Movement of *Phytophthora* spp. in Maryland's Nursery Trade

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Abstract

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Phytophthora spp. cause major losses in the nursery industry worldwide. However, a clear demonstration of the route of movement has not been previously shown. A survey of 10 Maryland nurseries was conducted over a 3-year period to investigate the presence of *Phytophthora* spp. on newly arrived plants, mainly from West Coast suppliers. Local nursery plants, irrigation water, and potting media were also sampled for *Phytophthora* spp. Isolates were identified using a combination of morphological characteristics and DNA sequencing. Species identified included *Phytophthora cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citrophthora*, *P. drechsleri*, *P. elongata*, *P. gonapodyides*, *P. hydropathica*, *P. irrigata*, *P. lacustris*, *P. multivora*, *P. nicotianae*, *P. pini*, *P. plurivora*, and *P. syringae*. *P. taxon pgchlamydo* was also isolated from irrigation water. Eight of the abovementioned *Phytophthora* spp. were isolated in association with incoming material, indicating that the movement of these pathogens continues to occur. Asymptomatic plant material was the main route of introduction of *Phytophthora* spp. to Maryland nurseries. Results also indicated that several *Phytophthora* spp. could be found in Maryland nurseries in association with infested potting media of asymptomatic plants. Although *P. ramorum* was not detected, our surveys underscore the significance of nursery practices that allow introductions of these significant plant pathogens to new geographic locations.

The most recent assessment of the wholesale value of nursery crops in the United States was estimated at \$4.65 billion in 2006 (United States Department of Agriculture [USDA] National Agricultural Statistics Service). Nursery crops consist of high-value plant species with intensive labor inputs and low tolerance for damage to plants. Therefore, economic losses can be severe if plant diseases develop (17,31). Multiple *Phytophthora* spp. are known as major pathogens of nursery plants (17). As the plant trade continues to increase globally, *Phytophthora* spp. continue to spread into new areas and threaten nurseries, landscapes, and forest ecosystems in the United States and other countries (45). Historically, nurseries have been responsible for, and continue to provide, one of the main routes of pathogen movement and introduction of exotic plant pathogens into native ecosystems (10,45,48,67,76).

One *Phytophthora* sp. that has gained attention for causing severe losses both in nurseries and forests is *Phytophthora ramorum* Werres, De Cock & Man in't Veld (57). This pathogen has been distributed with infected plants among nurseries in the United States, providing an opportunity for this pathogen to become established in new locations. In 2004, *P. ramorum*-infected camellia and rhododendron plants were shipped across the United States from Oregon and California nurseries (18,57). Incidents such as these have elevated concerns of *P. ramorum* spreading to other locations in the United States. This has provoked a wide range of surveys in both nurseries and forest ecosystems in an effort to detect and eradicate this organism (2,11,31,50,57,62,70,71,77). As a result of these surveys, a number of *Phytophthora* spp. new to the United States have been discovered. This includes several newly described species (1,13,14,20,30,31,54,55,60,62,63,71,77). Similar discoveries have also taken place in other countries. For example, *P. kernoviae* Brasier, Beales, and S.A. Kirk; *P. austrocedrae* Gresl. & E.M. Hansen; and *P. ramorum* represent recent finds in the United Kingdom (6,22,43). *P. lateralis* Tucker & Milbrath was recently discovered in France and the United Kingdom infecting and killing *Chamaecyparis lawsoniana* (A. Murray) Parl. (21,58). Additionally, *P. pinifolia* Alv. Durán, Gryzenh. & M.J. Wingf., a

previously unknown *Phytophthora* sp., was discovered in Chile after causing severe outbreaks of needle disease in *Pinus radiata* plantations (16). Similarly, *Phytophthora ramorum* and several new taxa were detected on nursery crops in Spain (48). Plant trade has been the main suspect in each these instances as the pathway for introduction.

In the United States, our attention has been directed toward the detection of *P. ramorum*. However, other *Phytophthora* spp. could be introduced, including listed species of regulatory concern in the United States (68). Many of these *Phytophthora* spp. are projected to cause significant losses in nurseries and natural ecosystems if they are ever introduced into the United States. The objective of this study was to investigate whether the introduction of *Phytophthora* spp. into Maryland nurseries is occurring through shipments of incoming plant material. We also identified *Phytophthora* spp. that are associated with or without any disease in selected Maryland nurseries in an effort to determine the species assemblages and potential of these pathogens to spread to new locations.

Materials and Methods

Study sites. Ten ornamental nurseries in Maryland were sampled between March and August 2010, 2011, and 2012. The nurseries ranged in size and production, from nurseries of over 1,000 acres to smaller, 50-acre sites. The nurseries were sampled multiple times each year, primarily in conjunction with new shipments arriving from West Coast nurseries.

