



Involvement of *Phytophthora* species in white oak (*Quercus alba*) decline in southern Ohio

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Summary

This study was initiated to investigate the possible role of *Phytophthora* species in white oak decline (*Quercus alba*) in southern Ohio at Scioto Trail State Forest. Surveys demonstrated the presence of four species of *Phytophthora* including one novel species. By far, the most common species was *P. cinnamomi*; *P. citricola* and *P. cambivora* were isolated infrequently. In few instances, *P. cinnamomi* was isolated from fine roots and necroses on larger roots. No special pattern of incidence was found, but *P. cinnamomi* was more commonly isolated from greater Integrated Moisture Index values suggesting moist lower bottomlands favour this *Phytophthora* species. When tree crown condition was examined relative to the presence of *Phytophthora*, no significant association was found. However, roots of declining *P. cinnamomi*-infested trees had 2.5 times less fine roots than non-infested and healthy trees, which was significantly different. The population densities of *P. cinnamomi* from declining trees were significantly greater than from healthy trees, suggesting increased pathogen activity that has the potential to cause dieback and decline and possibly the cause of a reduced fine root amount found on declining trees.

1 Introduction

Oak decline is mostly considered to be the result of the interactions between abiotic and biotic factors (BALCI 2002; THOMAS et al. 2002; JÖNSSON 2006). The role of *Phytophthora* as a primary factor causing root mortality and triggering tree decline has been evaluated in multiple studies (BALCI and HALMSCHLAGER 2003a,b; BRASIER et al. 1993; BRASIER and JUNG 2003; DELATOUR 2003; GALLEGRO et al. 1999; HANSEN and DELATOUR 1999; HARTMANN and BLANK 2002; JÖNSSON 2006; JÖNSSON et al. 2005; JÖNSSON-BELYAZIO and ROSENGREN 2006; JUNG et al. 1996, 2000; ROBIN et al. 1998; SÁNCHEZ et al. 2002; TANTER et al. 2000; VETTRAIANO et al. 2002). In some instances, a direct relationship was found between the presence of *Phytophthora* and decline or mortality of oak trees or seedlings (ROBIN et al. 1998; TANTER et al. 2000; RODRÍGUEZ-MOLINA et al. 2005). In other cases, their presence could not be directly associated with the health status of trees (HANSEN and DELATOUR 1999; HARTMANN and BLANK 2002; BALCI et al. 2007) or circumstantial evidences were found (BALCI and HALMSCHLAGER 2003a,b; JÖNSSON et al. 2005; JUNG et al. 2000; VETTRAIANO et al. 2002). However, when *Phytophthora* species isolated from rhizosphere soil samples around the base of trees were tested in artificial inoculation studies, they were able to cause significant damage to the stem or roots of seedlings (JUNG et al. 1996, 1999, 2002; MARÇAIS et al. 1996; ROBIN et al. 1998, 2001; MAUREL et al. 2001;

Received: 3.2.2009; accepted: 31.7.2009; editor: A. Vannini

BALCI and HALMSCHLAGER 2003b; BIANCO et al. 2003; BRASIER and JUNG 2003; JÖNSSON et al. 2003; JÖNSSON 2004; BALCI et al. 2008a).

Since 2002, extensive white oak (*Quercus alba* L.) decline and mortality has been observed in oak forests across southern Ohio. The profile of white oak crown dieback and mortality initially consisted of single trees or small groups of trees on lower slope and bottomland sites. The mortality was especially severe at the Scioto Trail State Forest (STSF) in southern Ross County. A series of stressors including severe drought in late 1998 and throughout 1999 combined with insect defoliators such as the forest tent caterpillar (*Malacosoma disstria* Hübner), common oak moth [*Phoberia atomaris* (Hübner)] and half-wing geometer [*Phigalia titea* (Cramer)] adversely affected tree health through 2004. Mortality accelerated in 2003–2005 and secondary agents such as *Armillaria* spp., the two-lined chestnut borer [*Agrillus bilineatus* (Weber)], and hypoxylon canker [*Biscogniauxia atropunctata* (Schwein.) Pouzer] were frequently observed in association with dead and dying trees. During this period, over 908.5 m³ of white oak sawtimber was salvaged at Scioto Trail State Forest.

In June 2004, soils around five white oaks were sampled at declining and non-declining white oak stands to determine the presence of *Phytophthora* species (BALCI et al. 2007). This limited sample, conducted as part of a larger survey of soil-borne *Phytophthora* species, revealed the presence of *P. cinnamomi* in soils associated with declining white oaks. Different trees at the same sites were sampled in June 2005 and identical results were obtained. Thus, we initiated this study to examine the incidence and relationship of *Phytophthora* to white oak decline at the Scioto Trail State forest.

