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Phytophthora Species Associated with Diseased Woody Ornamentals in Minnesota Nurseries

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ABSTRACT

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Phytophthora species are responsible for causing extensive losses of ornamental plants worldwide. Recent international and national surveys for the detection of *P. ramorum* have led to the finding of previously undescribed *Phytophthora* species. Since no previous *Phytophthora* surveys have been carried out in Minnesota, surveys of ornamental nurseries were performed over 4 years to isolate and identify the *Phytophthora* species causing diseases of woody plants in Minnesota. Species were identified by direct sequencing of internal transcribed spacer (ITS) rDNA, β-*tub*, and mitochondrial *cox*I genes. Species associated with diseased ornamental plants include *P. cactorum*, *P. cambivora*, *P. citricola*, *P. citrophthora*, *P. hedraiandra*, *P. megasperma*, *P. nicotianae*, and the previously identified but undescribed taxon *P.* Pgchlamydo. The most common species encountered were *P. cactorum*, *P. citricola*, and *P. citrophthora*. Two additional isolates obtained did not match known species. One was similar to *P. alni* subsp. *alni*, and the other appeared to be a new species and is referred to as *P.* sp. MN1. In addition, species are reported for the first time from several hosts. Results indicated that several *Phytophthora* species were more widespread in the nursery industry than previously thought, and undescribed species were causing disease in Minnesota ornamental nurseries.

Phytophthora species negatively impact ornamental nurseries throughout the world (8,19,20,43,52). In addition to causing large losses of nursery stock, exotic *Phytophthora* species also threaten natural ecosystems, where they have caused catastrophic damage (10,24,45). Recently, several new *Phytophthora* species have been described (10,14,16,32,52), indicating that our taxonomic knowledge of this genus is incomplete and that there are likely many undiscovered species that remain to be identified. Moreover, the detection of new hybrids, such as *P. alni* subsp. *alni* (14), indicates that new *Phytophthora* species are evolving, possibly in nursery locations where related but geographically isolated species come in contact. When non-native *Phytophthora* spp., such as *P. ramorum*, are discovered in nurseries and in the landscape, it is very important to contain their spread as quickly as possible. It is equally important to define the natural geographic ranges of established *Phytophthora* species so quarantines are not unnecessarily enforced on native pathogens or widespread exotics.

Prior to this report, no surveys of *Phytophthora* species had been carried out in Minnesota despite the problems caused to the state's $2.1 billion/year nursery and landscape industry (40). However, in other states and countries, numerous disease surveys have established the *Phytophthora* species that frequent nurseries and plantations (8,20,36,43,49,50) as well as forests (4–6,25).

Although no *Phytophthora* surveys have been carried out in Minnesota nurseries, several species have been isolated from studies on other crops. They include *P. cactorum* (3), *P. citricola* (35), *P. erythroseptica* (48), *P. infestans* (22), *P. megasperma* (21), and *P. sojae* (54). The *P. megasperma* isolated from alfalfa (*Medicago*) fields in Minnesota was likely *P. medicaginis* (26). The same species have been found in Wisconsin (17,21) as well as an additional species, *P. cryptogea* (20). Most recently, Balci et al. (4) isolated *P. europaea*, a *P. quercina*–like organism, and two unknown *Phytophthora* species from forest soils in Minnesota and Wisconsin.

Several techniques have been used to identify isolates obtained from *Phytophthora* surveys. Morphological characteristics have been primarily used in the past (8,36), but this form of species identification is not always reliable due to the morphological variability and overlap of species' characteristics within the genus (9,19). Direct sequencing of the internal transcribed spacer regions (ITS-1 and ITS-2) of conserved ribosomal DNA (rDNA) allows investigators to compare sequences and has been employed successfully for species identification in several recent studies (5,6,25,31,49). However, ITS sequences do not clearly resolve all *Phytophthora* species (15,37), so other investigators have used additional genome regions, including the nuclear beta-tubulin (β-*tub*) gene and mitochondrial encoded cytochrome *c* oxidase (*cox*) I and II genes, to differentiate between them (33,37).