Sampling method and material. We sampled plants shipped from other states and plants that were part of the inventory of the local nursery. For incoming plant material, samples were collected within 24 h of arriving at the nurseries and prior to being repotted or irrigated. Plants targeted during sampling included randomly selected plants (i) that showed disease symptoms and (ii) that appeared healthy and had no noticeable disease. In addition, piles of recycled potting media were collected. To isolate *Phytophthora* spp. from recirculating ponds, four rhododendron leaves (*Rhododendron maximum*) were placed in a mesh bag and deployed in recirculating ponds. Leaf baits were collected from native vegetation in West Virginia. Four leaves were placed in a mesh bag and set in recirculating ponds for 5 to 10 days. At the end of the baiting period, leaves were collected and transported back to the laboratory in a cooler.

Plant genera previously listed as high-risk hosts for *P. ramorum*, such as *Rhododendron* and *Pieris*, were the focus for this study.

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However, other plant genera were also sampled if plants expressed symptoms typical of *Phytophthora* infection or high-risk hosts were not present. When symptomatic plants were sampled, they were evaluated for symptoms typical of *Phytophthora* infection (e.g. leaf necrosis, stem cankers, and shoot blight). Samples of plant tissue containing necrotic lesions were placed in 1-gal. plastic bags and transported to the laboratory in a cooler. For asymptomatic plants, potting media was collected from the pots or root balls. We collected only the roots from bare-root plants.

In addition to surveying newly arrived plants in each nursery, blocks of existing plants were surveyed for disease symptoms and sampled as described above. Disease incidence was estimated by counting the total number of symptomatic and asymptomatic plants in each shipment or block within each nursery.

Isolation. Plant tissues sampled for *Phytophthora* spp. were tested using direct plating, whereas potting media and water samples were evaluated using baiting methods. Necrotic tissue samples were processed within a day of sampling. Sections taken from the edges of necrotic lesions were plated on a selective agar medium containing clarified V8 juice (cV8), pimaricin at 10 µg/liter, ampicillin at 200 µg/liter, rifampicin at 10 µg/ml, pentachloronitrobenzene at 25 µg/liter, nystatin at 50 µg/liter, and hymexazol (PARPNH) at 50 µg/liter (2,65). If necrotic lesions appeared to be relatively fresh, their margins were excised, blotted dry, and placed in petri plates containing of cV8-PARPNH. Otherwise, plant tissue was washed with tap water, surface disinfested in a 0.57% NaOCl solution for 2 min, rinsed in distilled water, blotted dry, then plated on cV8-PARPNH.

To isolate *Phytophthora* spp. from potting media, 200 to 300 g of potting medium was placed in a plastic container and approximately twice the amount of distilled water was added. Any organic matter floating on the surface of the water was either removed or a layer of cheesecloth was placed on the surface of the water to prevent organic debris from coming in contact with the baits. Roots collected from bare-root plants were also baited for *Phytophthora* spp. by flooding cut root sections in a plastic container. For baits, leaflets of *Quercus robur* or young leaves of *R. maximum* were used and proved to be sensitive baits to attract a diverse assemblage of *Phytophthora* spp. (2,17,37,47,50,62). Leaves were placed on the surface of the water and incubated for 3 to 5 days at 18°C. Following incubation, each leaf was examined for the presence of discoloration or lesion development. With oak leaflets, discolored leaves were also microscopically examined for *Phytophthora* sporangia (37). Leaves with discoloration or lesions were rinsed with tap water, followed by distilled water, then blotted dry and plated on cV8-PARPNH (2,65). Similarly, baits collected from recirculating ponds were rinsed and blotted dry, and 7 to 10 arbitrarily selected, water-soaked necrotic lesions were plated on cV8-PARPNH as described above.

For all isolation methods, emerging colonies of putative *Phytophthora* spp. were subcultured on cV8 agar. Isolates identified as *Phytophthora* spp. were transferred to cornmeal agar slants and maintained at 8 or 15°C.

Isolate identification. For molecular identification, isolates were grown in potato dextrose broth (Difco) for 5 to 10 days at 22°C. The broth was removed with a pipet and mycelia were lyophilized for 12 h. For each isolate, approximately 0.1 mg of lyophilized mycelium was transferred to 0.2-ml PCR strip tubes. Genomic DNA was extracted by adding 10 µl of Lyse and Go polymerase chain reaction (PCR) Reagent (Thermo Scientific Pierce) to each tube and cycling the samples at 95°C for 2 min, followed by 4°C for 2 min. Each cycle was repeated once. The internal transcribed spacer (ITS) region was amplified and sequenced (MCLAB) using primers ITS6 and ITS4 (75). Low-quality sequences were eliminated and the procedure was repeated with new material. Sequences were aligned and edited using Geneious Pro 5.5.6 Software (Biomatter Ltd.) and compared with known DNA sequences in the National Center for Biotechnology Information database using BLAST searches. For isolates initially identified as part of the *P. citricola* complex based on ITS sequences, additional

sequencing was conducted using primers TUBUF2 and TUBUR1 (41) to delineate these isolates to the correct species.