2 Material and methods

2.1 Study site and history of weather pattern

The study area is located in the Southern Unglaciated Allegheny Plateau Section of the Eastern Broadleaf Forest Province, which is characterized as a mature, dissected plateau of high hills, sharp ridges and narrow valleys (MCNAB and AVERS 1994). The study was conducted in the 3800 ha Scioto Trail State Forest in southern Ross County about 11 km south of Chillicothe, OH, USA (39°13'N; 82°56'E). In most areas, the forest is dominated by mature white oak, which comprises 50–70% of the overstory basal area of trees >10 cm diameter at 1.4 metres (d.b.h.). Common associates, depending on the site, include sugar and red maples (*Acer saccharum* Marsh., *A. rubrum* L.) comprising 10–20% of the basal area, northern red oak (*Q. rubra* L.), black oak (*Q. velutina* Lam.) and chestnut oak (*Q. montana* Willd.) which together comprise about 5% of the basal area. Other associates include yellow poplar (*Liriodendron tulipifera* L.), blackgum (*Nyssa sylvatica* Marsh.), hickories (*Carya* spp.), and ashes (*Fraxinus* spp.). Large saplings (trees >1.4 m tall and <10 cm d.b.h.) in the understory, excluding stems <2.5 cm d.b.h., were dominated by red and sugar maples in most areas; however, very mesic sites had large numbers of green ash (*F. pennsylvanica* Marsh.) and spicebush (*Lindera benzoin* L.).

Precipitation averages about 1014 mm annually (30-year average) and the mean temperature is 11.6°C. An unusual weather pattern occurred starting from 1999. The area received considerably lower annual rainfall in 1999 (818 mm) when compared to the 30-year average (1014 mm). The abnormalities occurred during the months of May, June and July. The rainfalls in 1999 for these months were 20, 43 and 59 mm respectively. In 2000 (82, 92 and 159 mm) and 2001 (267, 114 and 107 mm), considerably more rain was received during the growing season. In 2002, 2003 and 2004, a similar weather pattern occurred during these months and the annual rainfall was above the 30-year average.

2.2 Experimental design and sampling for *Phytophthora*

In 2006, fifty-one transects were randomly selected throughout the study area and two sample plots were established per transect (total 102 trees sampled). One plot was located down slope at low altitude and the other located upslope at a higher altitude ca. 50–100 m distanced from the first tree. At each plot, one white oak was selected and the crown status was rated based on established crown ratings (BALCI and HALMSCHLAGER 2003a) and grouped in two categories; healthy trees were those with less than or equal to 25% crown transparency and declining trees were those with greater than 25% crown transparency.

The GIS-derived Integrated Moisture Index (IMI) was utilized for each site established in the Scioto Trail State Forest and the IMI value was calculated based on hill shade, curvature, flow accumulation and total water-holding capacity (IVERSON et al. 1997). The data were standardized to a 0–100 score to facilitate calculation of IMI. IMI values for each sampling site were classified into the following classes; 1 (7.00–36.00) = xeric, 2 (36.01–55.00) = intermediate and 3 (55.01–80.7) = mesic sites.

2.3 Isolation and identification of *Phytophthora*

2.3.1 Isolation from soil samples

Phytophthora was isolated from soil samples using an oak leaflet baiting method (JUNG et al. 1996). Once a white oak was selected, soil sub-samples from four cardinal directions at a depth of 30 cm was collected. The organic layer was removed prior to soil sampling and additionally any white oak roots were sampled separately from the soil monolith (30 × 30 × 30 cm). 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 5-day-old *Q. robur* or *Q. palustris* leaflets at a temperature of 20°C. After 3–5 days, discoloured leaflets were examined microscopically and those with sporangia typical of *Phytophthora* were plated on PARPNH selective medium (ERWIN and RIBEIRO 1996). When initial isolation attempts failed, soils were dried at room temperature and the isolation method was repeated using the same protocol.

