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Phytophthora Species Detected in Two Ozark Forests with Unusual Patterns of White Oak Mortality

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Abstract

Widespread decline and mortality of white oaks (*Quercus alba*) occurred in Missouri Ozark forests between 2011 and 2017. Symptoms included rapid crown death with bronzing of leaves, retention of dead leaves, crown dieback and thinning, and loss of large limbs within one year of death. Decline and mortality were associated with hillside drainages and fit descriptions of European oak forests predisposed to decline by pathogenic *Phytophthora* species. A survey was performed at two locations in 2014 and 2015 to assess the distribution of dead and declining white oaks, and the occurrence and distribution of *Phytophthora* species. Multiple *Phytophthora* species were detected, including *P. cinnamomi*, *P. cactorum*, *P. europaea*, and *P. pini*. *P. cinnamomi* was the most common and widely distributed species among plots at both locations. The detection of *P. cinnamomi* at the base of white oaks was not associated with poor crown vigor. However, more quantitative survey techniques are necessary to clearly evaluate this relationship. *P. cinnamomi* kills fine roots of white and red oaks in North America and has been associated with the decline of white oaks in the United States (Ohio) and other countries. Further studies are needed to determine the importance of *P. cinnamomi* in oak decline within the Ozark highlands.

White oak (*Quercus alba* L.) is one of the most important hardwood species in temperate forests of the eastern United States. Its range extends from southern Maine to northern Florida and as far west as eastern Oklahoma and Texas. White oak is especially important in the Ozark highlands where the abundance and basal area of trees in the white oak section (*Quercus* section *Quercus*) is greater relative to other forests in the eastern United States (Fei et al. 2011; Oak et al. 2016). White oak is the most abundant hardwood species growing in Missouri forestlands, and it is used for many applications including saw timber and specialty oak barrels; in addition, the annually produced acorns support large populations of wildlife (Piva and Treiman 2016).

Rapid white oak mortality and occasional post oak (*Quercus stellata* Wangenh.) mortality were first reported in late summer 2011, and again during May 2012. Foresters reported that other closely related tree species, including chinkapin oak (*Q. muehlenberghii* Engelm.), appeared vigorous even when growing next to dead white oaks. Dead white oak trees included large overstory trees as well as smaller oak trees located in upland valleys with ephemeral or intermittent drainages (Reed et al. 2016b). Numerous additional reports were received between July 2012 and September 2013 following a 9-month drought that began May 2012. State agencies received fewer reports between 2014 and fall 2016. During 2017, there was an increase in reports of new mortality.

Most reports of mortality were received from the Salem Plateau region of the Missouri Ozarks in southeastern Missouri (Reed et al. 2016a). Subsequent aerial surveys revealed oak mortality occurred in discrete areas within state conservation areas, the Mark Twain National Forest, and private lands. Within valleys in those affected areas, mortality occurred along drainages either uniformly or in pockets (Fig. 1).

Tree death followed two patterns. The first pattern was the rapid death of trees with full crowns within a few weeks of the first leaves turning brown (Fig. 1). The second pattern involved tree crowns that were already noticeably thin. The crowns of these trees died suddenly or one part of the crown died suddenly and the rest of the crown died the following growing season. Similar to the first pattern, these trees also retained their dead foliage. Trees typically shed their small twigs and limbs within a year after death (Fig. 1). Tree mortality occurred shortly after leaf development in the spring or in late summer months. *Biscogniauxia* spp. and *Armillaria* spp. were often associated with dead trees. *Armillaria* isolates from fans on tree roots were identified as *Armillaria gallica* and *A. mellea* (Reed et al. 2016a). The cause of the white oak mortality was unknown, so the disease was given the temporary name, rapid white oak mortality, based on the affected host and the main characteristic symptom, rapid tree crown death (Ostry et al. 2011).

Since the 1970s, oak death in the Missouri Ozarks has been primarily associated with oak decline. Decline is a general disease category used to describe a stress-mediated disease of complex etiology that consists of predisposing, inciting, and contributing factors (Manion 1981). In the Missouri Ozarks, it has primarily affected oaks in the red oak section (*Quercus* section *Lobatae*) (Bruhn et al. 2000; Fan et al. 2012; Oak et al. 2016; Pedersen 1998). Oak et al. (2016) describe the numerous surveys of decline incidence and severity that elucidated the etiology of red oak decline in the Ozarks (Oak et al. 2016). It is unknown if the same complex of predisposing, inciting, and contributing factors result in white oak decline, in part, because of the historically low levels of white oak decline occurring in the Ozarks (Fan et al. 2012). In addition, oaks in the white oak section are physiologically and ecologically distinct from oaks in the red oak section (Johnson et al. 2009).

In the Ozarks, decline of red oaks largely occurs 2 to 3 years after physiologically mature red oaks are stressed by severe or prolonged droughts (Fan et al. 2012). The largest numbers of declining red oaks are found in dense stands on exposed, upper backslopes and ridgetops with nutrient-poor, rocky soils (Fan et al. 2012). Large-scale, anthropogenic disturbances in the late 1800s and early 1900s followed by fire suppression favored the establishment and growth of dense red oak stands on these less favorable sites (Kabrick et al. 2008; Oak et al. 2016). However, red oak decline does occur on other

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site types and is just as severe when tree density is taken into consideration (Kabrick et al. 2008). Agents known to contribute to the decline of red oaks include *Armillaria* spp., *Biscogniauxia atropunctata*, *Agrilus* spp., and red oak borer (*Enaphalodes rufulus*) (Bruhn et al. 2000; Stephen et al. 2001; Wargo 1996). Drought is the primary initiator of decline in the Ozarks, but late spring frosts and insect defoliation can also incite decline.