The objective of the study presented here was to survey, isolate, and identify indigenous and exotic *Phytophthora* species present in Minnesota ornamental nurseries. Detecting *P. ramorum* was of key concern, since its host range includes several native and important plants in Minnesota (2,44,51). Species identification was carried out using direct sequencing of ITS rDNA, β-*tub*, and *cox*I genes and also by studying morphological characteristics. Although this work was done in Minnesota, it has nationwide importance since Minnesota nurseries ship ornamental plants to nearly every state (40).

MATERIALS AND METHODS

Study sites. Minnesota ornamental nurseries were surveyed in 2002 and 2003 solely to detect *P. ramorum*, a pathogen which has destroyed entire tracts of California oak forests (45) and threatens forests of the eastern United States (2). This work was conducted by the Minnesota Department of Agriculture, the University of Minnesota, and the USDA Forest Service. *Phytophthora* isolates were obtained only from symptomatic rhododendrons. Twenty-one nurseries in the fall of 2002 and 24 nurseries in the spring of 2003 were surveyed by Minnesota Department of Agriculture personnel. Samples collected during the surveys were identified to genus by staff in the Plant Disease Clinic, University of Minnesota. *P. ramorum* was not detected. *Phytophthora* isolates obtained were identified to species in this present study.

Fifteen ornamental nurseries in or near Minneapolis and St. Paul, MN, and one nursery in Duluth, MN, were sampled between April and September in 2004 and 2005. The largest nurseries were visited several times each year, whereas the smaller nurseries were sampled twice in the course of the study. Plants that were previously confirmed as hosts or species associated with *P. ramorum* (2) were the focus of this study. Nonhosts of *P. ramorum,* however, were sampled occa-

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sionally if disease symptoms typical of *Phytophthora* were observed. Plant tissue was the primary material used for isolation, but potting mix and irrigation water were sampled occasionally.

Diseased plant survey and isolation methods. Diseased tissues from host plants, which displayed symptoms indicative of *Phytophthora* infection (i.e., canker, dieback, leaf necrosis, wilt) were removed, placed in plastic bags, and stored at 4°C until isolations were made in the laboratory. Isolations were made within 48 h of collection. Small sections from the margins of leaf, stem, and root lesions were removed and submerged in P_{10}ARP-CMA, a *Phytophthora*-selective medium (30), or PARP-V8 selective medium (20). Diseased roots were washed in sterile tap water for 20 s and then blotted dry with sterile paper before submerging in selective media. Cultures were stored at room temperature (20 to 25°C) in the dark and were monitored for hyphal growth for up to 10 days following the initial isolation. Cultures were then transferred to P_{10}ARP-CMA or PARP-V8. During the 2004 growing season, potting mixes of host plants and surface water from irrigation water holding ponds were sampled and stored in plastic bags and sterile plastic containers, respectively, at 4°C until they were processed. Processing of the container mixes and water occurred within 48 and 24 h, respectively, of sampling. They were baited as described in Balci and Halmschlager (5,6). All isolates were transferred to V8 juice agar (19) slants and kept at 12°C for long-term storage.

DNA extraction, polymerase chain reaction (PCR), and species identification. After subcultures grew on selective media for 1 to 4 weeks, DNA was extracted from them with a Qiagen DNeasy Plant Mini Kit using the manufacturer's instructions. A portion of the 18S rRNA gene, ITS-1, 5.8S, ITS-2, and a portion of the 28S rRNA gene were amplified by PCR in a PTC-200 Peltier Thermal Cycler or a PTC-150 Minicycler using the universal primers ITS1 and ITS4 (53). PCR was performed using the following parameters: 5 min at 94°C for the initial denaturation; then 34 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. A final extension was run for 5 min at 72°C. PCRs of the *cox*I and β-*tub* genes were performed for some isolates using the parameters specified in Kroon et al. (33) in order to clarify their species identities. Successful reactions were verified by visualizing PCR products on 1.0% agarose gels in 1.0× TBE buffer (10× TBE = 0.9 M Tris-HCl, pH 8.0, 0.9 M boric acid, 10 mM EDTA). PCR products were stained with SYBR green I stain (Cambrex Corp.) and illuminated by a Dark Reader transilluminator (Clare Chemical Research, Inc., Dolores, CO).