2.3.2 Isolation from root samples

In addition to the soil baiting, root samples <1 cm including fine roots were collected to isolate *Phytophthora* from any necrotic lesions. White oak roots from each of the four monoliths (30 × 30 × 30 cm) around the base of a tree were pressure washed with water and evaluated visually for lesions. If necrotic, dark-brown lesions were found, they were divided in half and one sample was plated directly onto PARPNH selective medium. The second half of the necrotic tissue was surface sterilized for 1 min in ethanol (96%), followed by 5 min in sodium hypochlorite (NaOCl) with 4% available chlorine, dipped for 30 s in ethanol (96%), rinsed in sterile deionized water, dried on filter paper and plated on malt extract agar amended with 100 mg/l streptomycin sulphate to suppress bacterial growth (HALMSCHLAGER and KOWALSKI 2004).

2.3.3 Identification of *Phytophthora* using molecular and morphological tools

Isolates were identified to species by a combination of morphological and molecular identification tools. Isolates growing on V8 juice agar (20 g agar, 3 g CaCO₃, 100 ml Campbell's V8 juice and 900 ml distilled water) were examined for their morphological characteristics, growth pattern on Difco potato-dextrose agar (PDA) and for their

temperature-growth relationship (ERWIN and RIBEIRO 1996). Heterothallic isolates were paired with A1 and A2 tester strains of *P. cinnamomi* and *P. cambivora*.

For molecular identification, DNA was extracted from 10 to 20 mg of mycelium of representative isolates using the DNeasy[®] Plant Mini Kit (Qiagen, Valencia, CA, USA) following the protocol for total DNA isolation from plant tissue. The DNA was suspended in 100 μ l of TE buffer provided in the kit and stored at -20°C prior to PCR amplification. Isolates were identified following the generation of internal transcribed spacer region (ITS) and 5.8rDNA sequence using the PCR primers ITS4 and ITS6 (WHITE et al. 1990; COOKE et al. 2000) as described in BALCI et al. (2008b). ITS sequences were then compared to ITS sequence contained in the *Phytophthora* database (<http://www.phytophthoradb.org>) and GenBank (<http://www.ncbi.nlm.nih.gov>) using the BLAST program (ALTSCHUL et al. 1997).

2.4 Determining population level of *Phytophthora cinnamomi*

Based on the *Phytophthora* isolation results obtained from 102 samples in 2006, we randomly selected 45 infested and 18 non-infested trees to determine the population density of *P. cinnamomi* in rhizosphere soil in 2007. The population density was estimated by direct plating soil onto PCH medium (SHEW and BENSON 1982) amended with antibiotics (10 mg/l pimarinic acid, 200 mg/l ampicillin, 10 mg/l rifampicin, 25 mg/l PCNB, 50 mg/l nystatin and 50 mg/l hymexazol). Soils were collected in June–August 2007 as described above, transferred to the lab in a cooler and stored at 5°C . Soils were processed no later than 6 weeks after sampling. From an initial bulked soil sample, two 50 g soil samples were used to determine *P. cinnamomi* population density. In addition, two 10 g subsamples of soil were oven-dried to determine the per cent moisture for each soil sample. Each sample was blended with 200 ml deionized water for ca. 30 s and the suspension was poured through nested 20-cm-diameter sieves (125 μm over 38 μm mesh sizes). The suspension collected from each sample was then distributed to the surface of ten 9-cm-diameter petri plates containing PCH medium. Each plate was incubated for 3 days at 20°C in complete darkness, and then the soil suspension was rinsed from the agar surface and *Phytophthora* colonies counted. Finally, average colony number per 100 g of oven-dry weight of soil was determined by calculating the oven-dry weight of soil applied per plate from moisture-content determination. If no *Phytophthora* colonies were present, two additional soil samples were processed from the initial soil sample using the same protocol and the average of four soil samples calculated. In addition to direct plating, a 250 g soil subsample was used to bait *Phytophthora* as described above to verify the presence of *P. cinnamomi* on all soil samples used to determine population level.

2.5 Root sampling and analyses

In August 2007, based on isolations from rhizosphere soil samples around the base of trees in 2006, roots of ten trees that were classified as *Phytophthora*-free and ten trees as *Phytophthora*-infested were sampled. Trees were selected randomly and were equally distributed from lower or upper slope plots associated with *Phytophthora*-free or *Phytophthora*-infested trees. To verify the isolation result for *Phytophthora* from 2006, soils were collected and baited for *Phytophthora* as described above. Each tree was re-evaluated for crown status and roots (≤ 1 cm diameter) were collected from four cardinal directions at a distance of 1–1.5 m from the tree base of the tree. A monolith of $60 \times 30 \times 30$ (twice larger in length compared to 2006 soil and root sampling) was removed and all roots characteristic to white oak were sampled. Roots were kept at ca. 5°C in a cooler and processed within 1–2 months. Prior to processing, all roots were soaked in water for 1 h, pressure washed and any white oak roots collected were scanned on a flatbed scanner. Lengths of roots with a diameter of 0–1 mm (referred to as fine root hereafter)

were measured using the software WINRHIZO PRO 5.0 (Regent Instruments, Québec, Canada) and presented as the average total fine root length (cm) of ten trees in each health class (Fig. 3). Roots with larger diameters (3 to 1 cm) were evaluated for brown necrotic lesions and if present plated directly onto PARPNH selective medium without surface sterilization. Random fine roots from each tree also were plated on selective medium to isolate *Phytophthora*.