Following molecular classification, representative isolates of each species were examined and classified morphologically to confirm their identities. Production of sporangia was induced by taking approximately 10-mm² sections from the margins of 3- to 5-day-old colonies growing on cV8 and flooding them with nonsterile soil-extract water in 60-mm petri dishes. Soil-extract water was prepared by mixing 500 ml of distilled water with 250 g of forest soil, straining the soil extract through Miracloth (EMD Millipore), then filtering the suspension by vacuum through a Whatman number 1 filter. Cultures were kept at 22°C in ambient fluorescent light from the laboratory. If no sporangia formed within 24 h, the soil-extract water was discarded and replaced with fresh soil-extract water daily until sporangia were produced. Hyphal swelling production was also noted using soil-extract water. All other structures were investigated on cV8 agar cultures 1 month old or older. Asexual and sexual structures were compared using a tabular key that includes 117 species of *Phytophthora* (47). A more comprehensive Lucid key based on types and putative epitypes and neotypes was also used for both morphological and molecular characterization (Z. G. Abad, J. C. Bienapfl, Y. Balci, T. I. Burgess, M. D. Coffey, F. N. Martin, and S. Kang, *unpublished data*).

Mating type determination. Isolates of *Phytophthora* that did not produce any oospores (heterothallic) were paired with an A1 and A2 mating type of *P. cinnamomi* tester strains identified in previous studies (Y. Balci, *unpublished*). A 5-mm plug was taken from each heterothallic isolate and transferred to the edge of each of two 90-mm petri plates. A 5-mm plug of each A1 and A2 isolate was placed opposite each isolate and the cultures were incubated at 22°C for approximately 2 weeks. If oogonia, antheridia, and oospores were produced, their morphological features and dimensions were noted for species description.

Results

In total, 1,523 samples were collected from 51 plant species and cultivars in 10 nurseries. The samples yielded 589 *Phytophthora* isolates from symptomatic plant tissue, infested potting media, and irrigation water. Among these isolates we identified 16 *Phytophthora* spp. (Table 1). *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian was the most frequently isolated species, followed by *P. cinnamomi* Rands and *P. plurivora* T. Jung & T.I. Burgess (Table 1). Interestingly, all *P. cambivora* (Petri) Buisman isolates tested (18 isolates) were of the A1 mating type and all *P. cinnamomi* isolates (90 isolates) were of the A2 mating type. Morphological examinations of the other *Phytophthora* spp. corresponded with features typical for each respective species. Few isolates remained to be classified to correct species status. For example, some isolates were identified as *P. citricola* Sawada based on ITS sequences as well as morphology. However, additional sequencing targeting loci other than ITS was needed to classify them to species status and, thus, listed them as *P. citricola* complex (Table 1). Similarly, for a few isolates, a disagreement existed between the molecular and morphological classification. These isolates were listed as *Phytophthora* sp. (Table 2). No *Phytophthora* spp. of regulatory concern were isolated in our sampling, including *P. ramorum*.

Phytophthora spp. were frequently isolated from symptomatic plant tissue (17% of total samples) but we found isolates representing many of the species also associated with asymptomatic plants (11% of total samples), and they were often baited from potting media (Table 1). We found *P. citrophthora* associated with symptomatic and asymptomatic plants but it was most frequently isolated from symptomatic plants (Table 1). A similar trend was found with isolates of *P. nicotianae* Breda de Haan (Table 1). In contrast, although there were a limited number of isolates present, *P. elongata* A. Rea, Stukely & T. Jung and *P. taxon* pgchlamydo were only isolated from symptomatic plants (Table 1).

Disease symptoms on a particular plant associated with different *Phytophthora* spp. usually were similar. However, *P. citrophthora* and all species regarded as *P. citricola* were most often found as-

sociated with aboveground parts of the plants, causing foliar blight and tip dieback. In contrast, *P. cinnamomi*, *P. cambivora*, and *P. nicotianae* were found primarily infecting belowground parts of the plants associated with root rot, butt rot, and lesions expanding from root collar towards the stems.

Phytophthora isolates were most frequently isolated from *Pieris* and *Rhododendron* spp., followed by *Buxus* and *Ilex* (Fig. 1). The diversity of *Phytophthora* spp. isolated from a host or substrate was greatest from *Pieris* plants, with 12 of the 16 total *Phytophthora* spp. (Fig. 2; Table 1). Ten different *Phytophthora* spp.

were isolated from *Buxus*, with *P. nicotianae* being the most frequent (Table 1). Eight *Phytophthora* spp. were isolated from *Rhododendron* spp. (Fig. 3), and *Ilex* spp. yielded seven different *Phytophthora* spp. (Table 1). The remaining plant species sampled yielded one or two *Phytophthora* spp. each.