The symptoms and patterns of white oak mortality in the Missouri Ozarks were more similar to those associated with declines of white oak in Ohio, USA, and cork (*Q. suber*) and holm oak (*Q. ilex*) in the Mediterranean region of Europe (Balci and Bienapfl 2013; Balci et al. 2010; Camilo-Alves et al. 2013; McConnell and Balci 2014; Nagle et al. 2010). The root-rot pathogen *Phytophthora cinnamomi* Rands was determined to have contributed to or acted as a predisposing factor in these declines (Camilo-Alves et al. 2013; McConnell and Balci 2014). *P. cinnamomi* causes root and crown rots in more than 900 host plant species on six continents (Zentmyer 1980). Symptoms of *P. cinnamomi* mimic drought and are similar to those observed in this study (Camilo-Alves et al. 2013). Excess soil moisture promotes Phytophthora root rot by increasing propagule production, motility, and infection while negatively affecting plant roots (Weste and Taylor 1971; Zentmyer 1980). Soil saturation with water, long periods of water stress, or fluctuations between water saturation and water stress result in greater symptom severity and mortality in infected than uninfected hosts (Camilo-Alves et al. 2013; Corcobado et al. 2014; Zentmyer 1980).

Schwingle et al. (2007) reported *Phytophthora* species, including *P. cinnamomi*, present in soils at the base of several declining oak trees in Missouri forests. At that time, it was suggested that *Phytophthora* might be involved in oak decline, but no follow-up studies were initiated in the Ozarks. Consequently, we surveyed the distribution of high and low vigor white oak trees in two Ozark valleys to determine if decline and mortality patterns were associated with topographic slope positions that typically favor *Phytophthora* reproduction and spread. These are slope positions that receive more water from upslope and up valley (Weste and Taylor 1971). We also surveyed for the occurrence and distribution of *Phytophthora* species, especially *P. cinnamomi*, to determine if any species were widespread and abundant enough to be a potential concern. To explore the possibility that *P. cinnamomi* was involved in the decline, we examined the vigor of tree crowns to determine if trees with *P. cinnamomi* at their base differed in tree crown vigor from those without *P. cinnamomi.*

Materials and Methods

Site description. Two upland, forested valleys in the Missouri Ozarks exhibiting unique white oak decline and mortality patterns were selected for assessment of occurrence and distribution of *Phytophthora* species. One valley was located near the Harmon Springs Campground (HSC) in the Potosi ranger district of the Mark Twain National Forest. Hillslopes in the valley are composed of well-drained to somewhat excessively well-drained, gravelly silt loam and cobbly loam soil. The drainage way is mostly composed of a well-drained, gravelly silt loam that is classified as frequently flooding but not ponding. Elevation ranged between 290 m and 335 m.

The second valley was located approximately 55 miles south of the first valley and was located in Sunklands Conservation Area (SCA),