2.6 Statistical analyses

The relationship of *Phytophthora* presence or absence with crown status was evaluated using contingency tables. Logistic regression analyses were used to test the tree health status as well as the presence of *Phytophthora* in relation to altitude and IMI values. A log transformation was applied to population densities to achieve a normal distribution. *Phytophthora cinnamomi* population densities and fine root lengths of declining and healthy trees were analysed using analyses of variance (ANOVA) and the Tukey-Kramer honesty of significance test was used to evaluate the means. Analyses were performed using JMP[®] 5.0 software (SAS 2002).

3 Results

3.1 Species assemblage and isolation frequencies

Phytophthora was isolated frequently when baited from soil samples. Of the 102 soil samples collected in 2006, 70 gave positive isolation of *Phytophthora*. Four species were isolated from soil samples; *P. cinnamomi* (68 isolates); *P. citricola* (1 isolate); *P. cambivora* (1 isolate); and, an undescribed species (1 isolate). In one instance *P. cinnamomi* was isolated from the same soil sample with *P. cambivora*; in all other samples only one species was isolated from a soil sample. Usually *Phytophthora* was isolated after the first isolation attempt, but on 20 soil samples *P. cinnamomi* was isolated after soils were dried and reflooded.

When soils from 45 infested and 18 non-infested white oak trees were re-sampled in 2007 to confirm the presence of *Phytophthora* throughout the 2-year period, mostly similar results were obtained. Among the 45 *P. cinnamomi*-infested trees in 2006, only ten resulted in negative isolation in 2007 and *P. cinnamomi* was isolated from rhizosphere soil samples of all other trees for the second year using baiting as well as direct plating on PCH medium. For the 18 *Phytophthora*-free trees, as determined in 2006, when re-sampled in 2007, in only one instance was *P. cinnamomi* isolated using baiting and direct plating on PCH medium. All other trees remained *Phytophthora*-free in 2007.

Soil samples collected in 2007 from ten infested trees and ten non-infested trees for root studies gave the same results as 2006 for presence/absence of *Phytophthora*.

All isolates of *P. cinnamomi* belonged to the A2 mating type and *P. cambivora* belonged to the A1 mating type. Molecular analysis confirmed the morphological identification of all isolates. Sequence data of identified species can be accessed through the *Phytophthora* database (<http://www.phytophthoradb.org>): *P. cinnamomi* (PD-01145 through PD-01207), *P. cambivora* (PD-01869), *P. citricola* (PD-01870) and *P. sp1* (PD-01627). Description of the isolate *P. sp1* as *P. ohioensis* is proposed elsewhere.

3.2 Presence and population density of *Phytophthora* in relation to site factors and decline status of trees

Phytophthora was widespread through the area studied and no special pattern of incidence was observed based on the isolation results alone (Fig. 1). However, when presence of *Phytophthora* was analysed in relation to IMI values of the sites, they were found to be

