Phytophthora spp. Associated with Forest Soils in Eastern and North-Central U.S. Oak Ecosystems

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ABSTRACT

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A survey of soils associated with oak species was conducted in 2003 and 2004 in Indiana, Illinois, Maryland, Michigan, Minnesota, Pennsylvania, Ohio, West Virginia, and Wisconsin to investigate the occurrence of *Phytophthora* spp. Soils taken from around the base of healthy and declining oak trees were flooded with H_2O and *Quercus robur* leaflets were used as bait for *Phytophthora* spp. From 829 soil samples collected near trees, 21% were positive for *Phytophthora* spp., with 55% of the 125 sites surveyed yielding a *Phytophthora* sp. *Phytophthora cinnamomi* was the most frequently isolated species, representing 69.4% of the *Phytophthora*-infested sites surveyed. Other species, in decreasing order of isolation frequency were *Phytophthora* sp. 2, *P. citricola*, *P. europaea*, *P. cambivora*, *P. quercina-like* isolates, and *Phytophthora* sp. 1. No significant association was found between the presence of *Phytophthora* organisms and site characteristics such as latitude, elevation, soil pH, or the crown condition of the trees. However, in *P. cinnamomi*-infested sites, a significant association was found with the deteriorating crown status of *Q. alba* and the presence of *P. cinnamomi*. The absence of *P. cinnamomi* above the 40ºN latitude range also was noteworthy.

Additional keywords: oak decline, *Phytophthora cinnamomi*

Studies of the role that *Phytophthora* spp. play in forest health and their existence in diverse ecosystems have been the focus of research during recent decades. Surveys in many forest ecosystems have demonstrated the existence of a variety of *Phytophthora* spp. (3,4,19,24,31,33,35,49). Interest in *Phytophthora* organisms has intensified because of the destructive impact they caused to the native vegetation in areas where they have been introduced and become established. Examples include the introduction of *Phytophthora cinnamomi* Rands in Australia, Mexico, Spain, and Portugal (10,44,47); the impact of *P. lateralis* Tucker & Milbrath on Port Orford cedar (*Chamaecyparis lawsoniana* (A. Murray bis) Parl.) in western North America (25); and the invasion of *P. ramorum* Werres, De Cock & Man in't Veld in California woodlands and southwest Oregon, with its accompanying impact on the plant nursery industry in the United States and Europe (17,18,22,40). Studies of these problems also have led to the discovery of new species and distributions. During the course of oak decline studies in Europe, five new species of *Phytophthora* were found, including *P. europaea* E. M. Hansen & T. Jung, *P. pseudosyringae* T. Jung & Delatour, *P. psychrophila* E. M. Hansen & T. Jung, *P. quercina* T. Jung, and *P. uliginosa* E. M. Hansen & T. Jung (34–36). Other previously unknown species discovered include *P. nemorosa* E. M. Hansen and Reeser from mixed-hardwood forests of the central coast of California and southwestern Oregon (26). Likewise, *P. kernoviae* Brasier, Beales, and S. A. Kirk is associated with bleeding stem lesions of forest trees and foliar necrosis of ornamentals in the United Kingdom (9).

The existence of native or exotic species of *Phytophthora* in soils of eastern and central U.S. oak ecosystems is largely unknown. This lack of information and the potential threat of *P. ramorum* to eastern oak species provided the impetus for this study. The objective was to conduct isolations from soils sampled across a broad geographic range of oak and related forest types to provide baseline information on *Phytophthora* spp. that are associated with oak forest ecosystems in the eastern United States. Relationships among geographic locations, elevation, soil pH, and decline status of oak trees were evaluated with respect to the incidence of *Phytophthora* spp. that were isolated.