PCR products were prepared for DNA sequencing by mixing 1 µl EXOSAP-IT (USB Corp., Cleveland, OH) and 2.5 µl PCR product and performing the following thermocycler program: 15 min at 37°C and 15 min at 80°C. All sequencing was carried out in an ABI 377 automated DNA fragment analyzer at the University of Minnesota BioMedical Genomics Center. Complimentary forward and reverse DNA strands were aligned with the software program ChromasPro (version 1.22, Technelysium Pty. Ltd.) and optimized by manual alignment. The ends of the consensus ITS sequences were trimmed to remove 18S and 28S sequences according to sequence boundaries described previously (34). Consensus sequences were matched to those in GenBank for identification to species level using BLASTn searches (1). Isolates with BLAST search results accompanied with *E* values >0.0 or nucleotide identities below 99% were further investigated by sequencing *cox*I or β-*tub* genes as well as by studying morphological traits. It was not possible to align the sequences of some isolates due to ambiguous sequence data derived from one sequencing direction (usually the forward direction). The BLAST results for these isolates were also corroborated with published morphological data (19,47).

When morphological characteristics were necessary to corroborate BLAST results, gametangia and oospores were measured on V8 juice agar media after 1 week of growth. Sporangia were produced by placing a colonized V8 juice agar section from a 1-week-old colony in a glass petri dish, flooding with 40 ml of 1.5% soil extract (29), and incubating on a lab bench for 24 h. Their length:breadth ratios and shapes were compared with published accounts (19,47). The production of chlamydospores and hyphal swellings by isolates were two additional morphological traits that aided in corroborating species identity.

Table 1. *Phytophthora* species isolated in 2002 and 2003 from diseased leaves and stems of *Rhododendron*

Phytophthora species	No. of isolates	BLAST search identities[a]
P. cactorum	24	99–100%
P. citricola	10	99–100%
P. citrophthora	15	98–100%
P. hedraiandra	8	99–100%
P. taxon Pgchlamydo	3	99%

[a] These values represent the range of nucleotide identities in the ITS-1, 5.8S, and ITS-2 rDNA between the various isolates of the respective *Phytophthora* species and GenBank accessions. All BLAST search results presented were accompanied by an *E* value of 0.0. Isolates sharing a nucleotide identity with a voucher specimen below 99% were verified morphologically.

RESULTS

In the 2002 survey of *Rhododendron*, 155 samples were collected from 21 nurseries. Isolations yielded *Phytophthora* from 11% of the samples. In the 2003 survey, 302 diseased samples from 24 nurseries were collected. Of those samples, 18% were associated with *Phytophthora*.

Phytophthora species associated with diseased *Rhododendron* in 2002 and 2003 are presented in Table 1. *P. citricola* and *P. citrophthora* were isolated commonly in 2002 and totaled 91% of the identified *Phytophthora* species. They were also isolated in 2003, but not as frequently as *P. cactorum*, which made up 47% of all identified species. *P. hedraiandra*, a recently described leaf pathogen of a *Viburnum* species (16) and a newly introduced organism to the United States (46), was found in both years. *P.* taxon Pgchlamydo, a previously identified but undescribed taxon (12), and *P. nicotianae* were isolated only in 2003. ITS nucleotide identities from the presumed *P. nicotianae* isolates ranged from 88 to 97%, and species identification was not corroborated with morphological data because cultures did not survive in storage for a sufficient period.