Phytophthora isolates were also frequently baited from irrigation recirculating ponds and potting media (Fig. 1). Recirculated irrigation water yielded eight *Phytophthora* spp. and recycled potting media yielded seven. We isolated 13 species from media surrounding the plants (Table 1). Though *Phytophthora* spp. were

Table 1. *Phytophthora* spp. isolated from Maryland nurseries in 2010, 2011, and 2012 from symptomatic and asymptomatic ornamental plants, and baited from potting media and irrigation water

<i>Phytophthora</i> spp.	Host, substrate	Symptomatic or asymptomatic ^a	Sample	Number of isolates
<i>Phytophthora cactorum</i>	Recycled potting media	N/A	Bait	2
	<i>Buxus</i> sp.	Symptomatic	Dieback	2
	<i>Pieris japonica</i> 'Mountain Fire'	Symptomatic	Potting media	1
<i>Phytophthora cambivora</i>	<i>Pieris japonica</i> 'Mountain Fire'	Both	Potting media	13
	<i>Pieris japonica</i> 'Valley Valentine'	Asymptomatic	Potting media	4
	<i>Rhododendron</i> 'Roseum English'	Symptomatic	Potting media	1
<i>Phytophthora cinnamomi</i>	Recycled potting media	N/A	Bait	3
	<i>Buxus sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	1
	<i>Ilex</i> × <i>meserveae</i> 'Blue Maid'	Symptomatic	Potting media	8
	<i>I. × meserveae</i> 'China Girl'	Asymptomatic	Potting media	2
	<i>Itea virginica</i> 'Henry's Garnet'	Asymptomatic	Potting media	5
	<i>Pieris japonica</i> 'Mountain Fire'	Both	Dieback, potting media	31
	<i>P. japonica</i> 'Scarlett O'Hara'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Azaleas'	Symptomatic	Potting media	3
	<i>Rhododendron</i> 'Besse Howells'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Cunningham's White'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'Fashion'	Both	Potting media	4
	<i>Rhododendron</i> 'Gumpo White'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'Herbert'	Symptomatic	Potting media	3
	<i>Rhododendron</i> 'Karen'	Asymptomatic	Potting media	4
	<i>Rhododendron</i> 'Nova Zembla'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'PJM'	Symptomatic	Dieback	8
	<i>Rhododendron</i> 'Roseum Elegans'	Asymptomatic	Potting media	4
	<i>Rhododendron</i> 'Yaku Princess'	Both	Potting media	7
<i>Phytophthora citricola</i> complex	Recycled potting media	N/A	Bait	2
	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	1
	<i>Nandina domestica</i> 'Gulf Stream'	Asymptomatic	Potting media	1
	<i>Pieris japonica</i> 'Mountain Fire'	Both	Potting media	5
<i>Phytophthora citrophthora</i>	Recycled potting media	N/A	Bait	12
	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	1
	<i>Buxus</i> 'Green Velvet'	Asymptomatic	Potting media	4
	<i>Buxus</i> sp.	Both	Dieback, potting media	5
	<i>Ilex</i> × <i>meserveae</i> 'Centennial Girl'	Both	Dieback, potting media	31
	<i>Itea virginica</i> 'Henry's Garnet'	Asymptomatic	Potting media	1
	<i>Pieris japonica</i> 'Brouwer's Beauty'	Symptomatic	Dieback	3
	<i>P. japonica</i> 'Mountain Fire'	Both	Dieback, potting media	15
	<i>P. japonica</i> 'Scarlett O'Hara'	Asymptomatic	Potting media	11
	<i>Rhododendron catawbiense</i> 'Chionoides'	Symptomatic	Dieback	7
	<i>Rhododendron</i> 'Percy Wiseman'	Symptomatic	Potting media	2
	<i>Rhododendron</i> 'Roseum Pink'	Symptomatic	Potting media	1
	<i>Rhododendron</i> 'Yaku Princess'	Symptomatic	Dieback	27
	<i>Rhododendron</i> sp.	Symptomatic	Dieback	2
	Irrigation water	N/A	Bait	3
	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	1
	<i>Ilex</i> × <i>meserveae</i> 'Blue Maid'	Symptomatic	Potting media	1
<i>Phytophthora drechsleri</i>	<i>Rhododendron</i> sp.	Symptomatic	Dieback	1
	Irrigation water	N/A	Bait	111
	Recycled potting media	N/A	Bait	1
	<i>Acer rubrum</i>	Asymptomatic	Root	10
<i>P. elongata</i>	<i>Buxus</i> sp.	Asymptomatic	Potting media	1
	<i>Oxydendron arboreum</i>	Symptomatic	Dieback	1
	<i>Pieris japonica</i> 'Valley Valentine'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Hino Crimson'	Symptomatic	Potting media	1
	<i>Sephora japonica</i>	Symptomatic	Dieback	2
	<i>Tilia cordata</i>	Symptomatic	Dieback	1
	<i>Rhododendron</i> sp.	Symptomatic	Dieback	2
	Irrigation water	N/A	Bait	14
	<i>Pieris japonica</i> 'Scarlett O'Hara'	Asymptomatic	Potting media	1

(continued on next page)

^a N/A = not applicable.