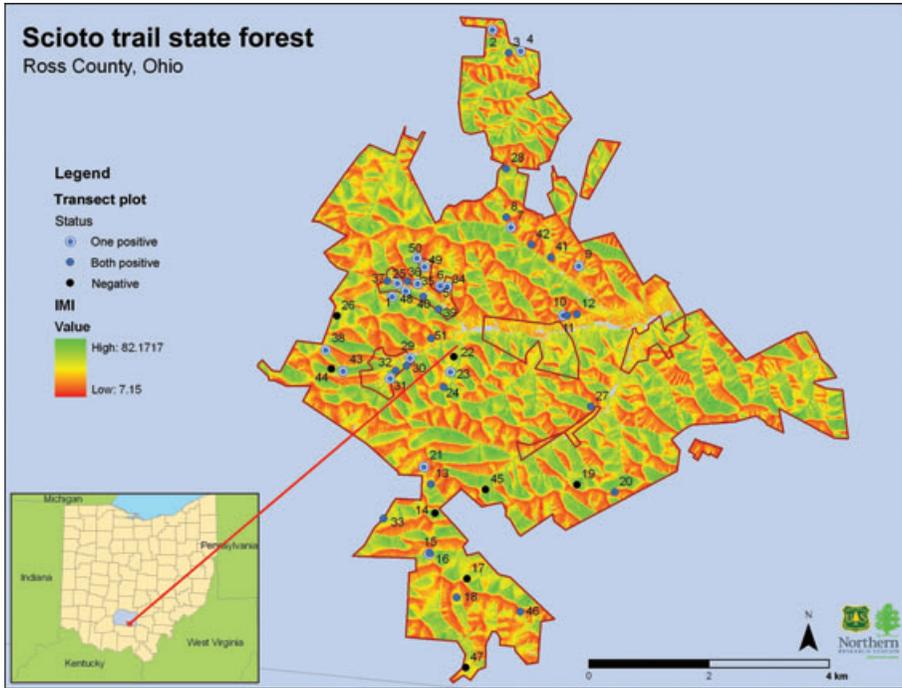


Fig. 1. Isolation results for *Phytophthora* throughout the study site. Each circle represents a transect that consists of two sites: one located down slope and other in upper slope. The GIS-derived Integrated Moisture Index (IMI) was utilized for the study area and was calculated based on hill shade, curvature, flow accumulation, and total water-holding capacity. Lower IMI values (red to orange) represent areas with little moisture and greater IMI values (yellow-green) increased amounts of moisture.

more common on sites with greater IMI values ($\chi^2 = 4.568$; $p = 0.032$) (Fig. 2a). A similar and significant trend ($\chi^2 = 3.897$; $p = 0.048$) was also observed when health status of trees was analysed with IMI values (Fig. 2b).

Sampling sites averaged 255 m a.s.l (206–321 m). There was no significant relationship between the presence of *Phytophthora* and the altitude of the site ($\chi^2 = 1.486$; $p = 0.222$).

Considering all 102 trees sampled in 2006, *Phytophthora* was isolated from 27 out of 43 healthy and 43 out of 59 declining trees. However, no significant association was found between the presence of *Phytophthora* and the crown status of trees (Fischer's exact test; $p = 0.289$).

There was a considerable difference in average population level of *P. cinnamomi* in infested sites (Table 1). The greatest population levels always were found in soils sampled from declining trees. Average population levels of *P. cinnamomi* found in soils associated with declining trees was about seven times greater than in soils associated with healthy trees (Table 1). The difference in population level was also significant when it was analysed in relation to the decline status of trees ($F = 7.543$; $p = 0.008$).

3.3 Root analysis

Roots collected from a declining or healthy tree usually had several small ($L < 1$ cm) lesions. However, no *Phytophthora* was isolated from necrotic tissues or from fine roots of healthy or declining roots of 102 white oaks collected in 2006. The only instance when

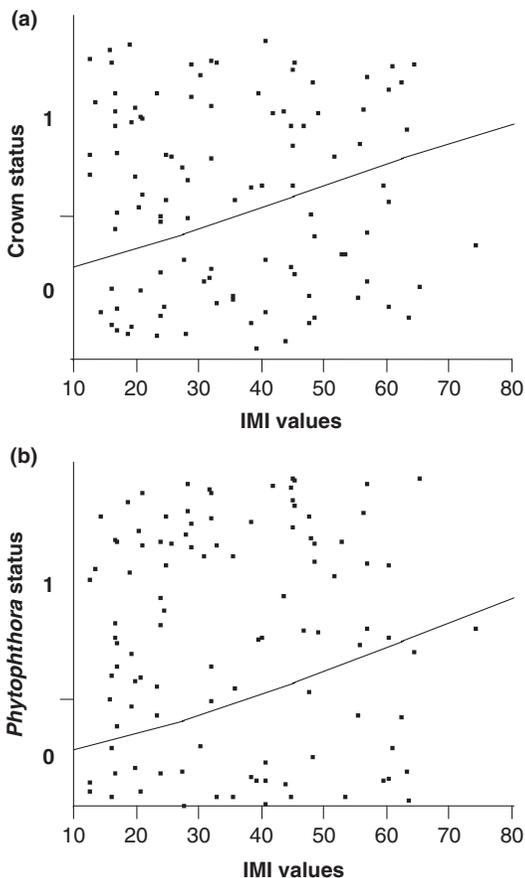


Fig. 2. Logistic fit of Integrated Moisture Index (IMI) values to (a) crown status of trees (healthy = 0; declining = 1) and (b) status of *Phytophthora* (absent = 0; present = 1). Greater IMI values indicate mesic sites (see methods).