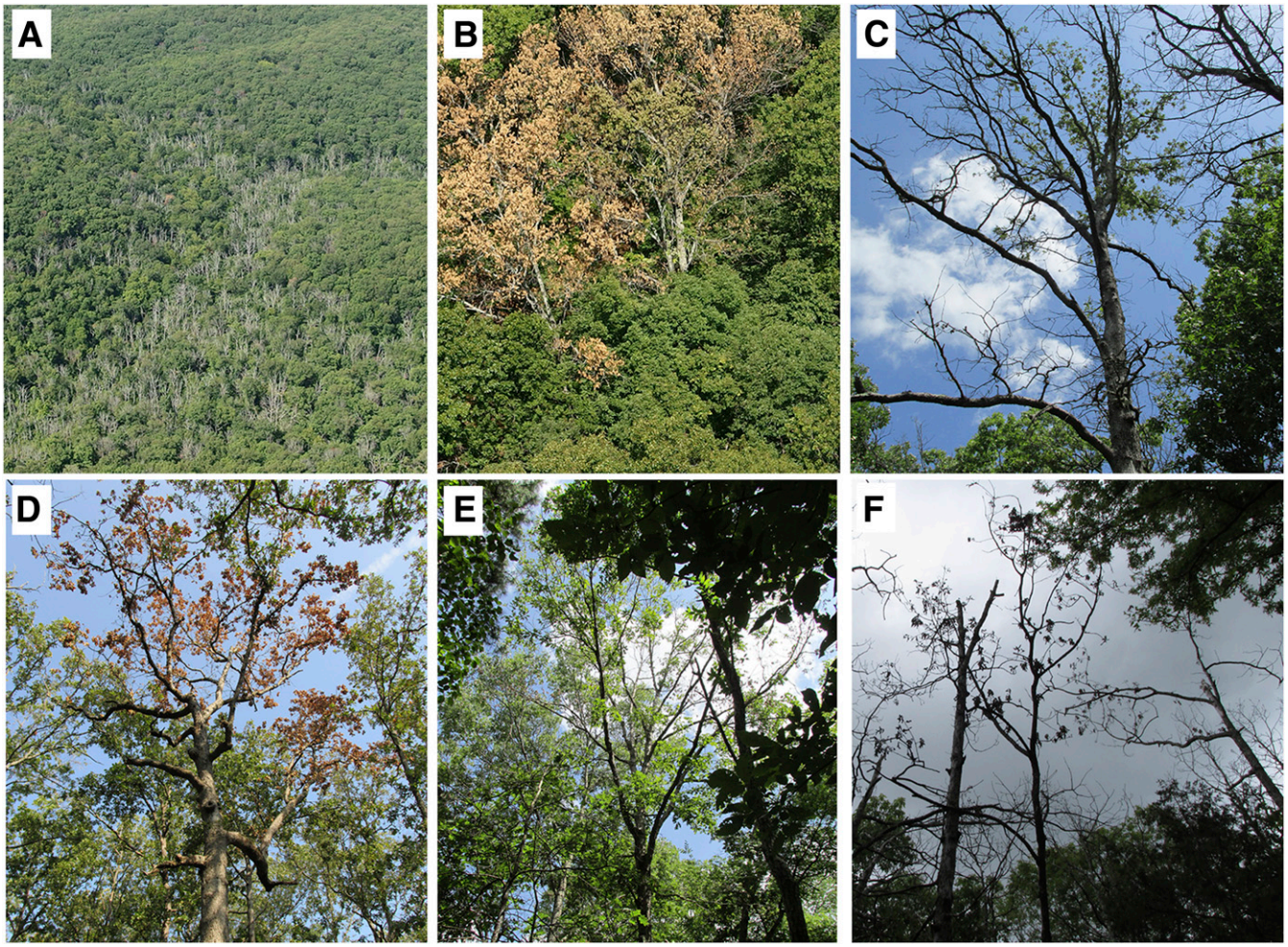

Fig. 1. Rapid white oak mortality associated with drainages and lower slopes of the Missouri Ozarks (**A**). Symptoms include a rapid crown dieback (**B**) as well as partial die-back of tree crowns that is often followed by tree death the following year (**C**). Other symptoms include retention of brown leaves (**D**), crown thinning (**E**), and rapid loss of dead branches (**F**).

adjacent to the Current River. Hillslopes in the valley are composed of a mixture of somewhat excessively well-drained silt and sandy loam soils that are gravelly, very gravelly, or stony. The drainage way is mostly composed of an excessively well-drained, gravelly, sandy loam that does not flood or pond. Elevation ranged between 250 m and 347 m at SCA.

Hardwood taxa commonly found at these sites include trees in the white oak section (Quercus section Quercus: white oak, chinkapin oak, post oak (*Quercus stellata* Wangenh.), red oak section (Quercus section Lobatae: northern red oak (*Quercus rubra* L.), scarlet oak (*Quercus coccinea* Muenhcc.), black oak (*Quercus velutina* Lam.), as well as hickory species (*Carya*) and dogwood species (*Cornus*). Small numbers of shortleaf pine (*Pinus echinata*) and eastern red cedar (*Juniperus virginiana*) occur at both locations.

Plot establishment. In each of those valleys, three adjacent forest stands, largely undisturbed by recent forestry activities, were selected for assessment of *Phytophthora* occurrence in soils. Geographic coordinates for 120 plot centers were randomly generated in each valley using the ArcMap application versus 10.2 (ESRI, Redlands, CA). A 0.08-ha plot was established at each coordinate that did not overlap with an already established plot, trail, or road. Of the 120 coordinates in each valley, 43 of the coordinates at SCA and 42 of the coordinates at HSC met the requirements for plot establishment. The geomorphological model of Ruhe (Ruhe 1960) was used to describe the location of each plot within the landscape (Fig. 2).

Rating crown vigor. During 2014, the upper two-thirds of the crown of each living intermediate, codominant, or dominant white oak tree in a plot was visually rated for decline using a scale designed by McConnell and Balci (McConnell and Balci 2014). Classes were defined as follows: class one, no decline symptoms, less than 10 to 15% crown transparency; class two, slight decline, some branch dieback and small gaps in lateral branch system, 15 to 35% transparency; class three, moderate twig and branch dieback, large gaps in lateral branch system, chlorosis and wilting of leaves, growth of epicormics shoots, 35 to 55% transparency; class four, severe branch dieback, many gaps in crown, chlorosis and loss of most leaves, many epicormic shoots, 55 to 75% crown transparency; and class five, dying tree, over 75% defoliation. Dead trees were rated as class six. Tree crowns with dead leaves were scored for transparency as if the dead leaves were removed. Crown vigor ratings for all white oak trees in a plot were averaged, and the plot classified as high vigor, 1 to 2.5; medium vigor, 2.6 to 3.5; or low vigor 3.6 to 6.

Soil sampling procedure. Vigor plots selected for *Phytophthora* sampling had to have at least three living white oak trees. This selection criterion was chosen because populations of pathogenic *Phytophthora* can decline in soils after host death, and it was deemed necessary to sample multiple trees in each plot to accurately detect if *Phytophthora* species were present in such well-drained soils (Zentmyer 1980). Of the 42 and 43 vigor plots at HSC and SCA, the number of high and low vigor plots sampled for soil baiting were 9 and 12 at HSC and 13 and 12 at SCA, respectively.

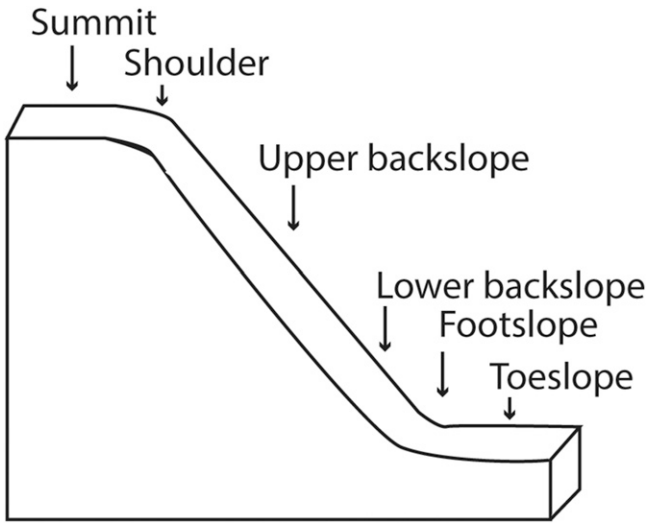

Fig. 2. Geomorphological elements of a hillslope include the flat summit, the convex shoulder, the linear upper and lower backslopes, the concave footslope, and the toeslope consisting of deposited materials from up-valley and uphill.

During 2014, soil samples for *Phytophthora* isolation by soil baiting were gathered from the bases of three randomly selected living white oak trees within high and low vigor plots. Four soil pits (30 × 30 × 25 cm) were dug at cardinal points, 1 m from the base of each sampled tree. The soil in each pit was mixed after rocks, pebbles, and plant debris were removed. Approximately 400 g of soil from each of the four pits was placed in a single, sealable 1-gallon food-storage bag. Bags of soil were stored in a cooler maintained at 8°C until processing (McConnell and Balci 2015).

