inherent stochastic nature of hybridization as compared with their parental species, as shown for hybrid taxa *P. alni* ssp. *alni* (6) and *P. cactorum* × *hedraiandra* (31).

In addition to the *Phytophthora* spp. that cause obvious disease symptoms on their hosts, the capacity for latent, symptomless infection by several species further confounds their detection and

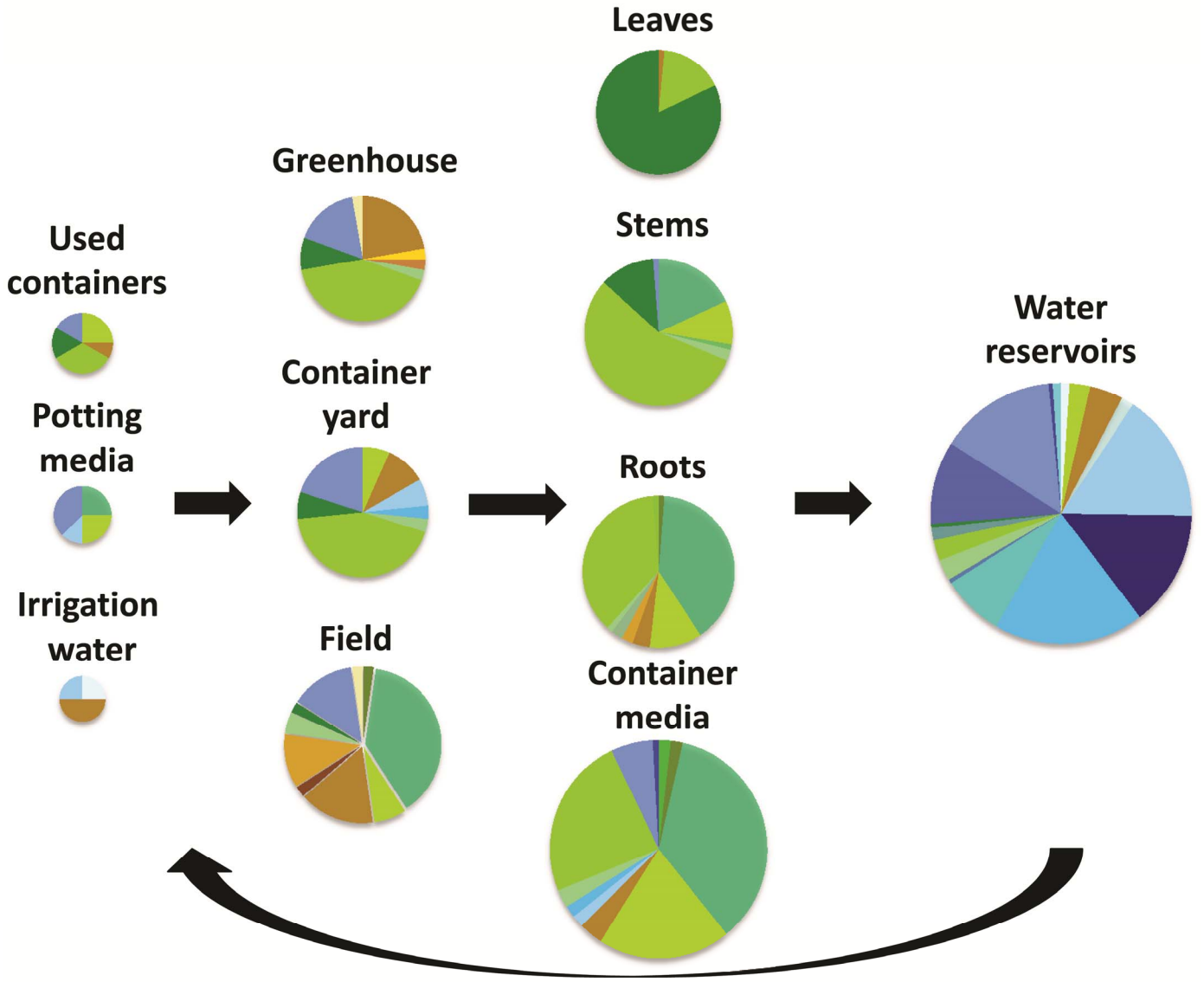

Fig. 4. Pie chart diagram illustrating the relative number of isolates (represented by the circle diameter) and species diversity (color) of *Phytophthora* recovered from different sources from the nurseries. Data are from all four nurseries and all 4 years combined.

potentially contributes to their unwitting spread with nursery plants. *P. inflata* , the exotic species *P. ramorum* (10,44,52) and *P. kernoviae* (13,14), can all persist as symptomless colonizers of roots, and *P. ramorum* can survive in soil and potting media, in the absence of a host, for at least 32 months (55).

