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Relative susceptibility of oaks to seven species of *Phytophthora* isolated from oak forest soils

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Summary

Isolates of *Phytophthora cambivora*, *P. cinnamomi*, *P. citricola*, *P. europaea*, *P. quercetorum* and two unidentified species were tested for their pathogenicity to eastern US oak species by root and stem inoculations. Experiments were conducted during two different periods and included 1-, 2- and 20-year-old oaks grown under greenhouse and field conditions. Species of *Phytophthora* were pathogenic in varying degrees to the oak species tested. All species were pathogenic to fine and taproots of at least one oak species. The fine root damage caused by the species of *Phytophthora* ranged from 9 to 55% when compared to the controls. Roots were more susceptible during the fall inoculation period than the summer. With exception of *Phytophthora* sp1 and *P. quercina*-like, all species of *Phytophthora* were pathogenic to oak stems with *P. cinnamomi* and *P. citricola* being the most aggressive. *Quercus montana* and *Q. rubra* were the most susceptible oak species to stem inoculation. Lesion sizes were considerably larger when 20-year-old trees were inoculated. Generally, no significant differences in lesion sizes were detected in greenhouse tests when the summer and fall inoculation periods were compared. However, on 2-year-old field-grown seedlings, lesion sizes were considerably smaller or not significantly different from controls during the fall inoculation period, suggesting lower, late season temperatures may restrict lesion development.

1 Introduction

Oaks are one of the most diverse groups of tree species in North America and represent an economically important group of hardwoods (McShea and Healy 2002). Of the various threats to the health of oak trees, species of *Phytophthora* have received special attention in recent years. Their involvement in decline and mortality of oaks has been the focus of a variety of studies in different parts of the world (Brasier et al. 1993; Robin et al. 1998; Gallego et al. 1999; Hansen and Delatour 1999; Jung et al. 2000; Tainter et al. 2000; Sánchez et al. 2002; Vettraino et al. 2002; Balci and Halmschlager 2003a,b; Delatour 2003; Jönsson et al. 2005; Jonsson-Belyazio and Rosengren 2006). In the western USA, *Phytophthora ramorum* Werres, DeCook & Man in't Veld has been associated with extensive oak mortality (Rizzo et al. 2002; http://www.suddenoakdeath.org) and could pose a serious treat if introduced to eastern oak forests (Venette and Cohen 2006). Fortunately, efforts to isolate this exotic pathogen in central and eastern US oak forests have failed. However, seven species of *Phytophthora* have been identified from healthy and declining trees as part of studies to determine the species of *Phytophthora* present in oak ecosystems (Balci et al. 2007). These *Phytophthora* species, which included well known and newly described ones, reside in oak forest soils without causing noticed aboveground symptoms on their hosts.

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However, among them, the frequently isolated species *P. cinnamomi* Rands and *P. citricola* Sawada are notorious plant pathogens that cause disease on numerous plant species, including stem cankers of oaks in the field (Mirchetich et al. 1977; Erwin and Ribeiro 1996; Robin et al. 1998; Tainter et al. 2000; Wood and Tainter 2002; Garbelotto and Hüberli 2006). Species of *Phytophthora* isolated from rhizosphere soil samples in Europe included several new species that have been suggested to be involved in oak decline by mainly causing root damage (Balci and Halmschlager 2003a,b; Brasier et al. 1993; Delatour 2003; Gallego et al. 1999; Moreira et al. 2000; Sánchez et al. 2002; Jönsson 2004; Jonsson-Belyazio and Rosengren 2006; Jung et al. 1996, 2000; Vettraino et al. 2002). Despite the wide occurrence of *P. cinnamomi* throughout eastern USA (Crandall et al. 1945; Campbell and Hendrix 1967; Balci et al. 2007), its role in oak decline still remains uninvestigated. The role of these species of *Phytophthora* play in the health of oaks needs to be evaluated and may contribute to our understanding of the general risks posed by *Phytophthora* spp. Therefore, we have tested the seven isolates of *Phytophthora* previously identified from soils of eastern US oak forests (Balci et al. 2007), to determine their potential pathogenicity and relative virulence to several eastern oak species. Relative susceptibility of oak during different inoculation times (summer *vs.* fall) and conditions (greenhouse *vs.* field), plant age (young *vs.* mature) and tissue type (root *vs.* stem) were evaluated by artificial inoculations.

2 Materials and methods

2.1 Isolates tested

The seven species of *Phytophthora* used in this study are selected randomly among the representative isolates of *Phytophthora*, which were isolated during a forest soil survey in 2004 and 2005 (Table 1) (Balci et al. 2007). *Phytophthora quercetorum* is a newly recognized species occurring over a wide range of oak forests in eastern USA (Balci et al. 2008). The *P. quercina*-like isolates matched *P. quercina* by sequence data; however, they differed from *P. quercina* culturally in oogonial features, growth pattern on PDA and a much slower growth rate. Similarly, the classification of *Phytophthora* sp1 via morphological features did not warrant its identification as *P. europaea*, although sequence data were closest to *P. europaea* (Balci et al. 2007). Isolates of *P. cinnamomi* and *P. cambivora* were of the A2 compatibility type as determined by pairings with known A1 and A2 compatibility-type isolates.

2.2 Soil infestation test

Two separate inoculations were conducted to assess the susceptibility of fine roots and taproots during different periods. The first inoculation period was from 24 March to 26

Table 1. **Isolates used in this study. All Phytophthora species were isolated in an oak forest soil survey in 2004 and 2005**

Isolate	*Phytophthora* spp.	Host	State	ATCC No.
OH 4/4	*P. cambivora*	*Q. velutina*	Ohio	MYA-4089
WV-Gr1/1	*P. cinnamomi*	*Q. rubra*	West Virginia	MYA-4085
OH 6/5	*P. citricola*	*Q. rubra*	Ohio	MYA-4087
WV BM 1/10	*P. europaea*	*Q. alba*	West Virginia	MYA-4088
MN 023	*P. quercina*-like	*Q. rubra*	Minnesota	MYA-4090
WV BSC 1/3	*Phytophthora* sp1	*Q. rubra*	West Virginia	MYA-4091
MD 9/2	*P. quercetorum*	*Q. rubra*	Maryland	MYA-4086

ATCC, American Type Culture Collection.