MATERIALS AND METHODS

Field study. Soils were sampled in Illinois (IL), Indiana (IN), Maryland (MD), Michigan (MI), Minnesota (MN), Ohio (OH), Pennsylvania (PA), Wisconsin (WI), and West Virginia (WV). Soils were sampled in spring 2004 (May to July) and fall 2004 (September to November). A few sites in MD and WV also were sampled in fall 2003. In each state, eight sites usually were sampled during the spring and fall periods. When possible, half of the sites were chosen from declining and half from nondeclining stands. Crown status of trees was assessed as follows: class 1, no symptoms of decline, crown transparency less then 10 to 15%; class 2, slight damage, dieback of some branch tips and small gaps in the lateral branch system of the crown, crown transparency 15 to 35%; class 3, moderate damage, apparent transparency in all parts of the crown, dieback of twigs and branches and large gaps in the lateral branch system, yellowing and wilting of leaves, epicormic shoots often present, crown transparency 35 to 55%; and class 4, severe damage, considerable transparency of crown and many large gaps, many dead twigs and branches, leaves mainly restricted to shoot tips, yellowing of leaves and many epicormic shoots, crown transparency 55 to 75% (3,4). Sites were chosen to avoid areas impacted by recent storm damage, major defoliation, or oak wilt caused by *Ceratocystis fagacearum* (T. W. Bretz) J. Hunt. Although most stands contained a diverse population of tree species, oak generally predominated. Trees were greater than 40 years of age and most were 70 to 100 years old. In each state, sampling sites were identified with respect to oak forest types and were distributed as much as possible among these different types. At each site, four soil subsamples were taken in cardinal directions and at a distance of 1 to 1.5 m from the base of each selected tree. After removing the organic layer, soils were sampled down to a depth of about 30 cm. The four subsamples were bulked to produce a total sample of approximately 2 kg. Generally,

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five trees were sampled per site. When no *Phytophthora* spp. were isolated during spring sampling, the same site was resampled in fall; however, different oak trees were sampled. Soil pH was measured for each composite soil sample using a 3:1 mix of soil and 0.011 $CaCl_2$ (= pH [$CaCl_2$]) or distilled water (= pH [H_2O]) and measured on a Thermo Orion pH meter.

Isolation. In the laboratory, each sample was mixed thoroughly; a single 250-g subsample was flooded with 500 ml of distilled water and baited by floating 3- to 7-day-old *Quercus robur* leaflets (32). Baited samples were kept at temperatures of 17 to 20ºC. After 3 to 5 days, discolored leaf samples were examined microscopically (×200) and those with sporangia typical of *Phytophthora* spp. were plated on PARPNH selective medium (V8 juice agar [V8A] media amended with pimaricin at 10 µg/liter, ampicillin at 200 µg/liter, rifampicin at 10 µg/ml, pentachloronitrobenzene [PCNB] at 25 µg/liter, nystatin at 50 µg/liter, and hymexazol at 50 µg/liter) to establish cultures (3,4). When initial isolation attempts failed, soils were dried at room temperature for 2 weeks and the isolation method was repeated using the same protocol. Soil samples from IN, MD, OH, PA, and WV were processed at West Virginia University (WVU) and those from IL, MI, MN, and WI were processed at the United States Department of Agriculture (USDA) Forest Service laboratory in St. Paul, MN using the same procedure described above. Randomly chosen soil samples were exchanged between laboratories to compare isolation results.

Identification. Isolates were identified to species using morphological features by comparing them with authenticated isolates and species descriptions (20). For morphological identification, the following features were measured: size and type of sporangia, oogonia, antheridia, chlamydospore production, occurrence of hyphal swellings, colony morphology on potato-dextrose agar (PDA), and growth rate on V8A. Heterothallic isolates were crossed with known mating types of *P. cinnamomi* and *P. cambivora*. Representative isolates also were identified at the Pennsylvania State University, Department of Plant Pathology (Y. Balci and S. Kang, *unpublished data*). This was done by sequencing the internal transcribed spacer region of the rDNA and comparing the sequences with those in GenBank using BLAST searches. The sequence data and cultures of representative isolates have been deposited at GenBank and at the American Type Culture Collection, respectively (Table 1).

Data analysis. The relationship between the existence of *Phytophthora* spp. and decline status of oak trees as well as the latitude was analyzed using logistic regression analyses and tested in a contingency table using Fisher's exact test. The differences of pH and elevation in *Phytophthora* spp.-infested and free sites were tested using two-tailed Student *t* tests. Analyses of all data were performed using the software program JMP 5.0 (43).