The first collection of soil samples occurred between 20 May and 15 June 2014. During June 2015, an additional soil sample was collected from a fourth white oak tree in each plot where *P. cinnamomi* was not detected in 2014. In the event that a plot contained only three living white oak trees, a second soil sample was collected at the base of one of the three trees sampled in 2014.

Isolation of *Phytophthora* spp. from soil. Soil samples were baited for *Phytophthora* within 1 to 2 months of collection (McConnell and Balci 2015). For each sample collected, 300 g of soil was placed in a 19.5 × 14 × 9 cm container and flooded with 400 ml of distilled water. Ten *Quercus robur* leaf discs (6 mm diameter) were floated on the surface of the water for 48, 96, or 120 h (Balci et al. 2007; Jung et al. 1996; Schwingle et al. 2007). After each time period, four leaf discs were removed from the water surface, blotted, and embedded in a *Phytophthora*-selective PARPNH medium (Pimaricin, Ampicillin, Rifampicin, PCNB, Nystatin, Hymexazol) in petri dishes (McConnell and Balci 2014). Petri dishes with embedded leaf discs were incubated at room temperature in the dark, and checked for daily colony growth for 5 days.

Leaves for leaf discs were taken from 1-year-old seedlings grown in pots and watered at the soil line with municipal water. Prior to soil baiting, leaves were determined not to be infected with *Phytophthora* by floating leaf discs from these seedlings on water and embedding them in PARPNH media.

Each colony growing from a leaf disc was subcultured to V8 juice agar amended with ampicillin (200 part per million). Subcultured isolates with colony morphology and reproductive structures typical of the genus *Phytophthora* were transferred into Van Tigham cells in V8 juice agar and then subcultured on water agar. Each culture was then hyphal tipped and transferred to V8 juice agar for 2 or more weeks before grouping by colony morphology and reproductive structures (Erwin and Ribeiro 1996). Morphological characteristics noted once every 24 h for 4 days included colony size, growth habit, and mycelium pattern (Jeffers 2015). Reproductive structures noted between 5 and 14 days included the size, shape, placement, and abundance of chlamydospores, oogonia, and antheridia as well as the presence or absence of abundant collaroid hyphae.

Isolate identification and morphological confirmation. One isolate representative of the *P. cinnamomi*–suspect morphotype was selected per plot for molecular identification. All remaining *Phytophthora* isolates representing other morphological types were also selected for molecular identification. Each isolate was grown on a sterilized cellophane sheet laid on the surface of V8 juice agar in a petri dish. The mycelium was scraped off the surface of the cellophane and placed in a 2.0-ml cryovial with o-ring. The cryovial with mycelium was stored overnight in a –80°C freezer prior to DNA extraction.

DNA was extracted from frozen mycelium using the Easy-DNA gDNA Purification Kit (Thermo Fisher Scientific, Waltham, MA). The internal transcribed spacer region was amplified for all chosen isolates using primers ITS4 and ITS5 (White et al. 1990). Each 50-µl PCR reaction contained 5× HS Red PCR Reaction Buffer (Bioline, Tauton, MA), 20 pmol primer, 1.25 U MyTaq HS Red DNA polymerase, and 100 ng template DNA. The PCR reaction was performed with an initial temperature of 94°C for 5 min followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 48°C,

and 1 min extension at 72°C. The final extension step was 5 min at 72°C.

The cytochrome oxidase gene (COX II) and connected spacer region were amplified with primers FM35 and FmPhy-10b for a subset of the chosen isolates to confirm species identities (Martin 2000; Martin et al. 2004). The COX II PCR was performed with an initial temperature of 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 47°C for 1 min, and extension at 72°C for 1 min and 30 s. The final extension step was 5 min at 72°C. The PCR products were treated with EXO-SAP IT (Applied Biosystems, Foster City, CA) as directed by the manufacturer and then sequenced using a Big Dye Terminator (Applied Biosystems, Foster City, CA) with cycle sequencing chemistry. High quality sequences were aligned and edited using Finch TV 1.4.0. (Geospiza Inc., Seattle, WA) and DNASTAR (DNASTAR Inc., Madison, WI) software. Edited sequences were compared with known sequences in the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/) and the *Phytophthora* database (Park et al. 2008) (http://www.phytophthoradb.org/). A threshold of 99% percentage identity was used for species-level identification (Supplementary Table S1). Aligned nucleotide sequences were deposited in GenBank (accession no. MF737141 to MF737162, MG882346 to MG882351).

The identity of species were confirmed using characteristics of the hyphae, sporangium, oogonium, and antheridia (Erwin and Ribeiro 1996; Gallegly and Hong 2008). Sporangia were induced by partially submerging agar blocks in nonsterile soil extract solution or distilled water (Jeffers 2015). Although the heterothallic *P. cinnamomi* var. *cinnamomi* isolates were not mated, all morphological features other than oogonia and antheridia were observed. *Pythium* species isolated during the study were treated in the same manner but are not discussed in this article.

Statistical analyses. ANOVA (PROC GLM) was used on ranked vigor data from the study sites HSC and SCA to determine if white oak vigor differed among the upper back, lower back, and foot/toeslope positions. Slope position was considered a fixed effect. Comparisons among means were made using Fisher's least significant difference. Fisher's exact test (PROC FREQ) with a two-sided probability was used to determine if *P. cinnamomi* detection was associated with high or low vigor plots. The Wilcoxon exact test (PROC NPAR1-WAY) was used to test if trees with or without *P. cinnamomi* detected in soils near their root crown differed in vigor. All statistical tests were performed using SAS 9.4 (SAS Institute, Cary, NC).