primarily isolated from potting media, *P. gonapodyides* (H.E. Petersen) Buisman, *P. hydropathica* C.X. Hong & Gallegly, and *P. plurivora* T. Jung & T.I. Burgess were associated with both irrigation water and potting media (Table 1). The species encountered in irrigation water included *P. drechsleri* Tucker; *P. gonapodyides*; *P. hydropathica*; *P. lacustris* Brasier, Cacciola, Nechw., T. Jung & Bakonyi; *P. plurivora*; *P. riparia* Reeser, W. Sutton & E.M. Hansen; and *P. syringae* (Kleb.) Kleb. *P. gonapodyides* was found in association with roots from a West Coast shipment of *Acer rubrum* trees, as well as other symp-

tomatic hosts. The only other species found in irrigation water that was also found in association with disease was *P. plurivora* (Table 1). In contrast, species in potting media were commonly found in association with disease. They included *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cambivora*, *P. cinnamomi*, the *P. citricola* complex, *P. citrophthora*, *P. elongata*, *P. gonapodyides*, *P. taxon pgchlamydo*, *P. multivora* P.M. Scott & T. Jung, *P. nicotianae*, *P. pini* Leonian, and *P. plurivora*.

Nine different *Phytophthora* spp. were isolated from plant tissue and potting media on arrival at Maryland nurseries (Table 2). They

Table 1. (continued from preceding page)

<i>Phytophthora</i> spp.	Host, substrate	Symptomatic or asymptomatic ^a	Sample	Number of isolates
<i>Phytophthora lacustris</i>	Irrigation water	N/A	Bait	1
<i>P. multivora</i>	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	1
	<i>I. × meserveae</i> 'Blue Maid'	Symptomatic	Potting media	2
	<i>Pieris japonica</i> 'Mountain Fire'	Both	Dieback, potting media	16
	<i>P. japonica</i> 'Valley Valentine'	Asymptomatic	Potting media	2
	<i>Rhododendron catawbiense</i> 'Chionoides'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Besse Howells'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Cunningham's White'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Sweet 16'	Symptomatic	Potting media	3
	<i>Rhododendron</i> 'Yaku Princess'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> sp.	Symptomatic	Dieback	2
<i>Phytophthora nicotianae</i>	Recycled potting media	N/A	Bait	2
	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	17
	<i>Buxus</i> sp.	Both	Dieback, potting media	25
	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	1
<i>Phytophthora pini</i>	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	2
	<i>I. × meserveae</i> 'Blue Maid'	Asymptomatic	Potting media	2
	<i>I. × meserveae</i> 'Centennial Girl'	Both	Potting media	5
	<i>Pieris japonica</i> 'Mountain Fire'	Both	Potting media	17
	<i>R. catawbiense</i> 'Chionoides'	Symptomatic	Dieback	2
	<i>Rhododendron</i> 'Sweet 16'	Symptomatic	Potting media	1
	<i>Rhododendron</i> 'Yaku Princess'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> sp.	Symptomatic	Dieback	8
<i>Phytophthora plurivora</i>	Irrigation water	N/A	Bait	3
	<i>I. × meserveae</i> 'Blue Maid'	Symptomatic	Potting media	2
	<i>I. × meserveae</i> 'Centennial Girl'	Asymptomatic	Potting media	6
	<i>Pieris japonica</i> 'Mountain Fire'	Both	Potting media	30
	<i>P. japonica</i> 'Scarlett O'Hara'	Asymptomatic	Potting media	2
	<i>R. catawbiense</i> 'Chionoides'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Nova Zembla'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Scintillation'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Sweet 16'	Symptomatic	Potting media	3
	<i>Rhododendron</i> 'Yaku Princess'	Symptomatic	Dieback	2
	<i>Rhododendron</i> sp.	Symptomatic	Dieback	2
<i>Phytophthora riparia</i>	Irrigation water	N/A	Bait	3
<i>P. syringae</i>	Irrigation water	N/A	Bait	1
<i>Phytophthora</i> sp.	Irrigation water	N/A	Bait	1
	Recycled potting media	N/A	Bait	2
	<i>Acer palmatum</i>	Asymptomatic	Potting media	7
	<i>A. rubrum</i>	Asymptomatic	Root	3
	<i>B. sempervirens</i> 'Suffruticosa'	Symptomatic	Potting media	2
	<i>Buxus</i> sp.	Both	Dieback, potting media	12
	<i>Camellia sasanqua</i> 'Yuletide'	Asymptomatic	Potting media	1
	<i>Cornus sericea</i> 'Baileyi'	Asymptomatic	Potting media	2
	<i>Hybiscus syriacus</i>	Symptomatic	Dieback	1
	<i>I. × meserveae</i> 'Centennial Girl'	Asymptomatic	Potting media	1
	<i>Kalmia latifolia</i> 'Elf'	Asymptomatic	Potting media	1
	<i>Pieris japonica</i> 'Cavatine'	Asymptomatic	Potting media	2
	<i>P. japonica</i> 'Mountain Fire'	Both	Dieback, potting media	6
	<i>Platanus occidentalis</i>	Asymptomatic	Potting media	4
	<i>Quercus palustris</i>	Asymptomatic	Potting media	4
	<i>Q. rubra</i>	Asymptomatic	Potting media	3
	<i>R. catawbiense</i> 'Chionoides'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Anah Kruscke'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'Cunningham's White'	Asymptomatic	Potting media	4
	<i>Rhododendron</i> 'Hino Crimson'	Symptomatic	Potting media	2
	<i>Rhododendron</i> 'Karen'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'PJM'	Symptomatic	Potting media	1
	<i>Rhododendron</i> 'Roseum Elegans'	Asymptomatic	Potting media	3
	<i>Rhododendron</i> 'Roseum Pink'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Scintillation'	Symptomatic	Potting media	1