July 2006 (spring–summer) and the second from 15 August 2005 to 30 December 2006 (fall). Six oak species were used for the experiment (Table 2). A soil infestation test was conducted on oak seedlings grown in 5-l plastic pots under greenhouse conditions. The potting mix consisted of 1 : 1 : 1, v/v/v non-sterile medium composed of peat, vermiculite and sand. Inoculum for these tests was prepared by growing isolates of *Phytophthora* in flasks containing sterile rice grains (Holmes and Benson 1994). To establish inoculum, 10 6-mm-diameter cork borer plugs were taken from 1-week-old cultures of each species of *Phytophthora* grown on a V8 juice agar (V8A) (Erwin and Ribeiro 1996). Plugs were transferred to an Erlenmyer flask containing a 50-g mixture of rice grains and 36 ml deionized water. Flasks containing rice grains and water were autoclaved twice prior to insure sterilization. After 4 weeks of incubation at 20°C under diffuse light, 10 g of the colonized rice grains were introduced into each potting mix to a depth of 10 cm. A glass rod was used to create a hole at three sites for the inoculation. Similarly prepared inoculum was applied a second time 1 month later to ensure colonization. The control seedlings received only sterile rice grains containing V8A plugs. Following inoculation, pots with a particular species of *Phytophthora* were placed in a large container, flooded and allowed to drain freely and thereafter watered weakly to maintain constant moisture in the potting-mix. A minimum of 10 seedlings were grown in a single pot and inoculated with each isolate. The pots were incubated in greenhouse for 4 months, and at the end of the incubation period, roots were pressure washed with water to remove the potting mix. Roots were evaluated for fine root damage and taproot infection.

To evaluate fine root damage, roots were placed in trays, flooded and scanned with an optical scanner. Root lengths were calculated for 0- to 1-mm-diameter root class (referred to hereafter as fine roots) using the software WinRhizo Pro 5.0 (Regent instruments, QC, Canada).

To evaluate taproot infection, roots were scored for the severity of lesion development on tap and coarser lateral roots (>2 mm) by using an index, where 0 = none to 25% lesion development; 1 = root lesion 25–50%, tap and lateral roots infected and localized necrotic spots visible; 2 = root lesion 50–75%, tap and all major lateral roots infected, dark sunken extending lesions on taproot; and 3 = lesion >75%, taproot infected and girdled up to the root collar.

Re-isolation of species of *Phytophthora* to PARPNH selective medium (Erwin and Ribeiro 1996) was attempted from each seedling with lesions and brownish discolouration on lateral and taproots as well as from randomly selected fine roots. Selective medium contained V8A amended with 10 μg/l pimaricin, 200 μg/l ampicillin, 10 μg/ml rifampicin, 25 μg/l pentachloronitrobenzene, 50 μg/l nystatin and 50 μg/l hymexazol. An oak leaflet baiting technique was used to prove the presence of *Phytophthora* (Jung et al. 1996). A single 250-g subsample taken from each pot was flooded with 500 ml of distilled water and baited by floating 3- to 7-day-old *Q. robur* or *Q. palustris* leaflets. Baited samples were kept at temperatures of 17 to 20°C. After 3–5 days, discoloured leaf samples were examined microscopically (200×) and those with sporangia typical of *Phytophthora* were plated on PARPNH selective medium. The temperature in the greenhouse typically was about 20 ± 2°C with exception during the summer period (mid-June–August), which resulted in much greater room temperatures for some days (24 ± 6°C).

2.3 Inoculation of stems of oak seedlings

Two separate experiments were conduced: one in greenhouse and the second under field conditions. For greenhouse experiments, 1-year-old seedlings sown from acorns collected from the field or obtained from a seed company were used. For field inoculations, 2-year-old bare root plants were obtained from state nurseries and potted in greenhouse. All seedlings were grown in 5-l plastic pots and were watered twice (summer) or once (fall)

Table 2. **Mean total lengths of oak roots with 0–1 mm diameter ± SD after soil was infested with seven *Phytophthora* species for the two inoculation periods**

Quercus spp.	Inoculation period	*Phytophthora* spp.							
		P. cambivora	*P. cinnamomi*	*P. citricola*	*P. europaea*	*P. quercetorum*	*P. quercina*-like	*Phytophthora* sp1	Control
Q. bicolor	I[1]	1573 ± 886	2256 ± 1223	2127 ± 628	2142 ± 869	2091 ± 878	nt	2398 ± 1021	1485 ± 854
	II[2]	1389 ± 904	1572 ± 1098*	2176 ± 1407	1549 ± 622	2003 ± 1907	870 ± 566*	nt	1914 ± 1219
Q. macrocarpa	I	1701 ± 692	2528 ± 669	1575 ± 876*	1285 ± 421*	1285 ± 421*	nt	1399 ± 908*	2411 ± 1328
	II	1228 ± 1124*	1045 ± 737*	1286 ± 724	1151 ± 562	1140 ± 422	1063 ± 740*	1625 ± 716	1430 ± 1103
Q. montana	I	1166 ± 865	1731 ± 874	1878 ± 819	1131 ± 659	1148 ± 593	nt	1479 ± 936	1156 ± 711
	II	992 ± 667	773 ± 452	954 ± 494	775 ± 506	900 ± 635	725 ± 529	674 ± 286	660 ± 485
Q. palustris	I	1982 ± 1223*	1915 ± 441*	1867 ± 1069*	2256 ± 1021	2329 ± 1097	nt	2171 ± 1151	2475 ± 988
	II	1703 ± 806*	980 ± 424*	2504 ± 2194	1353 ± 858	1431 ± 324*	1422 ± 742*	nt	1869 ± 2531
Q. robur	I	2696 ± 1434	3099 ± 1571	1608 ± 1584*	1326 ± 628*	2467 ± 1257	nt	1679 ± 1635*	2942 ± 2821
	II	1574 ± 1159*	2159 ± 1917	1514 ± 1512*	1686 ± 1204	1702 ± 1311*	1961 ± 1606	nt	2517 ± 2059
Q. rubra	I	1355 ± 460*	1893 ± 1069	1466 ± 896*	nt	969 ± 378*	nt	1445 ± 225	1757 ± 630
	II	989 ± 617	930 ± 788*	996 ± 859*	747 ± 538*	855 ± 372	987 ± 612	608 ± 311*	1109 ± 810