Our study shows a distribution of plant-associated *Phytophthora* taxa similar to other nursery studies (12,26,32,37,41, 45,50,51,56,57), where *P. cactorum, P. cambivora, P. citricola* sensu lato*, P. citrophthora, P. cryptogea*, and taxon Pgchlamydo were commonly encountered. *P. citricola* sensu lato has recently been split into multiple species (*P. citricola* III*, P. multivora, P. plurivora*, and *P. pini*) (27,29), preventing direct comparisons across studies for this group. Some differences are apparent as well. Our study included roots as well as foliage and, therefore, strictly soilborne species such as *P. cinnamomi,* frequently isolated in our study, would not be detected in several of the other studies that investigated leaves and stems only. *P. nicotianae* was found only in the hot and humid climates of the U.S. Midwest, U.S. Southeast, Spain, and Australia. *P. ramorum* was found in many of the other nurseries, not surprisingly because several studies were undertaken to survey for this pathogen in North America and Europe; however, it was not found in the nurseries in Oregon where our study was conducted. In terms of water-associated *Phytophthora* taxa, our study was similar to other studies (15,41,54) in having *P. gonapodyides, P. syringae*, and members of the *P. citricola* sensu lato complex; however, *P. lacustris* (formerly known as taxon Salixsoil) (39) was not reported from water in other nurseries. Taxon Pgchlamydo was also isolated more frequently in our study than in other nursery studies. The number of taxa reported here is similar to that reported for western Oregon forests and streams, in which 32 taxa were recovered (24), and from tanoak forests, soil, and streams in southwestern Oregon and Alaska (47,48).

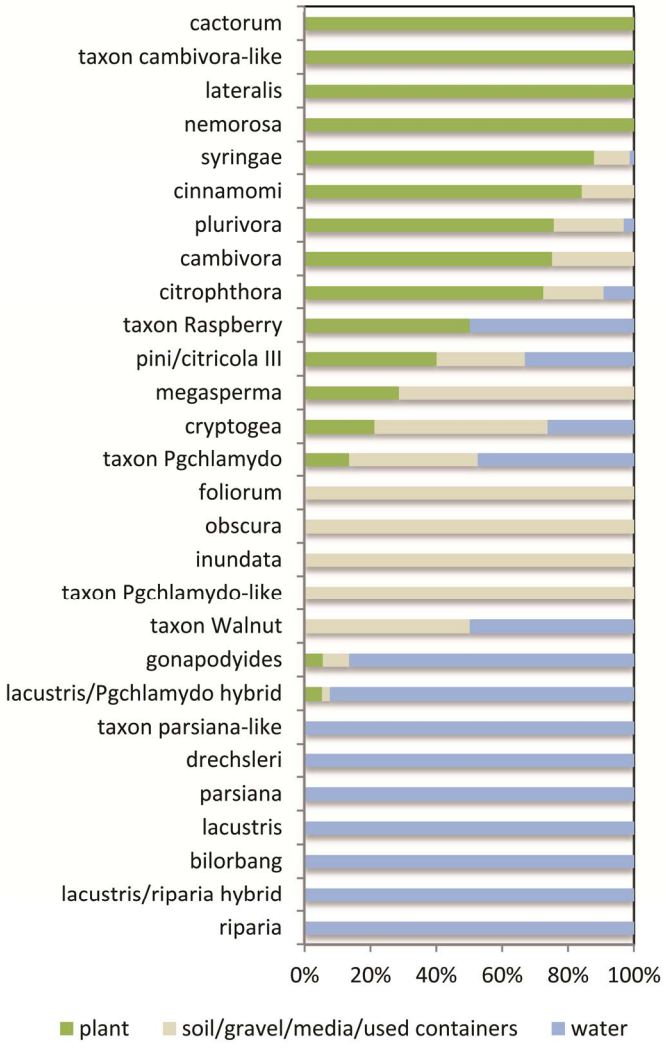

Fig. 5. Proportion (%) of *Phytophthora* isolates recovered from plants; soil, gravel, media, or used containers; and water.