Results

Crown vigor characterization. The crown vigor of 654 white oaks was rated in 43 plots at HSC and in 42 plots at SCA. Average vigor ratings of white oak trees at HSC and SCA were 3.3 ± 0.02 and 3.5 ± 0.03 SE, respectively, and did not differ significantly. Plots entirely composed of healthy, declining, or dead white oaks were present at both locations with vigor scores for plots ranging from 1.7 to 6.0 at HSC and 1.4 to 6.0 at SCA. White oak trees in plots on foot/toe slopes at HSC and SCA had greater scores and therefore were less vigorous than white oak trees in plots on lower backslopes and upper backslopes. (HSC, $F = 7.81$, df = 2, 35, $P = 0.0016$; SCA, $F = 17.12$, df = 2, 39, $P < 0.0001$; Fig. 3).

Occurrence and distribution of *Phytophthora* species. Soil samples were collected from 138 white oaks within 21 plots at HSC and 25 plots at SCA during 2014. White oaks sampled included dominant, codominant, intermediate, and suppressed trees (Supplementary Table S2). Soil samples were mostly collected from the base of trees with crown vigor ratings of two or three (Supplementary Table S3). Isolates of *Phytophthora* were collected from 14 of 21 plots and 2 of 7 plots at HSC during 2014 and 2015, respectively. At SCA, isolates of *Phytophthora* were collected from 9 of 25 plots and 2 of 17 plots at SCA, respectively. *P. cinnamomi* was the only species isolated from soils around white oak trees at both HSC and SCA. *P. cactorum* (Lebert & Cohn) J. Schröt and *P. pini* Leonian were isolated from soils around white oak trees only at SCA, and *P. europaea* E.M. Hansen & T. Jung was isolated from soils around white oak trees only at HSC.

Isolates of *P. cinnamomi* were heterothallic and produced abundant collaroid hyphae, chlamydospores, and ovoid to ellipsoid sporangia (Gallegly and Hong 2008). Sporangia developed when distilled water was used but not when nonsterile soil extract was used.

P. cactorum isolates were homothallic with paragynous antheridia closely affixed to the oogonial stalk. Sporangia were observed to be papillate, ovoid, and arranged in a simple sympodium (Gallegly and Hong 2008). Similarly, *P. pini* isolates were homothallic with paragynous antheridia and plerotic oospores. Sporangia were noncaducous, ellipsoid, ovoid, or misshapen with double papillae, and were arranged in simple sympodia (Hong et al. 2011).

The remaining two *Phytophthora* isolates closely matched the ITS region and morphology of known isolates of *P. europaea*. Isolates were homothallic with paragynous antheridia affixed to the tapered stalks of the oogonia. Oogonia contained aplerotic oospores, were tapered and curved. Sporangia were nonpapillate, noncaducous, and were mostly found on long hyphal branches (Gallegly and Hong 2008). The COX2 region of these same two isolates most closely

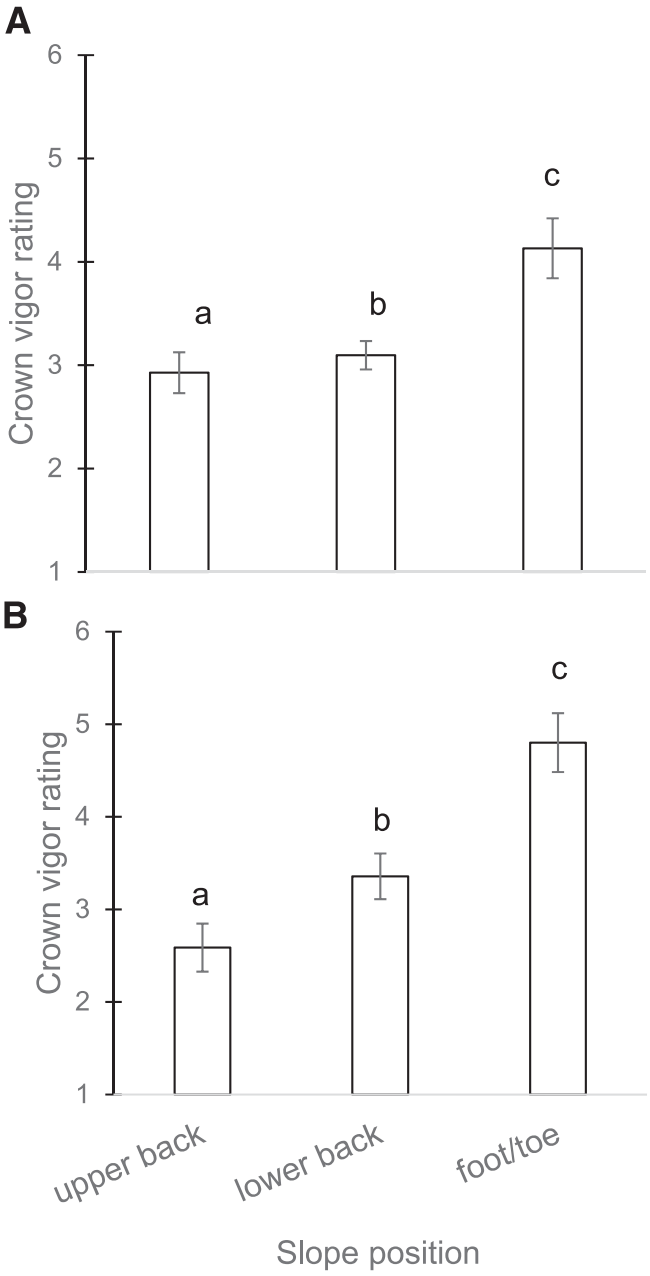

Fig. 3. Mean vigor ratings (±SE) for all surveyed white oak trees located on different slope positions at Harmon Springs Campground (**A**) and Sunklands Conservation Area (**B**) during May 2014. Means followed by the same letter are not significantly different based on Fishers LSD ($P = 0.05$).

matched the COX2 region of an unnamed *Phytophthora* isolate PD_02039. The DNA sequence of isolate PD_02039 most closely matched *P. europaea* isolate PD_00082. Here we name these two isolates *P. europaea*.