During 2004, 186 diseased plants known to be hosts or plants associated with *P. ramorum* were sampled from 12 nursery sites. Of those samples, 15% yielded *Phytophthora* isolates. Sampling in June yielded significantly higher proportions of *Phytophthora* isolates (23%) than did sampling in July through September. In 2005, 392 diseased plants known to be hosts or plants associated with *P. ramorum* were sampled from 10 nursery sites (nurseries were sampled up to seven times throughout the growing season), and of this total, 18% yielded *Phytophthora*. Like the 2004 survey, sampling in June yielded significantly higher proportions of *Phytophthora* isolates (32%) relative to sampling in other months. Several *Phytophthora* species isolated in 2004 and 2005 were derived from plants which had not been previously reported as hosts for that particular *Phytophthora* species (Table 2).

Two *Phytophthora* species were isolated from the same plant sample several times in 2004 and 2005. *P. citricola* and *P. citrophthora* were isolated from the same plant sample in six separate instances. *P. cactorum* and *P. citricola* came from the same sample on five separate occasions. *P. cactorum* and Unknown 1, *P. cactorum* and *P.* taxon Pgchlamydo, and *P. citricola* and *P. nicotianae*, respectively, were isolated from the same sample once.

As in the 2002 and 2003 surveys, *P. cactorum*, *P. citricola*, and *P. citrophthora* were the most commonly isolated *Phytophthora* species during the 2004 and 2005 surveys, making up 16, 55, and 17% of the total number of species identified, respectively (Table 2). *P. cactorum* and *P. citrophthora* were also baited from potting mixes containing plants that harbored these respective *Phytophthora* species on diseased aboveground tissue. In contrast,

Table 2. *Phytophthora* species isolated in 2004 and 2005 from diseased leaves, stems, and roots of ornamental plants