RESULTS

Seven *Phytophthora* spp. were isolated from 69 (55%) of the 125 sites that were included in the spring and fall sampling periods (Tables 1 and 2). The spring sampling period yielded twice the number of *Phytophthora* isolations as the fall period (Table 2). In all, 36 sites where no *Phytophthora* spp. were isolated in the spring period were resampled in the fall; of those, 9 sites yielded *Phytophthora* spp. Soils surrounding 829 trees were sampled and 171 (21%) yielded a *Phytophthora* isolate (Table 2). Usually, *Phytophthora* spp. were obtained on the first isolation attempt, although 23 (13.5%) additional isolations were made as a result of the second baiting routine. All of the *P. quercina*-like isolates and about one-third (five isolates) of *Phytophthora* sp. 2 were isolated during the second baiting. Generally, one species was isolated from a site. However, two species were isolated at five sites and three species were isolated at three sites. A single species generally was isolated from each soil sample, except on five occasions, when two species were isolated. For soil samples

Table 1. Information on representative isolates

Isolate	*Phytophthora* spp.	Oak species	Sampled from	Geographic coordinates and state[a]	GenBank accession no.	ATCC no.[b]
OH 4/4	*Phytophthora cambivora*	*Quercus velutina*	Forest soil	N 81º 26′ 0.1″; E 39 º54′ 36″, OH	EF032478	MYA-4089
WV Gr1/1	*P. cinnamomi*	*Q. rubra*	Forest soil	N 39º 40′ 22″; E 79 º46′ 20.3″, WV	EF032480	MYA-4085
OH 6/5	*P. citricola*	*Q. rubra*	Forest soil	N 84º 3′ 52.8″; E 39 º29′ 24″, OH	EF032477	MYA-4087
BM 1/10	*P. europaea*	*Q. alba*	Forest soil	N 38º 36′ 03″; E 79 º35′ 24.3″, WV	DQ313222	MYA-4088
MN 023	*P. quercina-like*	*Q. rubra*	Forest soil	N 93º 33′ 10.5″; E 45º 26′ 14.1″, MN	DQ313224	MYA-4090
WV BSC 1/3	*Phytophthora* sp. 1	*Q. rubra*	Forest soil	N 38º 17′ 10″; E 79º 58′ 31.1″, WV	EF032479	MYA-4091
MD 9/2	*Phytophthora* sp. 2	*Q. rubra*	Forest soil	N 78º 24′ 26.7″; E 39º 42′ 0″, MD	DQ313223	MYA-4086

[a] OH = Ohio, WV = West Virginia, MN = Minnesota, and MD = Maryland.
[b] American Type Culture Collection.

Table 2. Assemblage and isolation frequencies of *Phytophthora* spp. in each state during spring and fall 2004[a]

	Sites sampled		Positive sites		Soil samples assayed		Positive soil samples		
State	Total	S/F	Total	S/F	Total	S/F	Total	S/F	*Phytophthora* spp.
Illinois	10	10/–	1	1/–	50	50/–	1	1/–	*Phytophthora citricola*, S
Indiana	14	8/6 (2)[b]	9	6/3	80	40/40	30	20/10	*P. cinnamomi*, S,F; *P. citricola*, F
Maryland	16	14/2 (5)	10	7/3	106	71/35	19	14/5	*P. cinnamomi*, S,F; *Phytophthora* sp. 2, S
Michigan	8	6/2 (6)	0	–/–	70	30/40	–	–/–	–
Minnesota	9	8/1 (7)	3	2/1	80	40/40	7	3/4	*P. europaea*, F; *P. quercina-like*, S
Ohio	14	8/6 (1)	11	6/5	75	40/35	25	16/8	*P. cinnamomi*, S,F; *P. citricola*, S,F; *Phytophthora* sp. 2, S,F; *P. cambivora*, S,F
Pennsylvania	9	8/1 (7)	5	2/4	80	40/40	8	3/5	*P. cinnamomi*, S,F; *P. europaea*, F; *P. cambivora*, S,F; *Phytophthora* sp. 2, S
Wisconsin	11	8/3 (5)	4	3/1	80	40/40	4	3/1	*P. quercina-like*, S; *P. europaea*, S; *Phytophthora* sp. 2, S,F
West Virginia	34	28/6 (3)	26	22/6	208	163/45	77	57/20	*P. cinnamomi*, S,F; *P. citricola*, F; *P. europaea*, F; *Phytophthora* sp. 1, F; *Phytophthora* sp. 2, S,F
Total	125	98/63	69 (55%)	48/23	829	514/315	171 (21%)	116/54	…

[a] Data are number of samples; S = spring and F = fall sampling periods.
[b] Number in parentheses indicates the number of spring sampling sites that resulted in no *Phytophthora* recovery and, thus, were resampled in the fall sampling period.

containing more than one species of *Phytophthora*, the colonies of different species were different in morphology on the selective medium, and were subcultured onto V8A. No *Phytophthora* spp. were isolated from the Michigan soil samples. Several sites in Illinois, Minnesota, and Wisconsin also gave low isolation frequencies (Table 2).