Table 1. Average population density of *Phytophthora cinnamomi* per 100 g oven-dried infested soil collected from healthy and declining white oak (*Quercus alba*) trees.

Tree health status ¹	n	Mean	SD	Minimum	Maximum
Healthy	15	5.9	5.6	0	16.2
Declining	30	45.1	71.4	0	272.7

¹Healthy trees were those with less than or equal to 25% crown transparency and declining trees were those with greater than 25% crown transparency.

Phytophthora was isolated from roots was when roots were extensively sampled (see methods) in August 2007 to compare the root status of ten infested and ten non-infested trees. In three instances, *P. cinnamomi* was isolated from roots of infested trees as determined based on soil isolations. In no instance was *Phytophthora* isolated from roots of any *Phytophthora*-free trees in 2006 or 2007.

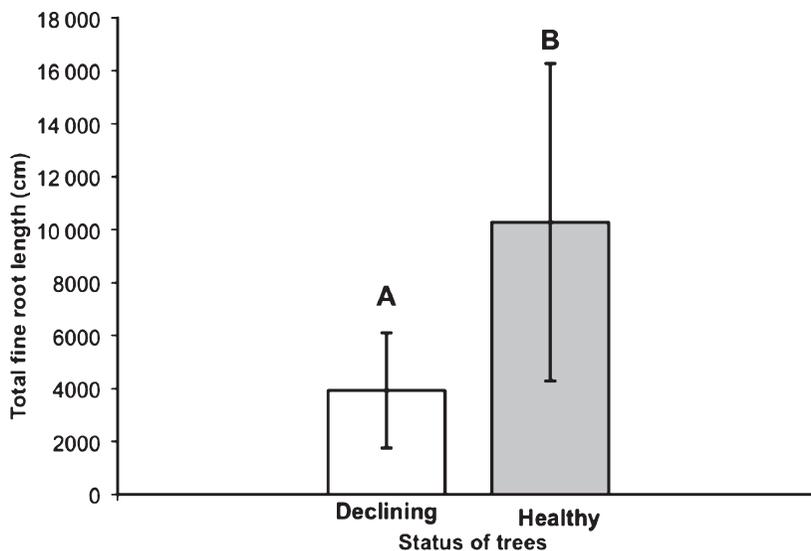


Fig. 3. Average and SD for total fine root length (<1 mm diam) of *Quercus alba* roots sampled from healthy (n = 10) and declining (n = 10) trees.

For the ten infested and non-infested trees, when roots were scanned, non-infested trees had significantly greater amounts of total fine roots compared to *Phytophthora*-infested trees ($F = 9.937$; $p = 0.005$) (Fig. 3). The average total fine root length of the sampled roots of infested trees was 3931 ± 2175 cm and 10282 ± 5990 cm for the non-infested white oaks (Fig. 3).

4 Discussion

Phytophthora cinnamomi was the most common and widespread species throughout Scioto Trail State Forest. In a related smaller study, we frequently isolated *P. cinnamomi* in Zaleski State Forest (Y. Balci and R. Long, unpublished data), located 52 km northeast of the study sites, suggesting the widespread occurrence of *P. cinnamomi* in southeastern Ohio oak forests. This pathogen also was commonly isolated from oak forests across the eastern USA below the 40°N latitude range (BALCI et al. 2007). It is unknown, how *P. cinnamomi* was introduced to the study site and became such wide-spread. *Phytophthora cinnamomi* is considered exotic in the USA and has been recognized as an important pathogen of a wide variety of forest plants since the 1930s (CRANDALL et al. 1945; ZENTMYER 1980; BALCI et al. 2007).

The IMI values gave better clues about the distribution of *Phytophthora* in STSF than slope/altitude separation. The occurrence of *P. cinnamomi* at STSF was not restricted by altitude. Although this simply might be due to the fact that the average altitude difference among the sampling sites (e.g. down vs. upper slope) was only about 20 m. The mean altitude of 255 m of the study site may not be detrimental on distribution of *P. cinnamomi*. In a previous study comprising a larger area, we never isolated *P. cinnamomi* above 825 m (BALCI et al. 2007). *Phytophthora cinnamomi* is widely reported in lower altitudes (ZENTMYER 1980), but no studies have conclusively examined its occurrence in relation to altitude.