Values that were significantly different from the controls according to Kruskal–Wallis test are indicated by an asterisc (*, p = 0.05). nt, not tested.
[1]First inoculation period April–August 2005.
[2]Second inoculation period August–February 2006.

throughout the experiment. Greenhouse- and field-grown seedlings were inoculated at two consecutive periods: period 1 (summer): 9 June to 24 August 2005, and period 2 (fall): 11 October to 14 December 2005. Oak seedlings used in these experiments are listed in Table 3. For each inoculation period and experiment (greenhouse or field), an average of 10 seedlings were used, which totalled over 450 seedlings for each of the treatment. Inoculum was prepared by growing the isolates of *Phytophthora* on V8A for 7 days in diffuse light at 20°C, and then removing 6-mm-diameter plugs with a cork borer from the advancing margin of the mycelium. The seedlings were inoculated by inserting a plug of inoculum between the bark and the cambial zone after a U-shaped cut was made aseptically approximately 3 cm above the root collar. The inoculation point was covered with sterile moist cotton, sealed with parafilm and covered with aluminium foil (Balci and Halmschlager 2003b). The control seedlings were inoculated similarly but with plugs of V8A. The evaluation of infection was based on the length of stem discolouration above the inoculation, excluding the wounding point, after a 2-month incubation period. Seedlings were also evaluated to determine if they were girdled or if they were dead. Re-isolation was attempted from necrotic lesions on PARPNH selective medium.

2.4 Inoculation of stems of field-grown oaks

Twenty-year-old trees used for the experiment were part of an oak plantation previously established at the Plant and Soil Sciences Farm of West Virginia University. The average stem diameters for *Q. rubra* L., *Q. robur* L. and *Q. montana* Willdenow were 13.5, and 10.5 cm for *Q. alba* L. trees. Four oak species were used for the experiment; however, the test was limited by the number of different oak trees available; all isolates were tested only on *Q. rubra* (Table 3). Inoculations were conducted only once in summer on 25 May 2005 and evaluated 2 months later. Trees were inoculated using the technique described by Tainter et al. (2000). Inoculum was prepared as described above for seedling experiments. Inoculations were made by removing 6-mm-diameter plugs from the advancing margin of the mycelium with a cork borer. Three inoculation sites were made on each tree, with two inoculated with the isolate tested and one with plugs of V8A serving as a control. Ten trees of each oak species were inoculated resulting in 20 inoculation sites per isolate. Inoculation was conducted 1.4 m above the ground by removing 0.6-cm-diameter bark plugs using a sterile cork borer. Inoculation points were made at equal distances (approximately 10 cm) around the circumference of the tree. After the inoculum was inserted and the bark plug replaced, the inoculation points were covered with sterile moist cotton and a 20-cm square of aluminium foil. The entire inoculation site was protected by covering with duct tape. The total length of phloem lesion was evaluated by removing the bark from the inoculation site and measuring the distance between the upper and lower lesion boundaries excluding the length of inoculation wound. Wood chips from the margin of the cankers were plated on PARPNH selective medium to confirm their colonization by the tested species of *Phytophthora*.

2.5 Statistical analysis

Data sets were checked for normal distribution and equal variance and transformed using log or square-root transformation. Stem lesion sizes with a normal distributions and equal variances were analysed using anova, and the Tukey–Kramer HSD test was used to separate the means among the species of *Phytophthora* as well as to rank oak species based on lesion sizes. For evaluation of taproot lesion scores as well as the fine root lengths, the Kruskal–Wallis non-parametric test was used. Standard least squares test with emphasis on effect leverage was used to analyse the effect of season on lesion sizes as well as rank oaks and *Phytophthora* for susceptibility and virulence during the evaluation of taproot data.

Table 3. **Mean lesion length (cm) caused by seven *Phytophthora* species following wound inoculation in different periods**

Inoculation experiments	*Quercus* spp.	*P. cambivora*		*P. cinnamomi*		*P. citricola*		*P. europaea*		*P. quercetorum*		Control	
		I[1]	II[2]	I	II	I	II	I	II	I	II	I	II
1-year-old seedlings grown in greenhouse	*Q. bicolor*	0.0 bc[3] B[4]	0.0 bc B	0.2* ab B	0.1* a B	0.3* a B	0.1* ab C	0.1 bc B	0.0 c D	0.1 bc A	0.0 c B	0.0 c	0 c
	Q. macrocarpa	0.1 bc B	0.2* bc B	0.1* ab B	0.2* ab AB	0.1* a B	0.3* a BC	0.1* ab B	0.1* b–d BC	0.1* ab A	0.1* c A	0 c	0 e
	Q. montana	1* a A	1* a A	0.3 bc B	0.4 bc A	0.6* ab B	0.7* ab B	0.4 bc A	0.3 bc A	0.2 bc A	0.2 c A	0.0 c	0.0 c
	Q. palustris	0.0 cd B	0.0 cd B	0.1* ac B	0.2* ab B	0.2* a B	0.2* a C	0.1 bd B	0.1 b–d CD	0.1* ab A	0.1 bc AB	0 d	0.0 cd
	Q. rubra	0.1 c B	0.2 bc B	1.4* b A	0.4* b A	2.2* a A	1.4* a A	0.3 c A	0.3 bc AB	0.2 c A	0.2 bc A	0.0 c	0.0 c
2-year-old seedlings grown in the field	*Q. alba*	1.5* ab BA	0 ab A	1.8* a A	1* a A	0.8* a–c AB	0.5* a B	1* a–c AB	0 ab A	0.7 b–d A	0 b A	0.0 d	0.0 b
	Q. montana	2* b A	0 bc A	3.5* a A	1* b A	1* bc A	1.5* a A	1* bc AB	0 c A	0.7* c A	0 c A	0 d	0 c
	Q. palustris	0.1 c C	0* b A	3.5* a A	0* a A	0 bc B	0. c B	1.1* b AB	0 bc A	0.5 bc A	0 c A	0.0 c	0.0 c
	Q. rubra	0.4 c BC	0 a A	2.3* a A	1 a A	1* b B	0.8 a AB	0.5* b B	0 a A	0.5* b A	0 a A	0 c	0 a
	Q. velutina	1.2* bc A–C	0 ab A	3.7* a A	0* a A	1* bc AB	0.4* a B	1.4* b A	0 ab A	0.9 b–d A	0 b A	0.	0.0 b
20-year-old trees in the field	*Q. alba*	nt		109* a AB		nt		8 b B		nt		1.1 b	
	Q. montana	nt		104* a AB		20 b B		15 b A		nt		1.4 b	
	Q. robur	30* b A		70* a BC		9 cd B		15* c A		nt		2.3 d	
	Q. rubra	17* b B		48* a C		41* a A		14 bc A		13 bc		1.5 c	