Soil samples exchanged between laboratories gave a different result for only one soil sample, where *Phytophthora* sp. 2 was isolated at the WVU lab but not at the Minnesota lab.

P. cinnamomi was the most commonly isolated species. It was isolated from 69.4% of the positive sites and represented 73.4% of *Phytophthora* isolates (Table 3). *P. cinnamomi* was not isolated from sites in IL, MI, MN, or WI. This species was only isolated at sites below 40ºN latitude (Fig. 1). When crossed with tester strains, all of the *P. cinnamomi* isolates proved to be the A2 mating type. Additional sampling in Kentucky (KY) at two oak sites during spring 2005 also yielded isolates of *P. cinnamomi* (Fig. 1).

The second most common species isolated was *Phytophthora* sp. 2. This species was isolated from 16.7% of the sites that yielded *Phytophthora* spp. and represented 8% of all isolates (Table 3). It occurred over a wide geographical area, including sites in MD, OH, PA, WI, and WV (Fig. 1). *Phytophthora* sp. 2 did not correspond to any previously described species.

P. citricola Sawada, the third most common *Phytophthora* sp. isolated, was associated with 11.1% of the sites that yielded *Phytophthora* spp. in four states (IL, IN, OH, and WV) and represented 5.2% of isolates (Fig. 1; Tables 2 and 3). The fourth most common species was *P. europaea* . This species was isolated from MN, PA, WI, and WV (Fig. 1; Tables 2 and 3). *P. europaea* was commonly isolated in the fall and only once in the spring sampling period from a WI site. *P. cambivora* (Petri) Buisman was the fifth most frequently represented species and was only isolated in OH and PA (Fig. 1; Tables 2 and 3). Among the isolates of this species, four were identified as the A2 mating type and three were identified as the A1 mating type.

Four isolates were identified tentatively as *P. quercina-like* because their sequence data was closest to *P. quercina*. However, they differed from *P. quercina* culturally in oogonial features, growth pattern on PDA, and much slower growth rate. *P. quercina-like* isolates were isolated only during the spring sampling period from sites in MN and WI and only following a second baiting procedure after soils had been air dried and reflooded.

A seventh isolate, designated *Phytophthora* sp. 1, matched the sequence data of *P. europaea*. Its morphology differed from *P. europaea* isolates by having smaller globose oogonia with no tapered bases, a different growth pattern on PDA, and different optimum and maximum temperature limits for growth. This isolate will be analyzed further to classify it into appropriate taxa. The *Phytophthora* sp.1 isolate was found only once in a fall sampling period from WV (Table 2).

P. cinnamomi was isolated more frequently from soils associated with *Q. alba* (27%) and *Q. montana* (32%) than from soils surrounding the other oak species. The soils associated with *Q. rubra* were the most commonly sampled, and yielded the most diverse group of *Phytophthora* organisms. However, *P. cinnamomi* was associated with only 10% of the samples from *Q. rubra*. No *Phytophthora* spp. were isolated from soils from around *Q. stellata* and *Q. falcata*, although relatively few trees of these species were sampled.

In total, soils around 461 healthy trees and 368 declining trees were sampled. Among the 171 *Phytophthora*-infested soil samples, *Phytophthora* spp. were found from soils collected around 95 healthy trees and from 76 declining trees. For the infested sites, there was no significant association between the presence or absence of *Phytophthora* spp. and the decline status of trees (Fisher's exact test, P = 1.000). However, at sites infested with *P. cinnamomi*, there was a significant association with the presence of *P. cinnamomi* and declining *Q. alba* trees (Fisher's exact test, P = 0.013, RR = 1.82, 95% CI = 1.06 to 3.15). This association was not found for *Q. rubra* (Fisher's exact test, P = 0.086) or *Q. montana* (Fisher's exact test, P = 1.000).