were found primarily isolated from potting media collected from asymptomatic *Pieris japonica* and *Rhododendron* spp. (Fig. 4; Table 2). Other isolates were recovered from bare-root plants. *Phytophthora gonapodyides* and unknown *Phytophthora* spp. were isolated from bare roots of new shipments of *Acer* spp. (Table 2). However, *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citrophthora*, *P. gonapodyides*, *P. multivora*, *P. pini*, and *P. plurivora* were also found in association with symptomatic plants upon arrival in Maryland nurseries (Table 2).

Discussion

Phytophthora spp. are commonly associated with nursery crops in the United States, as well as in other countries. The emergence of previously unknown *Phytophthora* spp. in nursery crops, such as *P. ramorum* and *P. alni* Brasier & S.A. Kirk, has increased efforts to investigate the occurrence of *Phytophthora* spp. in nurseries. This study is the first to evaluate nurseries in the Mid-Atlantic region of the United States for the presence of these pathogens. We identified 16 species of *Phytophthora* in Maryland nurseries or isolated directly from incoming plant material. Most importantly, they were frequently found in association with asymptomatic plants (e.g., potting media). This finding confirms the continued movement of *Phytophthora* spp. within the nursery trade and their potential spread to new environments. There is an urgent need to reevaluate current management strategies and enhance them, particularly by using approaches that take into account the critical

control points in nurseries where the risk of spread by this important group of plant pathogens can be mitigated (53).

Of the 16 *Phytophthora* spp. identified in Maryland nurseries, *P. citrophthora* was isolated most frequently, followed by *P. cinnamomi* and the group of species previously regarded as *P. citricola*. Almost all *Phytophthora* spp. found in this study, *P. citrophthora*, *P. cinnamomi*, *P. citricola*, and *P. nicotianae* in particular have been previously reported in nurseries in the United States (12,14,50,52,62,77). Some species, however, were less common. For example, *P. cambivora* is primarily known as a forest pathogen and is rarely found in association with nursery crops in the United States. It has been reported infrequently as a nursery pathogen in Oregon, California, Minnesota, and North Carolina (52,62,71,77) and has also been found at low frequencies in oak forests of the eastern United States (2). Interestingly, some of the *P. cambivora* isolates in our study were isolated from new shipments of *Pieris japonica* that originated in Oregon and were only isolated from plants in a single Maryland nursery. The other plants that yielded *Phytophthora cambivora* were also in the same Maryland nursery and had recently arrived from the same nursery.

It was not surprising to encounter *P. cinnamomi* because it is well established in natural environments on both the West and East Coast of the United States (2,15). This species was likely introduced with plant material brought by early European settlers, with reports dating back to the 1930s, when it was isolated from rhododendron plants (74). Our findings confirm that the nursery trade

Table 2. *Phytophthora* spp. isolated from Maryland nurseries in 2010, 2011, and 2012 associated with newly arrived symptomatic and asymptomatic ornamental plants

<i>Phytophthora</i> spp.	Host, substrate	Symptomatic or asymptomatic	Sample	Number of isolates
<i>Phytophthora cactorum</i>	<i>Pieris japonica</i> 'Mountain Fire'	Symptomatic	Potting media	1
<i>Phytophthora cambivora</i>	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	2
		Symptomatic	Potting media	2
<i>Phytophthora cinnamomi</i>	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	8
		Symptomatic	Potting media	13
	<i>Rhododendron</i> 'Besse Howells'	Symptomatic	Potting media	1
<i>Phytophthora citrophthora</i>	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	5
		Symptomatic	Potting media	3
	<i>Rhododendron catawbiense</i> 'Chionoides'	Symptomatic	Dieback	8
	<i>Rhododendron</i> 'Yaku Princess'	Symptomatic	Dieback	24
<i>Phytophthora gonapodyides</i>	<i>Acer rubrum</i>	Asymptomatic	Root	10
	<i>Oxydendron arboreum</i>	Symptomatic	Dieback	1
	<i>Sephora japonica</i>	Symptomatic	Dieback	2
	<i>Tilia cordata</i>	Symptomatic	Dieback	1
<i>P. multivora</i>	<i>Pieris japonica</i> 'Mountain Fire'	Symptomatic	Potting media	6
	<i>R. catawbiense</i> 'Chionoides'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Besse Howells'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Cunningham's White'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Yaku Princess'	Asymptomatic	Potting media	1
<i>Phytophthora pini</i>	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	5
		Symptomatic	Potting media	7
	<i>R. catawbiense</i> 'Chionoides'	Symptomatic	Dieback	2
	<i>Rhododendron</i> 'Yaku Princess'	Asymptomatic	Potting media	1
<i>Phytophthora plurivora</i>	<i>Pieris japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	4
		Symptomatic	Potting media	16
	<i>R. catawbiense</i> 'Chionoides'	Symptomatic	Dieback	1
	<i>Rhododendron</i> 'Yaku Princess'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'Scintillation'	Symptomatic	Dieback	1
<i>Phytophthora</i> sp.	<i>Acer palmatum</i>	Asymptomatic	Potting media	7
	<i>A. rubrum</i>	Asymptomatic	Potting media	1
	<i>Buxus</i> 'Green Velvet'	Asymptomatic	Potting media	4
	<i>Cornus sericea</i> 'Baileyi'	Asymptomatic	Potting media	2
	<i>Kalmia latifolia</i> 'Elf'	Asymptomatic	Potting media	1
	<i>Pieris japonica</i> 'Cavatine'	Asymptomatic	Potting media	2
	<i>P. japonica</i> 'Mountain Fire'	Asymptomatic	Potting media	4
	<i>Platanus occidentalis</i>	Asymptomatic	Potting media	4
	<i>Quercus palustris</i>	Asymptomatic	Potting media	4
	<i>Q. rubra</i>	Asymptomatic	Potting media	3
	<i>R. catawbiense</i> 'Chionoides'	Asymptomatic	Potting media	1
	<i>Rhododendron</i> 'Anah Kruscke'	Asymptomatic	Potting media	2
	<i>Rhododendron</i> 'Cunningham's White'	Asymptomatic	Potting media	3
	<i>Rhododendron</i> 'Scintillation'	Symptomatic	Potting media	1