Lesions that were significantly different from the controls are marked with an asterisc (*). nt, not tested.
[1]First inoculation period: June–August 2005.
[2]Second inoculation period: October–December 2006.
[3]Significant differences among the *Phytophthora* species within a single oak species and inoculation period. Isolates with the same letter do not differ significantly from each other according to Tukey's test (p = 0.05).
[4]Significant differences among the oak species within a single *Phytophthora* species. Oaks with the same uppercase letter do not differ significantly from each other according to Tukey's test (p = 0.05).

t-Test was used to evaluate the differences among the sizes of stem diameter of 20-year-old trees. Analyses of all data were performed using the software program JMP 5.0 (SAS 2002).

3 Results

To allow the readability and direct visual comparison of the results among experiments, the standard deviations were excluded in Table 3. *Phytophthora* sp1 and *P. quercina*-like isolates did not produce lesions that were significantly different from controls during the 1- and 2-year-old stem-inoculation experiments, thus they were excluded from Table 3 to increase the readability. These species were not tested on 20-year-old trees due to the limited availability of trees. There was statistically no difference among the stem diameter of trees used for the 20-year-old tree inoculations for each isolate, thus data were not corrected.

3.1 Soil infestation

3.1.1 Fine root damage

Because WinRhizo only calculated the quantity of fine roots within specific diameter classes, this procedure could not be used to measure root necrosis. However, WinRhizo showed that seedling inoculated with various species of *Phytophthora* usually had fever fine roots than the controls (Table 2); this was often notable when roots were evaluated visually. A dark brown or black discolouration was observed on fine roots of infected seedlings, although a separation of healthy from diseased fine roots was difficult based on colouration alone.

Generally, more fine roots were present during the summer inoculation period than the fall period when all oak species were considered ($p < 0.0001$). This trend also was evident and significant ($p < 0.0001$) with the control seedlings that produced, on average, 55% more fine roots during summer than in the fall.

The fine root damage caused by the tested species of *Phytophthora* was different during the spring or fall inoculations (Table 2). However, with the following oak–*Phytophthora* combinations, significant fine root damage occurred during both inoculation periods: *Q. robur* and *Q. rubra* inoculated with *P. citricola*, and *Q. palustris* inoculated with *P. cinnamomi* and *P. cambivora* (Table 2). The greatest fine root reduction was observed when *P. europaea* was used to inoculate *Q. robur* (55%) and *Q. macrocarpa* (47%) in the spring period. The least damage that still was different from the control seedlings were obtained when *Q. macrocarpa* (14%) and *Q. palustris* (9%) was inoculated with *P. cambivora* during the fall inoculation period.

When the susceptibility of the oak species were compared, the fine roots of *Q. bicolor* and *Q. montana* were the least susceptible to damage by various species of *Phytophthora* (Table 2). In contrast, the fine roots of the four other oaks were more damaged and similar in susceptibility to the tested species of *Phytophthora* (Table 2).

3.1.2 Taproot infection

Most species of *Phytophthora* were able to infect and produce lesions on the lateral and taproots. Lesions started generally on tip of taproot and expanded towards the collar of the seedling (Fig. 1a–c). Seedlings with severely infected taproots often produced new lateral roots above the necrotic area (Fig. 1a–c), which some of them also became infected (Fig. 1a). Brown necrotic areas frequently were visible at the point where the lateral or fine