Phytophthora organisms were isolated from soils that ranged in pH (H_2O) from 3.7 to 7.4 (3.2 to 7.1 for pH [$CaCl_2$]) and from elevations ranging from 11 to 1,119 m. When elevations among *Phytophthora*-infested and *Phytophthora*-free sites were compared, considering all *Phytophthora* spp., there was no significant difference in the means of elevations (t = 0.593, P = 0.554). Likewise, no significant difference existed considering only *P. cinnamomi*-infested sites and elevation (t = 0.940, P = 0.349). No significant differences were found for the mean pH (H_2O) values of *Phytophthora*-infested (5.1) and *Phytophthora*-free (5.04) sites (t = 0.782, P = 0.435). This also was true when pH (H_2O) of *P. cinnamomi*-infested sites was compared with *P. cinnamomi*-free sites (t = –1.916, P = 0.056). The absence of *P. cinnamomi* above 40ºN latitudes was significant (Fisher's exact test, $P < 0.001$) (Fig. 1), but no significant difference existed among the other *Phytophthora* spp. with respect to the 40ºN latitude range (Fisher's exact test, P = 1.000). *P. cinnamomi* was found in only one location in Pennsylvania above the 40ºN latitude range (Fig. 1).

DISCUSSION

Results of this study provide the first record of the broad range of *Phytophthora*

Table 3. Isolation frequencies of *Phytophthora* spp. from soil in relation to associated oak species and percent of *Phytophthora* spp. in *Phytophthora*-infested sites

		Number of positive trees		
***Phytophthora* spp.**	***Quercus* spp.**[a]	**Total**	**Spring/fall**	**Percent**
P. cinnamomi	*Quercus alba*	48	32/16	69.4
	Q. coccinea	3	1/2	
	Q. montana	26	21/5	
	Q. rubra	35	27/8	
	Q. velutina	15	10/5	
Phytophthora sp. 2	*Q. macrocarpa*	1	1/–	16.7
	Q. phellos	1	–/1	
	Q. platanoides	1	1/–	
	Q. rubra	9	4/5	
	Q. velutina	2	2/–	
P. citricola	*Q .alba*	2	1/1	11.1
	Q. coccinea	1	1/0	
	Q. muehlenbergii	1	1/–	
	Q. rubra	5	3/2	
P. europaea	*Q. alba*	3	3/–	8.3
	Q. phellos	1	–/1	
	Q. rubra	7	3/4	
	Q. velutina	2	–/2	
P. cambivora	*Q. alba*	1	1/–	6.9
	Q. muehlenbergii	1	1/–	
	Q. palustris	1	–/1	
	Q. rubra	2	2/–	
	Q. velutina	2	1/1	
P. quercina-like	*Q. ellipsoidalis*	1	1/–	4.2
	Q. rubra	3	3/–	
Phytophthora sp. 1	*Q. rubra*	1	–/1	1.4
Total	…	175	120/55	…

[a] Total number of oak species sampled were as follows; 175 *Q. alba*, 18 *Q. coccinea*, 20 *Q. ellipsoidalis*, 15 *Q. macrocarpa*, 81 *Q. montana*, 5 *Q. muehlenbergii*, 1 *Q. phellos*, 3 *Q. palustris*, 1 *Q. platanoides*, 382 *Q. rubra*, and 124 *Q. velutina*.

spp. that are associated with oak forest soils in the eastern and central United States. Study of oak forest soils in Europe yielded similar isolation frequencies, with about half of the sites yielding a *Phytophthora* sp. (3,4,19,27,31,33,49). Thus, *Phytophthora* spp. appear to be common soil inhabitants in these oak ecosystems. The higher isolation rate achieved in the spring sampling probably was due to the more favorable soil moisture and temperatures for the organism during this period of the year. In Australian forests, higher inoculum levels of *P. cinnamomi* were found in spring and early summer (51). The successful recovery of *Phytophthora* organisms in about one-third of the resampled sites and from 13.5% of soil isolations after the second baiting routine suggests the need for sampling throughout the year and a repeated isolation routine for a single soil sample to avoid false negative results on sites or soils initially assessed as *Phytophthora* free. Furthermore, to enhance detection, repeated isolation attempts are required for some species of *Phytophthora* such as *P. quercina* (3,4) and *P. cactorum* (Lebert & Cohn) J. Schröt. (29), because recovery was enhanced by repeated flooding after soils have been air dried. That also was true for *P. quercina-like* isolates which were isolated after the second baiting attempt in this study.