Phytophthora species	Host	Symptoms	No. of isolates	BLAST search identities[a]	GenBank accession[b]
P. cactorum	*Acer rubrum*	Leaf spot	1	100%	
	Acer tatricum	Leaf spot	1	100%	DQ486654
	Calycanthus floridus[c]	Leaf lesion	1	99%	
	Lonicera cvs.[c]	Dieback, stunting	2	99–100%	
	Malus 'Beacon'	Wilt	1	99%	DQ486655
	Malus cvs.	Canker, leaf spot	2	99–100%	
	Myrica pensylvanica[c]	Leaf spot	1	100%	
	Prunus maackii	Bleeding canker	1	100%	
	Pyrus calleryana	Leaf lesion	1	100%	
	Rhododendron 'Henry's Red'	Canker	1	100%	DQ486656
	Rhododendron 'Pahjola's Daughter'	Leaf lesion	1	100%	DQ486657
	Rhododendron cvs.	Dieback, leaf lesion	3	100%	
	Ribes alpinum	Dieback, leaf lesion	1	99%	
	Viburnum prunifolium[c]	Dieback, leaf margin necrosis	1	100%	
P. cambivora	*Quercus macrocarpa*	Leaf tip necrosis	1	100%	DQ486659
	Quercus macrocarpa	Discolored cambium	1	98%	
P. citricola	*Acer tatricum*	Leaf lesion	1	100%	DQ486660
	Acer spp.	Leaf lesion	2	100%	
	Forsythia 'Northern Gold'[c]	Leaf margin necrosis	1	100%	
	Hamamelis virginiana[c]	Dieback, leaf lesion	2	99–100%	
	Lonicera spp.[c]	Canker, dieback, root rot	3	98–100%	
	Magnolia stellata[c]	Leaf margin necrosis	1	100%	
	Pyrus calleryana[c]	Leaf lesion	1	99%	
	Quercus rubra	Leaf tip necrosis	1	100%	
	Rhododendron 'Mikkeli'	Leaf lesion	1	100%	DQ486661
	Rhododendron 'Rosy Lights'	Canker	1	99%	DQ486662
	Rhododendron sp.	Leaf lesion	1	100%	DQ486663
	Rhododendron cvs.	Canker, dieback, leaf lesion, petiole necrosis, root rot, wilt	37	99–100%	
	Rhus aromatica[c]	Leaf lesion	1	100%	
	Spiraea × *bum.* Froebel[c]	Leaf lesion	1	100%	
	Syringa vulgaris	Dieback, leaf lesion	5	99–100%	
	Tilia americana	Leaf lesion	1	100%	
	Viburnum prunifolium[c]	Leaf margin necrosis	1	99%	
P. citrophthora	*Dictamnus alba-purpureus*[c]	Crown and root rot	1	99%	
	Hamamelis virginiana[c]	Leaf spot	1	99%	
	Myrica pensylvanica[c]	Dieback	1	99%	
	Rhododendron 'Haaga'	Dieback, leaf lesion	2	99%	
	Rhus aromatica[c]	Leaf lesion	1	100%	DQ486664
	Rhus spp.[c]	Leaf and petiole necrosis, wilt	1	99%	
	Rosa sp.	Root rot	1	99%	
	Syringa 'Miss Ellen Willmott'[c]	Leaf spot	1	100%	DQ486665
	Syringa vulgaris 'Common Purple'[c]	Dieback	1	100%	DQ486666
	Syringa cvs.[c]	Dieback, leaf lesion	7	99–100%	
	Taxus cvs.	Dieback	2	99–100%	
P. hedraiandra	*Viburnum trilobum*	Leaf tip necrosis	1	100%	DQ486667
P. megasperma	*Hibiscus* sp.[c]	Stem rot, root rot	1	99%	DQ486668
P. nicotianae	*Euphorbia* sp.	Stem rot	1	100%	DQ486669
	Magnolia stellata	Leaf margin necrosis	1	100%	
	Rhododendron 'Mikkeli'	Canker, leaf lesion	2	100%	
	Rhus aromatica[c]	Dieback	1	100%	DQ486670
P. taxon Pgchlamydo	*Rhododendron* 'Henry's Red'[c]	Leaf lesion	1	100%	
	Taxus sp.[c]	Dieback	1	99%	DQ486671
P. sp. MN1	*Malus* 'Snowdrift'[c]	Leaf lesion	1	n/a[d]	DQ486672
Unknown 1	*Malus* 'Brauzam'	Leaf Spot	1	n/a[e]	
Unknown 2	*Acer platanoides*	Dieback, leaf tip necrosis	1	n/a[f]	DQ486658

[a] These values represent the range of nucleotide identities in the ITS-1, 5.8S, and ITS-2 rDNA between the various isolates of the respective *Phytophthora* species and GenBank accessions. All BLAST search results presented were accompanied by an *E* value of 0.0. Isolates sharing a nucleotide identity with a voucher specimen below 99% were verified morphologically.

[b] Accessions to GenBank were made for a representative number of isolates for each species. In addition, cultures representing these accessions will be deposited in the American Type Culture Collection (ATCC).

[c] These host genera are not proven hosts of the respective *Phytophthora* species.

[d] ITS and β-*tub* sequences of *P.* sp. MN1 did not match any species in GenBank well despite nonambiguous sequence data.

[e] Unknown 1 produced poor ITS sequence data that matched *P. drechsleri* and resulted in an *E* value of 2×10^{-82}. Its *cox*I sequence data matched *P. gonapodyides,* resulting in high nucleotide identity (99%) and an *E* value of 0.0. Morphologically, it represented *P. drechsleri* more so than *P. gonapodyides*.

[f] The ITS sequence of Unknown 2 matched that of a *P. fragariae* var. *rubi* GenBank strain with a high nucleotide identity (99%). The same isolate had high nucleotide identity (99%) with *cox*I of a *P. cambivora* GenBank specimen. Morphologically, the isolate matched both species.