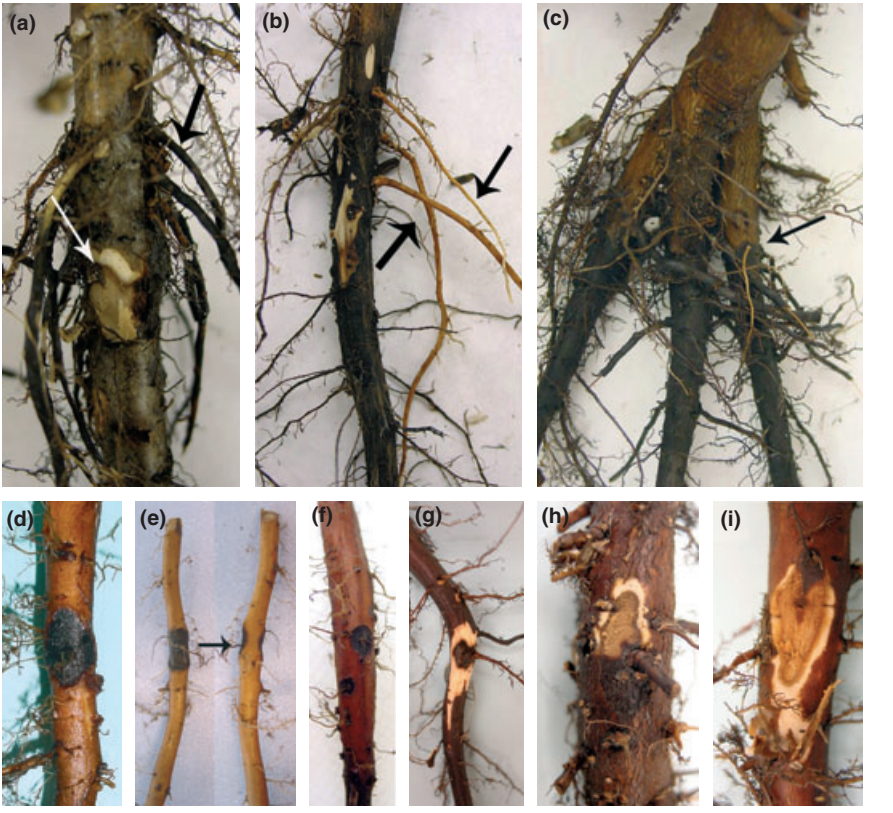

Fig. 1. Examples of infection caused on taproots of various oak seedlings by *Phytophthora* spp. (a) infection of taproots marked by a necrotic line (arrow) as well as infected new lateral roots (arrows); (b) differentiation of new lateral roots (arrows) allowing the seedling to survive; (c) expansion of lesions (arrow) upward the stem, all major taproots were infected and girdled; (d–f) black or brown necrotic tissue where the fine or lateral roots originate; (e) lesion girdling the taproot and (g–i) expansion of lesions starting from the point where smaller roots are attached to taproots

roots were originated (Fig. 1d–f). Lesions were expanding from such areas (Fig. 1g–i), and in some instances, they were about to girdle the taproot (Fig. 1e).

When taproots of the various oak species were evaluated for necrosis, the summer and fall inoculation results were variable and mostly inconsistent (Table 4). However, as with fine roots, generally more significant root damage occurred during the fall inoculation period. Overall, the effect of inoculation period was significant with all oak species tested ($p < 0.001$). However, in few instances, taproots of oaks were consistently susceptible during the two inoculation periods: *Q. macrocarpa*, *Q. robur* and *Q. rubra* inoculated with *P. cinnamomi*, *Q. macrocarpa* and *Q. robur* inoculated with *P. quercetorum*, and *Q. macrocarpa* with *Phytophthora* sp1 (Table 4). A few observations were notable. When oaks were ranked based on taproot infections, *Q. robur* ranked as the most susceptible species during both periods and *Q. bicolor* the least. Additionally, *Q. rubra* was damaged more during the fall period by all isolates of *Phytophthora* used in the study (Table 4).

When all species of *Phytophthora* were considered, the average lesion development index was 0.8 for the summer and 1.4 during fall inoculation period. *Phytophthora cinnamomi*

Table 4. **Mean taproot infections ± SD**

	P. cambivora		*P. cinnamomi*		*P. citricola*		*P. europaea*		*P. quercetorum*		*P. quercina*-like		*Phytophthora* sp1		Control	
Quercus spp.	I[1]	II[2]	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Q. bicolor	0.1[3] ± 0.4	1.8 ± 1.2*	0	0.6 ± 1.2*	0.2 ± 0.4*	0.4 ± 0.8	0.3 ± 0.5*	0	0.3 ± 0.5*	1 ± 1.1*	nt	0.3 ± 0.8	1 ± 0.8	nt	0	0
Q. macrocarpa	0.1 ± 0.4	2.1 ± 1.4*	2.7 ± 0.9*	2.0 ± 0.6*	0	0.4 ± 1	1.6 ± 1.3*	0.5 ± 0.9	2 ± 1.2*	1.1 ± 1.4	nt	0.4 ± 0.7	0.8 ± 0.9*	1.5 ± 1.4*	0	0
Q. montana	0	1.2 ± 1.3*	0.3 ± 0.5	3*	1.7 ± 1.2*	0	0.1 ± 0.3	1.1 ± 1.2*	0.8 ± 1.4	0	nt	0	3*	0.5 ± 0.5	0	0.1 ± 0.4
Q. palustris	0.2 ± 0.4	1.6 ± 0.9*	1.5 ± 0.8*	0	0.1 ± 0.4	2 ± 1.1*	0.4 ± 0.8	1.8 ± 1.5*	1.6 ± 1.1*	0.1 ± 0.4	nt	0.5 ± 0.8	1.4 ± 1*	nt	0	0.2 ± 0.5
Q. robur	1.2 ± 1.5	3*	2.2 ± 1.3*	2.3 ± 1.0*	1.4 ± 0.9*	0.9 ± 1.5	0	2.7 ± 1*	1.3 ± 0.8*	2.3 ± 1*	nt	2.4 ± 0.9*	1.9 ± 1.4*	nt	0	0.2 ± 0.5
Q. rubra	0	2.8 ± 0.9*	1.8 ± 1.3*	2.8 ± 0.6*	0	2.2 ± 1.2*	nt	3*	0	1.4 ± 1.4	nt	2.5 ± 0.9*	0.4 ± 0.7	2.4 ± 0.9*	0	0.1 ± 0.3

Isolates that produced significantly greater damages compared to the controls are marked with an asterisc (*, p = 0.05; Kruskal–Wallis test). nt, not tested.
[1]First inoculation period from April–August 2005.
[2]Second inoculation period from August–February 2006.
[3]Taproot infections were scored based on an index from 0 to 3 where: 0 = none to 25% lesion development; 1 = root lesion 25–50%, tap and lateral roots infected and localized necrotic spots visible; 2 = root lesion 50–75%, tap and all major lateral roots infected, dark sunken extending lesions on taproot; and 3 = lesion >75%, taproot infected and girdled up to the root collar.

usually caused the greatest root necrosis during the two inoculation periods as assessed by the lesion development index (Table 4). This species together with *Phytophthora* sp1 ranked as the most aggressive species during the spring, and similarly aggressive as *P. cambivora* during the fall inoculation period. A consistent pattern of pathogenicity from the summer period to the fall generally was not evident. For example, *P. cambivora* produced significant lesions during the fall inoculations, but usually none during the spring period (Table 4).

The tested isolates of *Phytophthora* were successfully re-isolated from the infested potting soil mix as well as from random chosen tap and fine roots. Recoveries of inoculated species were more successful from necrotic sections of infected taproots than from fine roots (data not shown). Some lesions on roots were formed with soil infestation trials inoculated with V8A but in no instances *Phytophthora* was isolated.