The frequent recovery of *P. cinnamomi* in our study corresponds with surveys conducted in the southeastern United States (13,53,55). The differences in species assemblage with those studies involves the occurrences of *P. cactorum* and *P. heveae*. The species that were isolated in this survey differed from similar *Phytophthora* oak forest surveys in Europe in that *P. cinnamomi* was the most frequently encountered species. In Europe, *P. quercina* commonly was isolated in oak forest surveys (3,4,19,24,27,31,33,49). The absence of *P. quercina* within our survey compared with European studies suggests a geographic limitation of this species to European oak forests. In oak forest studies conducted in Europe (3,4,19,24,27,31, 33,49) that were similar to our study, *P. citricola*, *P. europaea*, and *P. cambivora* also were present. Differences in species assemblage in European surveys compared with our survey mostly regard the occurrence of the unknown species: *Phytophthora* sp. 1, *Phytophthora* sp. 2, and *P. quercina-like* isolates. Species isolated in European oak forests (3,4,19,24,27,31, 33,49) but not isolated in our study included *P. cactorum*, *P. cryptogea* Pethybr. & Lafferty, *P. gonapodyides* (Petersen) Buisman, *P. megasperma* Drechsler, *P. pseudosyringae*, *P. psychrophila*, and *P. uliginosa*. With the exception of *P. gonapodyides*, which is widely distributed in streams of hardwood forests in the southern Appalachian mountains (28), and *P. cactorum*, which was isolated from forest soils in the southeastern United States (13), none of the other species have been reported from oak forest soils in the eastern United States. Our isolation results on species assemblage are consistent with similar studies (3,4,19,24,27,31,33,49, 53,55), where usually only one species was isolated from a sampled site. We believe more species would have been isolated if a more intensive survey was conducted in a sampling area, including different sites with different vegetation types and microenvironments. In this study, as in European surveys, a few sample sites were chosen from a large sampling area (usually the sampling area was several hundred hectares). Therefore, the species assemblage