P. cinnamomi was the species isolated most frequently from soils around white oak trees (Table 1). All other species were isolated from a single tree in one or two plots (Table 1). Baiting from additional soil collections baited during 2015 increased the detection rates for *P. cinnamomi* from 67 to 76% of plots at HSC and from 32 to 36% of plots at SCA. No other *Phytophthora* species were isolated from soil samples collected and baited during 2015.

P. cinnamomi was detected in soil at the base of white oak trees that were collected from foot and toeslopes as well as on lower and upper backslopes (Table 2). At HSC, detection frequencies for *P. cinnamomi* were greatest on foot and toeslopes and decreased toward the summit of the hillslope. No trend was apparent at SCA with moderate to low detection frequencies on foot and toeslopes, lower backslopes, and upper backslopes (Table 2). At HSC and SCA, *P. cinnamomi* was rarely detected in soils at the base of all sampled trees in a plot, resulting in greater detection frequencies at the plot level than the tree level (Table 2). Other *Phytophthora* species detected in soils located on the upper backslopes included *P. europaea* and *P. cactorum*. *Phytophthora* species detected in soils located on the lower backslopes of hillslopes included *P. cactorum* and *P. pini*.

Table 1. Frequency of *Phytophthora* species detected in soil collected during May and June 2014 and June 2015 at the base of white oak trees at Harmon Springs Campground (HSC) and Sunklands Conservation Area (SCA)

	No./(percentage) of plots[z]		No./(percentage) of trees[z]	
Taxa	**HSC**	**SCA**	**HSC**	**SCA**
Phytophthora cinnamomi	16 (76)	9 (36)	26 (37)	11 (12)
P. cactorum	0 (0)	2 (8)	0 (0)	2 (2)
P. europaea	1 (5)	0 (0)	1 (1)	0 (0)
P. pini	0 (0)	1 (4)	0 (0)	1 (1)

[z] Soil samples were collected at the bases of trees from 21 plots at HSC and in 25 plots at SCA. Three to four trees were sampled per plot for a total of 70 trees at HSC and 91 trees at SCA.

At HSC, *P. cinnamomi* was detected with equivalent frequency in both high and low vigor plots (Table 3, $P = 0.34$), but at SCA, *P. cinnamomi* was detected more often in high vigor plots. *P. cactorum* and *P. europaea* were only detected in soils collected from low vigor plots at SCA and HSC, while *P. cactorum* and *P. pini* were only detected in soils collected from high vigor plots at SCA. However, these *Phytophthora* species were detected too infrequently to evaluate any association with low or high vigor plots.

At HSC and SCA, increased white oak crown vigor was associated with detection of *P. cinnamomi* in surrounding soils (Table 4). At both locations, white oaks had more vigorous crowns, as indicated by lower scores, when *P. cinnamomi* was detected than when it was not detected (HSC, S = 619, $P = 0.0238$; SCA, S = 195.5, $P = 0.0020$). This trend was also observed among slope position and within vigor classes when only trees in plots with *P. cinnamomi* were considered (Supplementary Fig. S1).

Discussion

In the two upland valleys that we assessed, crown vigor of white oaks was poorest in those locations that typically result in the most rapid growth, the foot and toeslopes. White oak has a broad ecological amplitude. It is drought tolerant and is frequently found in dry, upland valleys, but it is also present in more mesic habitats as a transitional species (Abrams 2003; Johnson et al. 2009). In drier upland valleys such as the ones in this study, it grows most rapidly in areas with more moisture. These areas are the toe, foot, and lower backslopes of hillslopes, especially those with northern and eastern aspects that receive water from upslope and have lower evapotranspiration demands.

The pattern of poor vigor scores across the slope did not fit patterns of decline and mortality reported in the Missouri Ozarks for white oaks. Kabrick et al. (2008) found that the amount of mortality and decline for oaks in the white oak section did not differ among slope positions during a 10-year period. Others have reported mortality of oaks greatest on upper backslopes and slope shoulders (Fan et al. 2012; Voelker et al. 2008). None of these studies considered the

Table 2. Frequency of *Phytophthora cinnamomi* detection in soil samples from Harmon Springs Campground (HSC) and Sunklands Conservation Area (SCA) during 2014 and 2015, in relation to slope position

Slope position[y]	No. of plots sampled[z]	No./(percentage) of plots where *P. cinnamomi* was detected	No. of trees sampled[z]	No./(percentage) of trees with *P. cinnamomi* detected at base
HSC				
Foot/toe slope	4	4 (100)	12	8 (67)
Lower backslope	3	3 (100)	10	4 (40)
Upper backslope/shoulder	12	8 (67)	41	13 (32)
Summit	2	1 (50)	7	1 (14)
SCA				
Foot/toe slope	5	2 (40)	19	2 (11)
Lower backslope	7	1 (14)	27	1 (4)
Upper backslope/shoulder	13	6 (46)	45	8 (18)

[y] Slope position as defined by (Ruhe 1960) (Fig. 2).
[z] Three to four trees were sampled per plot for a total of 70 trees at HSC and 91 trees at SCA.