P. citricola was baited from potting mix that contained plants from which no *Phytophthora* isolates were recovered. In addition to being baited from potting mix, *P. citricola* was isolated from an irrigation water holding pond in 2004. Surveys of potting mixes and irrigation water were not conducted in 2005.

Unlike the 2002 and 2003 surveys, *Phytophthora* species identified from surveys in 2004 and 2005 were isolated from many host genera rather than from only *Rhododendron* species. Several species were identified in the 2004 and 2005 surveys that were not isolated in previous surveys (Table 2). *P. cambivora* and *P. megasperma* were two of these species. Two additional species not found in previous surveys were isolated from *Malus* (crabapple) cultivars. One (Unknown 1, Table 2), cultured from *Malus* 'Brauzam', had an ITS sequence that best matched *P. drechsleri* (accession L76549). However, the ITS rDNA was sequenced several times, resulting in ambiguous base-calling, and the BLAST search match to *P. drechsleri* produced an *E* value of 2×10^{-82} and a nucleotide identity of 99% (162/164). The *cox*I gene of Unknown 1 was sequenced, and it best matched *P. gonapodyides* (accession AY564181). The morphology of Unknown 1 was more typical of *P. drechsleri* than of *P. gonapodyides*, given its production of chlamydospores and hyphal swellings.

Another isolate (*P.* sp. MN1) cultured from a *Malus* species had highest ITS nucleotide identity with *P. quininea* (accession DQ275189). Nevertheless, this nucleotide identity was low (92% [434/472]) and the *E* value was high (10^{-171}), despite unambiguous sequence data. *P. ramorum* (accession AY616757) shared the next highest ITS nucleotide identity (*E* value = $5*10^{-136}$) with *P.* sp. MN1 after *P. quininea*. A sequence produced from the β-*tub* gene of this isolate compared most closely with that of a *P. insolita* GenBank submission (accession AY564073) (nucleotide identity = 95% [798/836], *E* value = 0.0). Both *P. quininea* and *P. insolita* are homothallic species (19), but *P.* sp. MN1 is heterothallic. Due to low ITS and β-*tub* nucleotide identities and different sexual reproduction character between our isolate and that of known species, *P.* sp. MN1 appears to represent an undescribed taxon.

Another species (Unknown 2, Table 2) not found in previous surveys was isolated from *Acer platanoides* (Norway maple). It closely resembled both *P. fragariae* var. *rubi* and *P. cambivora*. The ITS sequence derived from Unknown 2 shared 99% (768/770) nucleotide identity with a *P. fragariae* var. *rubi* GenBank submission (accession AF266761). However, the *cox*I gene of the isolate best matched that of *P. cambivora* (accession DQ202503), sharing 99% (869/879) nucleotide identity. Morphologically, Unknown 2 resembled *P. fragariae* var. *rubi* in being homothallic. It resembled *P. cambivora* in producing bullate oogonia and hyphal swellings.

DISCUSSION

Surveys completed during 2002 to 2005 revealed the most frequently isolated *Phytophthora* species from Minnesota ornamental nurseries were *P. cactorum*, *P. citricola,* and *P. citrophthora*. All three species are common in ornamental nurseries around the world and have been isolated from container mixes from nurseries in the southeastern United States (20), from ornamental nursery plants in Oregon (43) and Ohio (49), and from recycled irrigation water in ornamental nurseries in Germany (50). *P. citrophthora* was the most common *Phytophthora* species isolated from effluent holding ponds in three commercial nurseries in California, and *P. citricola* was also isolated there (36). Irrigation water in six Virginia nurseries contained *P. citrophthora* and *P. citricola* (27). In five nurseries in Poland, Orlikowski and Szkuta (42) isolated *P. citricola* from 88% of all diseased rhododendron tissues tested. Since these species are common to many areas of the world, the isolation of these species from Minnesota nurseries was not surprising. Not only are *P. cactorum*, *P. citricola*, and *P. citrophthora* found in nurseries, but they also are encountered in natural areas. Balci and Halmschlager commonly isolated *P. citricola* in surveys of Austrian (5) and Turkish (6) woodlands. In the United States, *P. citricola* was collected from oak forest soils in Illinois, Indiana, Ohio, and West Virginia (4) and from stream water in the southern Appalachian Mountains (28).