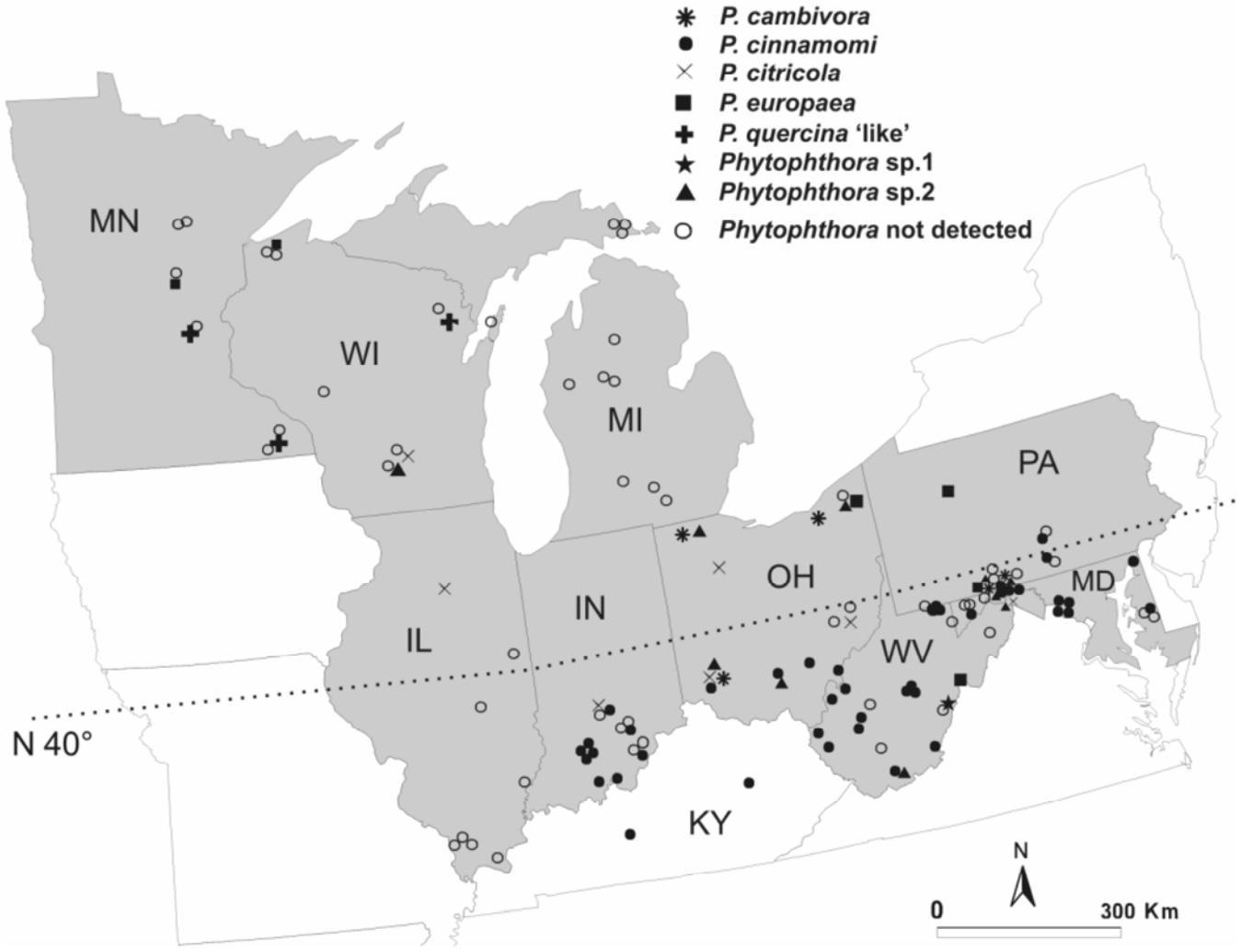

Fig. 1. Distribution of *Phytophthora* spp. isolated from soil throughout the survey area.

in this study may represent the most common species present within the study area and may under-represent the species diversity because of the low intensity of this sampling.

P. cinnamomi is considered exotic to North America and is believed to have been introduced more then a century ago (16,54). Whether this species suppressed the indigenous *Phytophthora* populations and thus resulted in higher isolation frequency is unknown. *P. cinnamomi* is widely distributed and is the most commonly isolated *Phytophthora* sp. in the southeastern United States and is extensively reported from forest soils, ornamental plant nurseries, and urban settings throughout the United States (13,16,20,21, 52,54,55). *P. cinnamomi* has been implicated as a factor in the littleleaf disease complex of pine trees (*Pinus echinata* and *P. taeda*), with root rot and mortality of sand pine plantations (*P. clausa*) in west Florida, and with the death of American chestnut (*Castanea dentata*) in the southeastern United States (5,16,46,54). The species also threatens the Christmas tree (*Abies* spp.) industry (7,54). *Phytophthora cinnamomi* has been reported to be associated with bleeding cankers on *Q. laurifolia* in central Florida (52) and trunk cankers of *Q. agrifolia* and *Q. suber* in California (38). Its impact on oak trees under forest settings was shown in central Mexico on *Q. glaucoides*, *Q. peduncularis*, and *Q. salicifolia* (47) and in Europe on *Q. rubra* and *Q. robur* by its association with ink disease (41) and decline of *Q. suber* and *Q. ilex* (10,39). Although the organism was widespread in our study, we found a significant association of *P. cinnamomi* only with declining *Q. alba*. We could not associate *P. cinnamomi* with declining *Q. rubra*, despite its frequent isolation from *Q. rubra* sites.