Water from reservoirs used for irrigation was the greatest source of *Phytophthora* diversity encountered in the nurseries. The two nurseries that used recycled water treated it with sodium hypochlorite before use in irrigation, presumably reducing the number of isolates and the diversity of *Phytophthora* taxa associated with it. The epidemiological importance of infested water is difficult to assess. Ordination showed that the community of *Phytophthora* taxa found in water is distinct from the community of plant-associated *Phytophthora* spp. (Fig. 3). The vast majority of *Phytophthora* isolates detected in untreated water belong to taxa that are known as aquatic opportunists; relatively few taxa are known plant pathogens (Fig. 5). Aquatic opportunists are mostly found in water but appear to cause plant disease under certain circumstances (25). *Phytophthora* taxon Walnut, for example, has recently been implicated in causing mortality of *Juglans regia* in a park in northern Italy (16), and *P. bilorbang* is associated with European blackberry decline in Western Australia (1). Even for known plant-pathogenic species, studies relating inoculum dose in irrigation water to disease incidence are notably lacking. However, a conservative approach, in which all recycled water is treated prior to use in irrigation, is likely warranted to prevent the establishment of potentially pathogenic *Phytophthora* taxa on plants.

The flow of materials through a nursery can be conceptualized as a variable network of pathways from propagation to the field and then back again through the reuse of materials (e.g., pots, potting mix, or water) or through propagation methods (Fig. 4). Similar to epidemiological systems, where a management goal may be to interrupt the life cycle of a pathogen, systems approaches to nursery management seek to identify pathways in which pathogens enter or circulate through a nursery system so that critical control points can be targeted to prevent flow (43). We characterized differences in the *Phytophthora* communities present in water reservoirs as well as soil and gravel as potential pathways (Table 3; Figs. 2 to 4). Although untreated water in reservoirs accounted for the most diverse assemblages of *Phytophthora* taxa (Table 2), many of these taxa are considered aquatic opportunists as opposed to plant pathogens (Figs. 3 and 5). This suggests that managing recirculated water in these nurseries may not be a high-priority critical control point unless plant pathogens are also recirculated. In contrast, containers and potting media were among the least diverse sources of *Phytophthora* isolates (Table 2) and the taxa observed were typically a subset of taxa found on plant materials (Table 4). This suggests that pathways, including containers and potting media, may maintain and disperse members of the *Phytophthora* communities but they are not a likely source of new pathogens to the nursery. Soil and gravel had diversities comparable with those of *Phytophthora* communities observed on plant materials (Table 2) and were composed of similar taxa (Table 4; Figs. 2 and 3), with some exceptions (Fig. 5). This implicates contact with soil and gravel as a likely source of *Phytophthora* taxa from the environment to plant materials; soil and gravel also provide a residual source of inoculum from one crop to the next. Therefore, soilborne inoculum may be considered a high-priority critical control point, where management activities may have the greatest impact on plant health within a nursery.

Several contamination hazards are likely to exist in most nurseries. These include general sanitation practices, handling of incoming plants for propagation or resale, placement of cull piles, use of contaminated water, handling of potting media, and soil

drainage issues. However, their relative contribution to disease is not known, making it difficult for growers to decide which critical control points to address and which Best Management Practices to implement (43). Our study focused on select hazards in four nurseries and, thus, provides only partial information necessary for development of systems approaches for control of disease. Furthermore, our study only examined presence and makeup of *Phytophthora* communities at perceived contamination hazards. Critical thresholds for each critical control point remain to be established. Understanding the presence and composition of *Phytophthora* communities in Oregon nurseries is a first necessary step in development of systems approaches for management of *Phytophthora* hazards.

ACKNOWLEDGMENTS

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