continues to facilitate its movement. In contrast, the most commonly encountered species in this study, *P. citrophthora*, was never reported from forest settings in the United States or in Europe. Conditions associated with the nursery environments are perhaps required for this pathogen to establish and cause disease. Similarly, *P. nicotianae* is common in nurseries but has not been found in forest ecosystems in the mid-Atlantic forests (2).

The persistence of *Phytophthora* spp. in the nursery environment was not linked to morphological features or sexual behavior observed in culture. For decades, our understanding of the survival and resilience of *P. cinnamomi* was primarily attributed to its thick-walled chlamydospores. However, a recent study exploring the survival mechanisms of *P. cinnamomi* in Australia's forest ecosystems indicated that hyphal aggregates in plant tissue are a more significant survival strategy. Further, oospores were routinely found in plant tissue despite the absence of the opposite mating type (e.g., inbreeding) (39). The large variety of *Phytophthora* spp. isolated from soilless potting media during our survey indicated similar survival mechanisms in other *Phytophthora* spp. Plant tissue in soilless potting media may provide additional opportunities for these organisms to survive.

We frequently isolated a species of *Phytophthora* previously identified as *P. citricola*. Taxonomic revisions of this group currently include six formally described taxa that are morphologically similar and closely related phylogenetically (23,38,56,64). Though a commonly listed species, in previous nursery surveys, *P. citricola* isolates were never characterized to correct species status in the United States (31,44,52,62,71,77). In our study, the most common species in this group was *P. plurivora*, followed by *P. pini*. The least commonly isolated species were *P. multivora* and *P. elongata*. They were associated with symptomatic plants as well as baited from infested potting media and irrigation water in four different nurseries. Currently, our knowledge regarding these four *Phytophthora* spp. is limited. We know little of their origin, geographic

distribution, or host range. Surveys in mid-central Atlantic oak forests identified only *P. pini* and *P. plurivora*. Therefore, *P. multivora* and *P. elongata* could be of concern if introduced into the U.S. oak forest ecosystems. Both of the species were described from Eucalyptus forests of Australia (56,64). Their occurrence in the U.S. nursery trade can perhaps be explained through international plant trade. However, similar to *P. cinnamomi*, these species may have been misidentified as *P. citricola* for decades and we are only now recognizing their presence.

Although we did not recover *P. ramorum* or any other *Phytophthora* spp. of regulatory concern in the United States, we have demonstrated the ongoing introduction of *Phytophthora* spp. with shipments of nursery stock. Generally, the *Phytophthora* spp. we isolated are known nursery pathogens; however, there is the possibility of new genotypes being introduced. This could include isolates of new races, hybrids, or new mating types with different levels of virulence, host range, and fungicide sensitivity (15,26,28,29,34,35,40,46,55,66,69,72). For example, in our study, all isolates of *P. cambivora* were of mating type A1, which was also noted in a study examining *P. cambivora* populations in Oregon associated with golden chinquapin tree mortality (*Chrysolepis chrysophylla*) (59). The authors suspected that *P. cambivora* was introduced to Oregon due to the prevalence of the A1 mating type. *P. cambivora* is found sporadically in eastern oak forests but is primarily dominated by the A2 mating type (2; M. McConnell and Y. Balci, *unpublished*). This species was also reported recently in association with root rot and stem cankers of Noble fir (*Abies procera*) in the Pacific Northwest (9). The consequence of this new introduction is unknown; however, this situation is of concern because *P. cambivora* is suspected to be one of the parental ancestors of the hybrid *P. alni*, which currently causes major decline of alder (*Alnus* spp.) in Europe (7,33,36). Therefore, new introductions could lead to the development of new genotypes or hybrids, which could under-

Fig. 1. Frequency of isolation of *Phytophthora* spp. from different hosts and substrates sampled in Maryland nurseries in 2010, 2011, and 2012.

mine management practices in areas where these organisms appear to be under control (3,4,24,51,70).