Our inability to isolate *P. cinnamomi* from forest soils above 40ºN latitude could be due to low winter temperatures or other suppressive soil conditions that simply may be unfavorable for its survival. The current range of *P. cinnamomi* matched areas with an average annual minimum temperature greater than –20ºC, which represents USDA plant hardiness zone 6a (15). The negative effect of low temperatures on *P. cinnamomi* populations (6,51,54), the pattern of pathogen spread in Europe (11), and development of *P. cinnamomi*-induced cankers on *Q. rubra* and *Q. robur* (37) has been demonstrated; however, less is known about the effect of temperature on pathogen survival in soil. Areas with low soil temperature may have inhibited pathogen survival and, thus, restricted the spread of *P. cinnamomi* to other geographic areas. *P. cinnamomi* did not survive in upper soil layers but remained viable at depths below 10 cm after freezing temperatures in artificially inoculated soils (54). Similarly, cold winter temperatures inactivated *P. cinnamomi*-infested root segments of *Abies fraseri* in naturally infested soils when soil temperature dropped to 0ºC or less (6). Furthermore, Roth and Huhlman (42) did not observe mortality of Douglas-Fir (*Pseudotsuga menziesii*) seedlings grown in *Phytophthora cinnamomi* infested soils when soil temperatures were lower than 15.5ºC. When compared with *P. cinnamomi*, the widespread occurrences of the other isolated *Phytophthora* spp. within the survey area suggest that they are better adapted to diverse environmental conditions such as temperature extremes.

Our data provides circumstantial evidence that supports an association between the presence of *P. cinnamomi* and declining *Q. alba* trees. Other studies also have demonstrated an association of *Phytophthora* spp. with declining oak trees in certain geographic areas (4,33,49). However, the aim of this study was to examine the incidence of *Phytophthora* spp. in oak ecosystems rather than to examine their role. Because of the involvement of a variety of biotic and abiotic factors in any oak decline syndrome (1,48), the association of *Phytophthora* spp. with crown condition ratings over a large survey area is not definitive evidence of a cause-and-effect relationship. Indeed, in some studies, no association was found with decline and frequency of *Phytophthora* spp. (3,14,19, 27,30,49).

P. citricola has a worldwide distribution and commonly was isolated in oak and chestnut (*C. sativa*) stands in Europe (3,4,19,24,33,49,50) and was isolated from trunk cankers on *Q. agrifolia* in natural oak woodlands in California and from streams in the southern Appalachian Mountains (21,28,38). It has been suggested that this organism may form a species or subspecies complex which could be separated into genetically and morphologically distinct types (1,12). Among the three *P. citricola* types identified in Austria and Turkey (3,4), isolates obtained in this study matched the type A isolates. Pathogenicity of *P. citricola* isolates on various oak species has been demonstrated (Y. Balci, *unpublished data*; 4,8,32,38), but no relationship was observed in our study with declining oak trees.

P. europaea previously was isolated in oak forests in Austria, France, and Germany (24,27,33). In the United States, this species has been isolated from leaves of bay laurel (*Umbellularia californica*) and stream water in California (D. Rizzo, *personal communication*), and Christmas tree plantation soils in Michigan (D. W. Fulbright, *personal communication*). Isolates from California and Michigan were identical in their morphology and internal transcribed spacer rDNA sequence analysis when compared with *P. europaea* isolates from this study (*data not shown*). Although its origin remains unknown, our findings suggest a much wider occurrence of this species in U.S. oak forests than in Europe (2).

P. cambivora was a commonly isolated species from European oak forest soils (19,27,32,33), and often was associated with soil and cankers of chestnut trees (*Castanea* sp.) (49,50). This species also was isolated from forest soils in Argentina (23). In the United States, it was mostly recognized as causing crown and root rot of fruit trees (19) and, recently, was found in south-central Oregon in soils and basal cankers on chinquapin (*Castanopsis* sp.) (45). This species appears to be common in forest soils and has the ability to cause stem lesions on particular hosts, including *Castanea* and *Fagus* spp. (1). However, it has never been found in association with any aboveground cankers on oak trees.

The occurrence of a variety of well-known as well as unknown isolates in oak forests of the eastern and north-central United States suggests that a diverse population of *Phytophthora* organisms exists. Their presence usually is not associated with symptoms on aboveground parts, although they may cause root damage. A time lag between factors may obscure the relationship between soilborne *Phytophthora* spp. and the initiation of aboveground decline symptoms. The existence and pathogenicity of exotic *Phytophthora* organisms in forest ecosystems likely are related to specific environmental conditions. As shown here for *P. cinnamomi*, the pathogen is widely distributed in diverse forest settings that favor its survival but not necessarily its virulence.

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