Table 3. Number of low and high vigor plots at Harmon Springs Campground (HSC) and Sunklands Conservation Area (SCA) where *Phytophthora cinnamomi* was detected

		No. of plots where *P. cinnamomi* was detected[z]		
Location	**Plot vigor rating[y]**	**Detected**	**Not detected**	**Fishers Exact Test, *P* value**
HSC	Low	8	4	0.34
	High	8	1	
SCA	Low	1	11	0.01
	High	8	5	

[y] Low vigor refers to an average crown score of 3.5 to 6. High vigor refers to an average crown score of 2.5 to 1.
[z] Soil samples were collected during May and June 2014 and June 2015.

influence of pathogens on white oak mortality patterns. We expect that the drought of 2012 contributed to the poor vigor scores we observed. Drought is the primary inciting factor causing decline in the Ozarks. However, the rapid death of drought-tolerant white oaks is atypical. Drought-related decline and mortality should have peaked 2 to 3 years after the drought, during 2014 and 2015 (Fan et al. 2012). At HSC and SCA, foresters reported dead and dying white oaks already present at these sites during 2013. This was confirmed by our finding dead trees during the spring of 2014 that had already shed their small twigs and branches. It is not clear if competition played a role in the development of white oak mortality. Death and decline of the largest, competitively dominant trees and not smaller trees contributed mostly to the new pattern of decline (Reed et al. 2016a).

Regardless, the relationship between decreasing vigor and lower topographical positions has not been reported previously for white oak in upland valleys of the Missouri Ozarks. Further, there has been no investigation of biological factors that contribute to decline and mortality of white oak in the waterways of these upland valleys. Coincidentally, foot and toeslope plots that had the poorest crown vigor scores are the locations most likely to provide adequate conditions for *Phytophthora* development, infection, and spread during wet years. These foot and toeslope plots in upland valleys also experience severe dryness during extended droughts. Although drought does not favor the reproduction of *P. cinnamomi* or subsequent infection, severe drought and fluctuations between saturated and too dry conditions result in the greatest pathogenicity (Corcobado et al. 2014). It is hypothesized that increased pathogenicity results from a complex of factors including chronic losses of fine roots that fluctuate between acute and slight depending on soil water content, especially early in the growing season, water shortages due to infection and drought, depletion of resources from root regeneration, nutrient deficiencies, and decreased photosynthetic capabilities (Corcobado et al. 2013; McConnell and Balci 2014; McConnell and Balci 2015).

In this survey, four *Phytophthora* species, *P. cinnamomi*, *P. europaea*, *P. cactorum*, and *P. pini*, were detected at two forested locations. Including those species detected by Schwingle et al. (2007), six species are now known to occur in declining Missouri oak stands. Other studies of North American and European forest soils have reported 7 to 11 *Phytophthora* species. However, those studies sampled more locations, and several used multiple baiting techniques (Balci et al. 2007; Hansen and Delatour 1999; Vettraino et al. 2002, 2005). It is likely that additional *Phytophthora* species occur in Missouri forests, especially around waterways. Use of additional baiting techniques may be necessary to fully describe all *Phytophthora* species occurring in Missouri forests (Hansen and Delatour 1999; Smith et al. 2007).

Similar to other forested regions (Hansen et al. 2012), little is known about the distribution or ecological role of *Phytophthora* species in the Missouri Ozarks. This is the first reported detection of *P. europaea*, *P. cactorum*, and *P. pini* in Missouri Ozark forests. The presence of *P. europaea* and *P. pini* in forests is not unusual. They have been reported in eastern U.S. and/or European forests (Balci et al. 2006, 2007; Darmono et al. 1991; Jung and Blaschke 1996; Jung et al. 2002). *P. cactorum* was reported infecting apple trees in New Franklin, Missouri (Howard County) in 1968, but reports of this common nursery pathogen in forests are less common (Balci and Bienapfl 2013; Gates 1972). The presence of a gravel road running the length of the study area along the drainage and vacation homes near the base of the drainage at SCA site may well be facilitating the introduction of *Phytophthora* species into the forest. Of these three species, *P. europaea* and *P. cactorum* are known to cause root necrosis and stem lesions on seedling and mature oak trees (Balci et al. 2008; Erwin and Ribeiro 1996). *P. europaea* has been associated with oak decline in Europe but is not considered a serious threat (Jung et al. 2002; Orlikowski et al. 2011). It is unclear if *P. pini* infects white oaks. In 2011, the species epithet *P. pini* was resurrected to replace *P. citricola* I within the *P. citricola* complex, so much of the information on its host range is likely confused with *P. citricola* reports (Hong et al. 2011).

P. cinnamomi was the only species associated with both locations in this study. *P. cinnamomi* can be an aggressive soil pathogen that attacks more than 5,000 woody plant species and is thought to have originated in Southeast Asia (Davison 1998). The pathogen is widely distributed in temperate and tropical regions and is known to be present in North America, Europe, Africa, Asia, and Australia. *P. cinnamomi* was also the most frequently detected species of *Phytophthora* in a recent survey of forest soils in the northeastern United States (Balci et al. 2007). Missouri and its neighboring states were not included in that survey. *P. cinnamomi* was detected previously in Missouri forest soils taken from a stand of declining oaks (Schwingle et al. 2007). The detection of *P. cinnamomi* at HSC and SCA and next to declining oak trees in other Missouri forests (S. E. Reed, *data not shown*) supports the possibility that it could be widespread enough to be a contributor to oak decline in the Ozarks.

P. cinnamomi was not limited to the waterways as we expected. Instead, *P. cinnamomi* was distributed from the top to the bottom of the hillsides sampled at HSC, including a slope facing northwest and another facing southeast. However, the trend was for greater *P. cinnamomi* detection at lower slope positions than upper slope positions. This pattern may be a consequence of increased reproduction, infection, and spread by *P. cinnamomi* in wetter areas. For example, propagules move downhill and laterally in areas that undergo flooding, ponding, or water saturation, whereas propagule spread is limited to downhill spread when these factors are not present (Weste and Taylor 1971). The USDA-NRCS soil map unit for HSC indicates that this waterway frequently floods but does not pond.

The pattern of *P. cinnamomi* being detected more consistently among plots than within plots, and less often at higher slope positions than at lower slope positions, indicates either that *P. cinnamomi* has a patchy distribution within the forest or that we did not always detect *P. cinnamomi* when it was present. False negatives occur when population densities are too low for detection with soil baiting (Vannini et al. 2013). The broad distribution of *P. cinnamomi* at HSC supports the latter suggestion.