3.2 One-year-old seedling inoculations

Direct stem inoculation of the 1-year-old oak seedlings resulted in variable results that were highly dependant on the species of *Phytophthora* employed and the host inoculated. Of all the species, *Phytophthora* sp1 and *P. quercina*-like did not cause any lesions that differed significantly from the controls regardless the oak species inoculated (data not shown). This was in dramatic contrast to *P. cinnamomi* and *P. citricola* that produced the largest necrotic lesions of any of the species of *Phytophthora* during both inoculation periods. In general, when isolates produced lesions on a particular host, little variations existed among the lesion sizes (Table 3). The lesion sizes were also usually similar in size during the two inoculation periods and consistent (Table 3).

When oak species sustained damage, they usually did not vary significantly in their susceptibility to *Phytophthora* infection during either inoculation periods (Table 3). The most susceptible oak species during the two inoculation periods, as measured by the size of lesions produced, were *Q. montana* and *Q. rubra* (Table 3). A few seedlings of *Q. montana* were girdled when inoculated with *P. cambivora*, *P. cinnamomi*, *P. citricola* and *P. europaea*, but mortality of seedlings did not occur during the 2-month period. In the case of *Q. rubra*, some seedlings were killed by inoculation with *P. cambivora* and *P. europaea*. Mortality or girdling of stem did not occur with the other oaks tested.

When re-isolations from necrotic tissue were attempted, the recovery of *Phytophthora* sp1, *P. quercetorum* and *P. quercina*-like usually required several attempts and often was unsuccessful. This was especially true when no lesions were produced. In contrast, *P. citricola* and *P. cinnamomi* were consistently recovered at high rates when compared to other species (data not shown). No *Phytophthora* was isolated in any of the control treatments inoculated with V8A.

3.3 Two-year-old seedling inoculations

For the most part, the results using field-grown 2-year-old seedlings mirrored those obtained for the greenhouse-grown 1-year-old seedlings. However, necrotic lesions generally were larger with the 2-year-old seedlings (Table 3). Mortality of few seedlings only occurred when *Q. montana* was inoculated with *P. cambivora*, *P. cinnamomi* and *P. citricola* (data not shown). Girdling of seedlings was noted on *Q. alba*, *Q. rubra* and *Q. velutina* inoculated with *P. cinnamomi*, but mortality did not occur during the 2-month experimental period.

Phytophthora cinnamomi produced significantly larger lesions than the controls during both inoculation periods on all oak species (Table 3). However, lesion sizes were considerably reduced when inoculated in the fall. Likewise, *P. citricola* produced significant lesions during both inoculation periods on all oak species except *Q. palustris* and on

Q. rubra during the second inoculation period. For this species, lesion sizes remained similar during both inoculation periods and not different statistically (Table 3). *Phytophthora cambivora*, *P. europaea* and *P. quercetorum* were able to cause significantly larger lesions than the controls, but only during summer inoculation period (Table 3).

When species of *Phytophthora* were ranked for their ability to cause infections on a particular oak species, generally *P. cinnamomi* was the most aggressive species during summer inoculations whereas *P. cambivora*, *P. citricola*, *P. europaea* and *P. quercetorum* usually did not differ significantly from each other by the size of the lesions they produced (Table 3). During fall inoculations, *P. cinnamomi* and *P. citricola* were similarly aggressive and produced significantly larger lesions when compared to the controls, whereas all other tested species did not cause any lesions during the fall period (Table 3).

Overall, the 2-year-old seedlings were more susceptible to *Phytophthora* infection during summer period than during the fall ($p < 0.001$). When ranked for their susceptibility, *Q. montana* was a slightly more susceptible oak, but when aggressive isolates such as *P. cinnamomi* was considered, all oak species were similarly susceptible (Table 3).

3.4 Twenty-year-old tree inoculation

Symptoms on the 20-year-old trees after the 2-month inoculation period included the formation of epicormic sprouts and bleeding within the infected area. No changes in crown conditions were noted after the 2-month inoculation period.

Phytophthora cinnamomi was the most aggressive species and consistently caused lesions that were significantly different from controls on all oak species tested. The largest lesions also were produced by *P. cinnamomi* on *Q. alba* and *Q. montana* (Table 3). With *Q. rubra*, *P. citricola* was equally aggressive as *P. cinnamomi* and produced lesions similar in size. *Phytophthora europaea* caused significant lesions that were different from controls only when stems of *Q. robur* were inoculated. When *Q. rubra* was inoculated by all species of *Phytophthora*, only *P. europaea* and *Phytophthora* sp2 were unable to produce lesions that differed significantly from the controls (Table 3). *Quercus robur* was also significantly affected by three of the tested species of *Phytophthora*.

4 Discussion

The series of inoculations that were conducted demonstrated the unique variation that occurs when different isolates of *Phytophthora* and hosts were compared. Generally, some species were more pathogenic and some hosts were more susceptible to infection. In some experiments, the period of the year played a significant role in the development of lesions and host susceptibility.

The soil inoculations demonstrated that many of the species of *Phytophthora* can cause root damage. This was first noted when fine roots were evaluated. Because we did not separate roots as infected or healthy by visual evaluation, inclusion of infected or dead roots during the scanning progress certainly resulted in an underestimation of the amount of fine root mortality. Further, replacement of dead fine roots by healthy ones may have progressed at different rates for different oak seedlings, further obscuring the effect of inoculation on fine root mass during the two experiments. However, even though root scans of the total fine roots were unable to determine actual fine root mortality, they did suggest that infection by some of the species of *Phytophthora* may have significantly diminished the total mass of fine roots produced. A longer incubation period may have better demonstrated the actual degree of fine root mortality.

In contrast, visual evaluation of necrosis on taproots gave better indication of the potential of the tested *Phytophthora* species to cause root damage. This evaluation method also provided clear evidence of the potential of different species of *Phytophthora* to cause

necrosis on lateral and taproots, which ultimately could be detrimental to fine root production if the pathogen continues to colonize the tap and lateral roots. In *P. cinnamomi*-infested sites when isolations where attempted from roots of mature *Q. rubra* trees (>60 years), similar necrotic spots on roots commonly were observed, which some of them resulted in the isolation of *P. cinnamomi* (Y. Balci, unpublished data).