There also was no spatial pattern of incidence found. In a larger study, investigating the incidence and site factors on *P. cinnamomi* in cork oak (*Q. suber* L.) stands in

Portugal, no spatial pattern or relationships were found with topography despite the common occurrence of *P. cinnamomi* (MOREIRA and MARTINS 2005). Moreover, the spatial incidence might be related to site moisture content as demonstrated with IMI values in this study. Moisture is known to be a critical factor in relation to infection by *Phytophthora* (ZENTMYER 1980). However, incidence distribution of *Phytophthora* in soil can be better explained by considering the interaction of aeration, soil physical and chemical properties and soil moisture (ERWIN et al. 1983; ERWIN and RIBEIRO 1996). Indeed occurrence of *Phytophthora* species in oak forest soils was found to be restricted by soil type, lower soil pH and nutrients such as magnesium and calcium (JUNG et al. 2000; BALCI and HALMSCHLAGER 2003a; JÖNSSON et al. 2005; MOREIRA and MARTINS 2005).

Phytophthora cinnamomi is reported to cause root rot and stem cankers on oaks (CRANDALL 1936, 1950; CRANDALL et al. 1945; MIRCETICH et al. 1977; WOOD and TANTER 2002; GARBELOTTO and HÜBERLI 2006). In STSF, we never found the organism associated with stem cankers, but only in association with roots and soils around the boles of white oak trees. This indicates that the impact of *P. cinnamomi* is likely restricted to roots of trees at STSF. One particular reason could be the climatic restrictions during winter that limit its development aboveground (MARÇAIS et al. 2004; BALCI et al. 2007).

At STSF, most of the dead or declining trees occurred on the higher quality bottomland and lower slope sites, and in fewer instances on upper slopes. This pattern of decline and mortality is characteristic for *Phytophthora*-associated problems in other ecosystems (SHEARER and TIPPETT 1989; JUNG et al. 2000; HARTMANN and BLANK 2002). A similar observation for mortality of oaks also was observed with *P. cinnamomi*-infested oak forests in Spain and Portugal where dieback of trees often occurred in big groups or foci along streams, valleys or other depressions and in shallow soils with fine texture (BRASIER et al. 1993; COBOS et al. 1993; SÁNCHEZ et al. 2002; MOREIRA and MARTINS 2005). Disease development in STSF may have progressed faster in poorly drained bottomland sites, which could favour pathogen activity and propagule formation and enhance the ability of *P. cinnamomi* to successfully colonize host trees.

A time lag is usually present from when trees start declining and die and when mortality becomes conspicuous to land managers. A large number of trees had already died when we initiated this study and easily accessible sites had been salvaged. By incorporating those trees into the study, it may have been possible to find a closer association between the presence of *Phytophthora* and the decline status of the trees. Thus, our inability to relate the presence of *Phytophthora* to tree crown health status might have been different depending on when the study was initiated (e.g. early stage of decline, mid or the end phase). In similar studies, no consistent results have been obtained that utilized crown status as a means to investigate the effect of *Phytophthora* on tree health (BALCI and HALMSCHLAGER 2003a,b; BALCI et al. 2007; DELATOUR 2003; HARTMANN and BLANK 2002; JÖNSSON et al. 2005; VETTRAIANO et al. 2002). However, as demonstrated with root sampling, a comparison of root status of trees instead of crown condition alone resulted in a better estimation of *Phytophthora* spp. impact. Studies investigating the root status of oaks demonstrated a significant correlation between crown transparency and root health (JUNG et al. 2000). Similarly, JÖNSSON-BELYAZIO and ROSENGREN (2006) demonstrated root reduction due to *P. quercina* on *Q. robur* trees despite determination that crown symptoms were inconclusive. Furthermore, symptom expression on oak seedlings in artificial soil infestation experiments occurred only when over half of the roots were killed (BALCI et al. 2008a), thus the effect of *Phytophthora* spp. may be under estimated based on crown status alone.

The significantly greater population levels found on declining trees across the study sites suggest that *P. cinnamomi* may have influenced root health by increased pathogen activity and thus contributed to a greater fine root loss. Our findings comparing ten infested and

ten non-infested trees supported this view since considerably lower amounts of fine root lengths were found for *P. cinnamomi*-associated trees compared to trees without *Phytophthora* in their rhizosphere soil. The significantly greater amounts of declining trees in sites with greater IMI values may have been the consequence of increased pathogen population/activity in mesic sites, resulting in greater root damage. In areas infested with *P. cinnamomi*, highly conducive specific site conditions were shown to contribute to rapid mass decline of Eucalyptus trees in Western Australia (ZENTMYER 1980; SHEA et al. 1983; SHEARER and TIPPETT 1989).