Interestingly, *P. cinnamomi* was not detected frequently throughout SCA, nor was there a trend toward more detections in low lying areas than higher areas. However, the spatial distribution of the detections suggests that *P. cinnamomi* is more widespread at SCA than revealed by the soil baiting. In this study, at SCA, *P. cinnamomi* was isolated most frequently on the upper backslope of a north facing hillslope at the start of the valley. The pathogen was also detected down valley, next to the main drainage, approximately 0.7 km from this hillside. *P. cinnamomi* was not detected between these two points even though water from the infected hillslope flows down past the dead trees along the main drainage and past the second detection point 0.7 km downstream. In addition, a gravel road extends from the hillside where *P. cinnamomi* was detected down into the valley and runs parallel with the drainage containing dead trees. The road is at a slightly higher elevation, so water flows from the gravel road, across the slope, and down into the drainage. Gravel roads and soil carried on the tires of vehicles and equipment have been associated with *P. cinnamomi* spread (Weste and Taylor 1971; Zentmyer 1980). Either

Table 4. Mean crown vigor rating (±SE) during 2014 for study trees at Harmon Springs Campground (HSC) and at Sunklands Conservation Area (SCA) based on *Phytophthora cinnamomi* detection

Detection	No. of trees	White oak crown vigor (±SE)[y]
HSC		
P. cinnamomi detected	24	2.3 ± 0.2 a[z]
No detection	39	2.8 ± 0.1 b
SCA		
P. cinnamomi detected	10	1.7 ± 0.1 a
No detection	64	2.7 ± 0.3 b

[y] Based on 2014 vigor ratings.

[z] Means followed by different letters are significantly different according to the Wilcoxon exact test: HSC, S = 619, $P = 0.0238$; SCA, S = 195.5, $P = 0.0020$; larger vigor scores indicate poorer crown vigor.

the gravel road or the natural flow of water in the valley should be acting as a vehicle for *P. cinnamomi* introduction to plots where no *P. cinnamomi* was detected.

The low detection rate on foot and toeslopes at SCA in comparison with HSC may be related to the soil characteristics. The silt loam and loam soils at HSC have a greater percentage of clay than the silt and sandy loam soil at SCA (21 to 35% versus 4 to 15%, respectively) and thus a greater capacity to hold water. Soil at HSC would favor increased propagule production, survival, and detection more than the soil at SCA (Zentmyer 1980). Also, lateral spread of *P. cinnamomi* spores would be favored by the frequent flooding of the drainage way at HSC (Weste and Taylor 1971). In contrast, the drainage way at SCA does not flood frequently, and some portions of the stream are belowground.

We found that white oaks trees with *P. cinnamomi* detected in soil at their base had crowns that were more vigorous than trees without *P. cinnamomi*. However, the small difference between mean crown vigor of trees with and without *P. cinnamomi* and the coarseness of the scale used to assess vigor call the meaningfulness of these differences into question. Sample size on affected slopes was restricted due to the unequal distribution of white oak trees and mortality among slope positions, as well as the shapes of the valleys (Reed et al. 2016a). Results of this study may have differed if sampling had occurred before most of the mortality occurred at these sites. Further, sampled trees with declining and healthy crowns were close together, and it can be assumed that vigorous and declining trees shared the same rhizosphere soil and similar *Phytophthora* populations (Jung et al. 2000).

With the current methodology, our exploratory analysis does not support the idea that *P. cinnamomi* is associated with the poor vigor of white oaks at either HSC or SCA. At these two sites, *P. cinnamomi* may be acting as a saprophyte or infecting other plant species, or the remaining oaks may be resistant to or tolerant of *P. cinnamomi* infection (Hardham 2005; Weste and Marks 1987; Zentmyer 1980). *P. cinnamomi* is not always a contributing or predisposing factor in oak decline (Camilo-Alves et al. 2013; Jung et al. 2000). Site factors such as soil nutrient levels, soil type, soil pH, and the presence of competitive microbes can limit *P. cinnamomi* pathogenicity by either bolstering tree vigor or negatively affecting *P. cinnamomi* reproduction and spore survival (Balci et al. 2010; Jönsson et al. 2005; Jung et al. 2000; Moreira and Martins 2005; Zentmyer 1980).

A more thorough investigation into the role of *P. cinnamomi* in oak decline in the Missouri Ozarks should be undertaken. Our study demonstrates that *P. cinnamomi* is the dominant *Phytophthora* species in some upland, Missouri Ozark forests. In dry, upland valleys, *P. cinnamomi* occurs on all parts of the hillslope and is not restricted to water drainages. *P. cinnamomi* is widely enough distributed in these valleys to be considered as a contributor to oak decline. Although a relationship between *P. cinnamomi* presence and poor crown vigor was not revealed in this study, further investigation using quantitative metrics of *P. cinnamomi* populations (Balci et al. 2010) in Ozark forests is necessary. Multiple studies have demonstrated that when appropriate site conditions and pathogenic *Phytophthora* species are present in oak stands, oaks have higher rates of crown transparency, less vigor, and/or fewer fine roots (Balci et al. 2008, 2010; Jung et al. 2000; McConnell and Balci 2014). *P. cinnamomi* is a predisposing agent in the decline and death of cork and holms oak in the Mediterranean region, and it has wreaked havoc in the dry sclerophyll forests of Australia. In addition, *P. cinnamomi* has been shown capable of causing stem lesions and fine root loss on white and red oak seedlings (McConnell and Balci 2015; Schwingle et al. 2007). Considering that damage to oak trees by *P. cinnamomi* has been documented, the role of *P. cinnamomi* in the decline of all oaks in the Ozarks needs to be more thoroughly investigated.

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