Seedlings produced more fine roots during the summer period than during the fall. This could be attributed to the warmer soil temperatures during the summer that are more conducive to root growth (Larson 1970). The fewer significant infections discovered during the summer period may indicate a better opportunity to overcome the infections caused by the species of *Phytophthora* due to increased production of healthy roots during this period. In contrast, the reduced root production during the fall inoculation period, and the greenhouse conditions, may have favoured the pathogen thereby resulting in greater root mortality during the fall. Root susceptibility may be markedly different during different seasons and influenced by factors such as the variation in the dynamics of fine root turnover and production of new secondary roots. When others have examined root health, susceptibility of oak roots to *Phytophthora* infection has been shown to be greater in nutrient-poor soils (Jönsson et al. 2003) and under prolonged stress factors such as drought or waterlogging (Shearer and Tippett 1989; Marçais et al. 1993; Robin et al. 2001; Sánchez et al. 2002).

Greenhouse and field inoculation of seedlings and trees demonstrated the pathogenicity of the species of *Phytophthora* to oak stems. Five out of the seven species proved pathogenic to the stems of oak species with *P. cinnamomi* and *P. citricola* consistently being the most aggressive. The seedling experiment in the greenhouse provided a reasonable estimate of the susceptibility of oak stem tissue and was supported by the inoculation of 2- and 20-year-old field-grown plants. Most species of *Phytophthora* that were tested on different age groups of the same host gave similar results relative to susceptibility. However, the lesions formed on 20-year-old trees were considerably larger when compared to lesions on 1- and 2-year-old seedlings, indicating that susceptibility of oak stem tissue to damage may increase with age. Increased susceptibility with tree age was also reported for *P. cinnamomi*-infected *Q. rubra* trees (Robin et al. 1992).

Our preliminary data provided support for a seasonal variation in susceptibility of stem tissue to *Phytophthora* infection as demonstrated with 2-year-old seedling experiments conducted in the field. In the field, variation in stem susceptibility to *Phytophthora* infection was already demonstrated for *Q. suber* (Luque et al. 2002), *Q. rubra* (Robin et al. 1994) or on other plants such as Eucalyptus (Shearer and Tippett 1989) and apple (Jeffers and Aldwinckle 1986).

The time of inoculation under field conditions was a significant factor relative to the size of lesions that were formed; lower late-season temperatures apparently restricted lesion development, so much so, that smaller or insignificant lesions were produced. However, under controlled conditions in the greenhouse, lesions remained similar in size. This suggests that seasonal changes may influence the aggressiveness of *Phytophthora* under field conditions. However, this generalization was not true for all species tested as was demonstrated with the 2-year-old seedling field trial with *P. citricola*. Lesions formed by this isolate remained similar in size and were not significantly different between the two inoculation periods. The ability of *P. citricola* to successfully infect the stem tissues even with lower late-season temperatures suggests a better adaptation to lower temperatures. Indeed, we have previously demonstrated the existence of *P. citricola* above the N 40° latitude range, which appears to be limiting to the range of *P. cinnamomi* (Balci et al. 2007). This further supports that the interaction between a host and *Phytophthora* can be influenced by environmental conditions as has been demonstrated *in situ* for *P. cinnamomi* on *Q. rubra* (Robin et al. 1994) and *Q. suber* seedlings (Luque et al. 2002).

Tested species of *Phytophthora* varied by their ability to successfully infect, colonize and cause necrosis of different tissue types. For instance, *P. quercina*-like and *Phytophthora* sp1 were pathogenic to root systems of oaks but not pathogenic to stem tissue. Similarly, *P. quercetorum* was weakly pathogenic to the stems of oak seedlings but more aggressive to the fine roots of seedlings. *Phytophthora quercina*, a common inhabitant of European oak forest soils, has been previously demonstrated to be pathogenic only towards roots of oaks but not to stem tissues of any oak species tested (Jung et al. 1996; Balci and Halmschlager 2003b; Bianco et al. 2003). Thus, these unknown isolates may be classified as root pathogens when artificially inoculated.

This study further confirms the potential pathogenicity of *P. cambivora*, *P. cinnamomi* and *P. citricola* isolates recovered from eastern US soils to roots and stems of several oak species. These species also were shown to be pathogenic on oaks in other seedling stem inoculations with variable levels of virulence (Jordan and Tainter 1996; Jung et al. 1996; Marçais et al. 1996; Robin and Desprez-loustau 1998; Tainter et al. 2000; Balci and Halmschlager 2003b; Bianco et al. 2003; Garbelotto and Hüberli 2006). *Phytophthora cinnamomi* and *P. citricola* are widely distributed throughout the world and are also known to cause stem lesions on oaks in forest settings (Mirchetich et al. 1977; Erwin and Ribeiro 1996; Robin et al. 1998; Tainter et al. 2000; Wood and Tainter 2002; Garbelotto and Hüberli 2006). However, *P. cambivora* is usually reported in association with diseases on *Castanea* sp. and *Fagus* sp., close relatives of oak (Vettraino et al. 2001, 2005; Balci and Halmschlager 2003b; Jung et al. 2005; Hartmann et al. 2006). Considering all stem-inoculation experiments, virulence of *P. cinnamomi* was similar to *P. citricola* and greater than *P. cambivora* and the other tested species of *Phytophthora*.

Phytophthora europaea was previously reported to be weakly pathogenic towards roots of *Q. robur* seedlings (Jung et al. 2002). However, in our experiments, *P. europaea* was highly pathogenic to *Q. robur* roots and moderately pathogenic to the stem and root system of other oaks. Variation in isolate pathogenicity has been demonstrated for *P. cinnamomi* (Robin and Desprez-loustau 1998; Linde et al. 1999), and might also exist among North American and European isolates of *P. europaea*. When classified based on lesion sizes on all stem inoculations, the aggressiveness of *P. europaea* during the experiments was similar to *P. cambivora*.

Quercus montana and *Q. rubra* ranked as the most susceptible oak species during stem inoculations of seedlings and trees. This finding corresponds to those when stem inoculations with *P. ramorum* were conducted on these two species, resulting in their ranking as highly susceptible species (Tooley and Kyde 2007). Other oak species did not express consistent susceptibility especially when a less aggressive isolate was used as inoculum. However, when virulent isolates such as *P. cinnamomi* and *P. citricola* was inoculated, little or no variation existed in susceptibility of seedlings or older plants.