The actual numbers of *P. cinnamomi* propagules in soils reported here can not be compared to other studies because the number of chlamydospores and its distribution in soil can be influenced by differences in soil type, soil pH, soil texture, soil depth, organic matter and bulk densities as well as sampling times among studies (MARKS et al. 1975; WESTE and VITHANAGE 1978; SHEARER and SHEA 1987). The population level of *Phytophthora* was variable among sites at STSF. The isolation results using baiting also revealed variation since different results were obtained when sites were re-sampled 1 year later, indicating *P. cinnamomi* populations fluctuate between years. A seasonal variation in population levels of *P. cinnamomi* also was detected in Hawaiian forest soil (KLIEJUNAS and NAGATA 1979), Eucalyptus forests in Australia (WESTE and VITHANAGE 1978; SHEARER and SHEA 1987; SHEARER and TIPPETT 1989) and also for other *Phytophthora* species (FLOWERS and HENDRIX 1972; SEWELL et al. 1974).

The greater mortality rates of white oaks compared to other oak species (e.g. *Q. montana* and *Q. rubra*) at the study area might be due to its greater susceptibility to *Phytophthora* infection. In a previous study that included eleven oak species only a significant relationship with the presence of *Phytophthora* and declining white oaks in the field was detected (BALCI et al. 2007). When *P. cinnamomi* was tested for pathogenicity on various oak species in an artificial inoculation experiment, white oak roots were among the most susceptible to *Phytophthora* infection (Y. Balci, W. MacDonald and K. Gottschalk, unpublished data). In the field, white oaks may have similarly experienced a greater root loss compared to other oaks. The absence of wide-spread mortality of other oak species across forests of Ohio may support the contention that white oaks are more susceptible to the factors causing oak decline.

White oak decline in STSF provides another example of an oak decline scenario where a root pathogen, a series of unusual environmental events, and a susceptible host resulted in decline or death of trees. The wide-spread occurrence of the exotic pathogen *P. cinnamomi* in eastern US oak forest has been demonstrated previously (BALCI et al. 2007). Its role in oak decline may have been underestimated because no aboveground cankers were present in most oak forests in eastern and north-central US. However, seedling and tree trials have demonstrated that *P. cinnamomi* and several other *Phytophthora* species are capable of causing substantial damage to oak roots if soil is infested artificially or when conditions are favourable in the field (BALCI and HALMSCHLAGER 2003a,b; BALCI et al. 2008a; GALLEGRO et al. 1999; GARBELOTTO and HÜBERLI 2006; JÖNSSON et al. 2005; JUNG et al. 2000; MARÇAIS et al. 1996; MAUREL et al. 2001; MOREIRA and MARTINS 2005; ROBIN et al. 1998, 2001; SÁNCHEZ et al. 2002; TAINTER et al. 2000; VETTRAINO et al. 2002). The role of *P. cinnamomi* in oak decline in eastern USA is not studied. However, multiple examples in southeastern US forests such as its involvement in the littleleaf disease complex (*Pinus echinata* and *P. taeda*), root rot and mortality of sand pine plantations (*P. clausa*) as well as with death of chestnut (*Castanea dentata*) along the Appalachian mountains demonstrates its potential as an aggressive root pathogen and its significant impact on plant health (CRANDALL et al. 1945; ZENTMYER 1980; BARNARD et al. 1993; TAINTER and BAKER 1996).

Our study provides evidence that declining white oaks may have experienced greater fine root loss due to increased pathogen activity. The surplus in precipitation during the growing season, after the drought in 1999, may have contributed to the spread and

infection of *P. cinnamomi* in infested mesic sites at a greater level and resulted in more fine root mortality. A long-term field study on mature trees examining the dynamics of root loss due to *P. cinnamomi* activity under different moisture regimes and soil characteristics will contribute greatly to our understanding of the decline and death of white oak that occurs in various settings. Its role in root health on various tree species along the eastern US oak ecosystems remains to be determined.

Acknowledgements

This research was made possible by funding from the USDA-Forest Service, Northeastern Area State and Private Forestry, Forest Health Monitoring Program, Evaluation Monitoring component (06-CA-11244225-316). We wish to thank Selin Balci, for her excellent assistance during the extensive lab routines. Special thanks are given to Stephanie Colwell, Jim Stein, Clayton Rico, Timothy Fox, and Joan Jolliff for conducting field sampling and data collection. We also like to thank both anonymous reviewers for their valuable suggestions that have greatly improved the manuscript.

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