Results from the current study suggest that the *P*. sp. MN1 belongs to an undescribed and unknown taxon. *P. quininea*, the species whose ITS rDNA most closely resembles that of *P.* sp. MN1, is a pathogen of *Cinchona* species in Peru, Bolivia, and Puerto Rico (19). *P. insolita*, the species whose β-*tub* gene most closely resembles that of *P.* sp. MN1, was discovered in soil from a citrus orchard in Taiwan and found to be pathogenic to apple and cucumber fruits in inoculation trials, but no natural hosts are known (19). This is the first report of this taxon, and its host range must be investigated to determine if it is a threat to natural ecosystems as well as to ornamental plants.

Two other unknowns were isolated in the 2004 and 2005 surveys. Though the identity of Unknown 1 remains debatable because of poor ITS sequence data, Unknown 1 is likely to be *P. drechsleri* and not *P. gonapodyides,* primarily because it produces chlamydospores (12,19). *P. drechsleri* was shown to be pathogenic on *Malus* roots and stems by Matheron et al. (38), and Unknown 1 was isolated from *Malus*. Further investigation of Unknown 1 is needed to establish its taxonomic status.

Unknown 2 was isolated from a variegated Norway maple tree, and it has an ITS profile most similar to that of *P. fragariae* var. *rubi*. However, *P. fragariae* var. *rubi* is a pathogen solely of *Rubus idaeus* (19). In addition, Cooke et al. (15) demonstrated that *P. fragariae* var. *rubi* could not reliably be differentiated from *P. fragariae* var. *fragariae* using ITS data. Subsequent investigations showed these two *Phytophthora* varieties could be differentiated by sequencing *cox*I and II genes (37). The *cox*I sequence of Unknown 2 shared highest nucleotide identity with *P. cambivora*. Morphologically, Unknown 2 resembles *P. fragariae* var. *rubi* in being homothallic and *P. cambivora* in producing bullate oogonia and hyphal swellings. The A2 mating type of *P. cambivora* occasionally produces oogonia in single culture (23). *P. cambivora* and a *P. fragariae*–like species have been shown to hybridize (11), and the resulting hybrids display morphological characteristics of *P. cambivora*, but they are homothallic. A standard hybrid between these two parent species was named *P. alni* subsp. *alni* (14). The fact that the isolate we collected from a diseased Norway maple has gene sequences similar to both *P. fragariae* var. *rubi* and *P. cambivora* and morphology similar to *P. alni* subsp. *alni* suggests it may be hybrid in origin. Since *P. alni* subsp. *alni* has proven to be a very damaging pathogen of *Alnus* (11,24), the presence of a similar organism in Minnesota is alarming.

The likelihood of *Phytophthora* hybrids developing in nursery settings depends, in part, on the two parental species occupying similar areas on the same host plant (11). We cultured 18 isolates sharing the same diseased tissue with a different *Phytophthora* species in our 2005 surveys (i.e., 2.3% of the 2005 samples). This demonstrates that *Phytophthora* species commonly occupy the same niche in nursery environments, and careful investigation of *Phytophthora* isolates should determine if they are suspected hybrids. This is exceedingly important since *Phytophthora* hybrids have been found to infect different hosts than parent species (11,18), and hybrids can cause widespread damage on these new hosts (24).