Our surveys suggest that potting media is the most important source of introduction and spread of *Phytophthora* spp. to and from Maryland nurseries. Many of our *Phytophthora* isolates were cultured from potting media collected from asymptomatic plants as they arrived at the nurseries. We also isolated a high diversity of *Phytophthora* spp. from a small sample of recycled potting media. By reusing leftover potting media, the nurseries may inadvertently expose new plants to *Phytophthora* spp. and risk the movement of those plants off site to new locations. The nurseries are also risking the possibility of losing more plants if they are susceptible to the

Phytophthora spp. in the recycled media. Employees at one nursery we sampled indicated that they lost approximately 3,700 *Viburnum* plants after using recycled media to repot the plants. Minimizing or eliminating the use of recycled potting media, as well as practices that target the soil phase, should reduce the risk of movement and introduction of *Phytophthora* spp.

We also recovered eight *Phytophthora* spp. from irrigation water. Some of the species appeared to be native to stream ecosystems, such as *P. gonapodyides*, *P. hydropathica*, *P. irrigata*, and *P. lacustris* (25,26,49; Y. Balci, unpublished). Although some of these species have been shown to be pathogenic in artificial inoculation experiments, they are rarely found associated with diseases and

Fig. 2. Frequency of isolation of *Phytophthora* spp. from *Pieris* sp. sampled in Maryland nurseries in 2010, 2011, and 2012.

their role as plant pathogens still remains to be determined (24,25). In Maryland nurseries, these species did not cause disease levels comparable with some of the more commonly found species such as *P. citrophthora*, *P. cinnamomi*, and *P. plurivora*. Irrigation water in nurseries represents a potentially significant pathway, where new *Phytophthora* spp. or populations could escape into the nursery, potentially infecting additional plants, and eventually escape to new geographic locations where they could cause additional problems (27,73). The opposite pathway from nurseries to the native environment is also possible with irrigation runoff. This has been observed with *P. ramorum* after it was introduced to nurseries in six states and could occur with other *Phytophthora* spp. we isolated in our study (8,19,20,32,42,61). Although this is a

concern for the possible introduction of *P. ramorum* or other *Phytophthora* spp. of regulatory concern, there is the risk that they could spread into the forests and cause additional disease outbreaks. In Maryland nurseries, only a few that we surveyed had irrigation water treatment systems in place, making pathogen establishment and spread among nursery plants even more likely to occur.

Our focus was to determine the movement and introduction of *Phytophthora* spp.; however, we also found two new fungal diseases. In one nursery, there was an outbreak on hundreds of *Hibiscus syriacus* 'Lavender chiffon', where plants exhibited symptoms of stem cankers and severe dieback. We determined that the causal agent was *Fusarium sambucinum*, which is not a known pathogen

Fig. 3. Frequency of isolation of *Phytophthora* spp. from *Rhododendron* sp. sampled in Maryland nurseries in 2010, 2011, and 2012.

Fig. 4. Frequency of isolation of *Phytophthora* spp. from different hosts sampled upon arrival in Maryland nurseries in 2010, 2011, and 2012.

of *H. syriacus* (Y. Balci, unpublished). In two other nurseries, we observed thousands of *Pieris japonica* 'Mountain Fire' plants exhibiting symptoms of shoot dieback, stem cankers, and plant death. We determined that the causal agent was *Phomopsis amygdali*, which is primarily a peach and almond pathogen in the United States (5). In both cases, the infected plants arrived in shipments from West Coast nurseries and demonstrate new introductions of two fungi that were previously not known as nursery pathogens.

The presence of *Phytophthora* spp. in the nursery trade has been well documented (12–15,17–20,24,31,44,53,55,62,68,70,71,77). In our study, we provided direct evidences of the ongoing introduction of *Phytophthora* spp. into the Mid-Atlantic region of the United States. More importantly, we have verified that *Phytophthora* spp. can be introduced in potting media and in association with various asymptomatic hosts. Several studies have shown the potential pathways and practices that can reduce or eliminate spread of pests and pathogens (53). One very significant pathway, as shown in this study, was the potting media, which is only regulated in international U.S. plant trade. Although major efforts and regulations are in place to eliminate introduction of *Phytophthora ramorum* into eastern U.S. nurseries, in few locations was this quarantine pathogen detected. Clearly, current protocols used by nurseries are vulnerable and do not ensure eliminating transmission of plant pathogens into new environments. Our findings demonstrate that introduction of plant pathogens to and from Maryland nurseries is still continuing and, unless a system-based approach that uses various pest risk management measures throughout the plant production system becomes the norm in the nursery sector, they will continue to facilitate potentially very damaging pathogens.

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