The previously identified but yet undescribed species *P.* taxon Pgchlamydo was isolated from new hosts, *Rhododendron* spp. and a *Taxus* sp., in 2003 and 2005 from Minnesota nurseries. Brasier et al. (13) suggested that isolates previously declared *P. gonapodyides* belonged to a separate taxon because they produced chlamydospores and spherical hyphal swellings that were unlike *P. gonapodyides*. This conclusion was supported by another study that found those same isolates, named Group K isolates, differed from known species in their mitochondrial DNA, restriction fragment length polymorphism (RFLP), and isozyme profiles

(39). Brasier et al. (12) first introduced the designation *P.* taxon Pgchlamydo for these chlamydospore-producing isolates and showed that besides differing morphologically, they vary in ITS sequence from *P. gonapodyides*. *P.* taxon Pgchlamydo has been isolated from Douglas fir (*Pseudotsuga menziesii*) roots in British Columbia, Noble fir (*Abies procera*) roots and stems in Oregon, cherry (*Prunus*) roots in Cheltenham, UK, and water in France (12,13). Brasier et al. (12) suggested that 10 other isolates placed in Group K by Mills et al. (39) are isolates of *P.* taxon Pgchlamydo. They were isolated from *Prunus* species in New York and Michigan and *Malus pumila* in New York (39). It is thought that *P.* taxon Pgchlamydo is most often found in river water or wetland and forest soils rather than ornamental crops (12). Indeed, *P.* taxon Pgchlamydo isolates were isolated recently from stream water in *Austrocedrus chilensis* forests in Patagonia, South America (25). However, isolates of *P.* taxon Pgchlamydo were also recently baited from roots of alder trees in three nurseries in Bavaria (31). Results from our survey suggest *P.* taxon Pgchlamydo may be more widespread in the horticultural community than previously thought. Fulfillment of Koch's postulates on *Taxus* and *Rhododendron* are needed to confirm that *Rhododendron* and *Taxus* are new hosts.

Our identification of cultures isolated in 2002 and 2003 surveys produced the first report of *P. hedraiandra* in the United States (46). All *P. hedraiandra* isolates were associated with diseased rhododendrons, and Koch's postulates using *Rhododendron* 'Mikkeli' as the host were successfully completed (46). *P. hedraiandra* closely resembles *P. cactorum* genetically and morphologically (16). *P. cactorum* also infects *Rhododendron* (19), and more work must be done to investigate how the host range of *P. hedraiandra* differs from that of *P. cactorum*. Here, we report an additional association of *P. hedraiandra* from *Viburnum trilobum* roots. *P. hedraiandra* is a proven pathogen of *Viburnum* species in Italy and Spain causing collar and root rot as well as leaf lesions (7,41).

Species such as *P. cambivora* and *P. megasperma* were not found commonly in our survey of Minnesota nurseries, but they have been isolated in other ornamental nursery surveys (36,43,50). *P. cambivora* and *P. megasperma* were not encountered more often, probably because they are primarily root pathogens (19) and sampling in our study focused on symptomatic aboveground plant parts.

Fortunately, *P. ramorum* was not found in Minnesota nurseries over the past 4 years. This, however, does not indicate the pathogen has not been introduced, since it may have gone undetected. The discovery of *P.* sp. MN1 in a Minnesota retail nursery indicates that this taxon is likely to be found in other locations in the United States. Furthermore, its relatedness to exotic *Phytophthora* species suggests its host range and etiology need to be investigated promptly. This is the first report of *P. cambivora*, *P. citrophthora*, *P. nicotianae*, and *P.* taxon Pgchlamydo in Minnesota. The host ranges of *P. hedraiandra*, *P.* sp. MN1, *P.* taxon Pgchlamydo, and Unknown 2 will be investigated further. In addition, we report many potential new hosts for various *Phytophthora* species, and pathogenicity trials must be carried out to verify pathogenicity on these hosts. Surveys in Minnesota and in other regions of the United States must continue, and new *Phytophthora* species and hybrids that have potential for economic and ecological disaster must be discovered and their etiology determined.

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