Despite the fine root loss and taproot infection that occurred with some oak seedlings, no mortality or noticeable dieback symptoms of aboveground parts of seedlings were visible during any of the soil-inoculation experiments. The extent of root loss for a particular oak seedling that leads to aboveground symptom development and mortality is not known, but appears to be over 55%, as no mortality occurred below this value in our study. In other studies, an adequate nutrient uptake was recorded with an average fine root mortality of 26% on *Q. robur* seedlings inoculated with *P. quercina* (Jönsson et al. 2003; Jönsson 2004), and mortality only occurred on *Q. ilex* seedlings after about 67% root loss when inoculated with *P. cinnamomi* (Maurel et al. 2001). This may also support the field observations where direct associations of crown symptoms with the presence of the species of *Phytophthora* in forest soils could not be demonstrated (Balci et al. 2007). Similarly, in European oak forest soils, despite the presence of various species of *Phytophthora*, a direct association with decline was not demonstrable but only shown to be circumstantial

(Brasier et al. 1993; Robin et al. 1998; Gallego et al. 1999; Jung et al. 2000; Hartmann and Blank 2002; Sánchez et al. 2002; Vettraino et al. 2002; Balci and Halmschlager 2003a,b; Jönsson et al. 2005; Jonsson-Belyazio and Rosengren 2006). Root infection was suggested to be localized and progressive under natural conditions (Shearer and Tippett 1989; Crombie and Tippett 1990), hence trees can suffer root loss for a number of years with a progressive infection but the involvement of other detrimental biotic or abiotic factors may be required before aboveground symptoms are expressed or death occurs. A better understanding of the impact of low levels of root infection on plant health and the dynamics of a totally healthy root system under forest settings is necessary to determine the impact of *Phytophthora* on mature trees.

We have demonstrated that the seven species of *Phytophthora* isolated from oak forest soils can be pathogenic to oak stem and root tissue when introduced artificially. Because of the limiting effect of cold winter temperatures in eastern and north-central USA, stem lesions may be significantly influenced by late-season temperatures and the tested species likely restricted to root parts of the plant. Therefore, the susceptibility of an oak species needs to be evaluated over extended time periods so that the environmental components that influence the aggressiveness of these organisms and host susceptibility under field conditions can be evaluated. Determining the long-term effects of *Phytophthora* on tree health under natural settings remains a difficult task.

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References

Balci, Y.; Halmschlager, E., 2003a: Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. For. Pathol. **33,** 157–174.

Balci, Y.; Halmschlager, E., 2003b: *Phytophthora* species in oak ecosystems in Turkey and their association with declining oak trees. Plant Pathol. **52,** 694–702.

Balci, Y.; Balci, S.; Eggers, J.; MacDonald, W. L.; Juzwik, J.; Long, R. P.; Gottschalk, K. W., 2007: *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. **91,** 705–710.

Balci, Y.; Balci, S.; Blair, J. E.; Park, S.-Y.; Kang, S.; MacDonald, W. L. 2008: *Phytophthora quercetorum* sp. nov., a novel species isolated from eastern and north-central U.S. oak forest soils. Mycol. Res.; in press, doi: 10.1016/j.mycres.2008.02.008

Bianco, M. C.; DiBrisco, D.; Luisi, N.; Lerario, P., 2003: Pathogenicity of *Phytophthora* species on *Quercus* seedlings. In: Phytophthora in Forests and Natural Ecosystems. 2nd Int. IUFRO Working Party 7.02.09 Meeting, Albany, W. Australia, September 30–October 5, 2001. Ed. by McComb, J. A.; StJ Hardy, G. E.; Tommerup, I. C. (Murdoch Univ. Print), pp. 148–153.

Brasier, C. M.; Robredo, F.; Ferraz, J. F. P., 1993: Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathol. **42,** 140–145.

Campbell, W. A.; Hendrix, F. F., 1967: *Pythium* and *Phytophthora* species in forest soils in the southeastern United States. Plant Dis. Rep. **51,** 929–932.

Crandall, B. S.; Gravatt, G. F.; Ryan, M. M., 1945: Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology **35,** 162–180.

Crombie, S. D.; Tippett, J. T., 1990: A comparison of water relations, visual symptoms, and changes in stem girth for evaluating impact of *Phytophthora cinnamomi* dieback on *Eucalyptus marginata*. Can. J. For. Res. **20,** 233–240.

Delatour, C., 2003: Phytophthoras and Oaks in Europe. In: Phytophthora in Forest and Natural Ecosystems. 2nd Int. IUFRO Working Party 7.02.09 Meeting, Albany, W. Australia, September 30–October 5, 2001. Ed. by McComb, J. A.; StJ Hardy, G. E.; Tommerup, I. C. (Murdoch Univ. Print), pp. 78–88.

Erwin, C. D.; Ribeiro, O. K., 1996: Phytophthora Diseases World-Wide. St Paul, MN, USA: American Phytopathological Society Press, 562 pp.

Gallego, F. J.; Perez de Albaga, A.; Fernandez-Escobar, R., 1999: Etiology of oak decline in Spain. Eur. J. For. Path. **29,** 17–27.

Garbelotto, M.; Hüberli, D., 2006: First report on an infestation of *Phytophthora cinnamomi* in natural oak woodlands of California and its different impact on two native oak species. Plant Dis. **90,** 685.

Hansen, E. M.; Delatour, C., 1999: *Phytophthora* species in oak forests of north-east France. Ann. For. Sci. **56,** 539–547.

Hartmann, G.; Blank, R., 2002: Occurrence and site relations of *Phytophthora* spp. in declining oak stands in north-western Germany. Forst. Holz. **57,** 539–545.

Hartmann, G.; Blank, R.; Kunca, A., 2006: Collar rot of *Fagus sylvatica* caused by *Phytophthora cambivora*: damage, site relations and susceptibility of broadleaf hosts. Progress in Research on *Phytophthora* Diseases of Forest Trees. In: Third Int. IUFRO Working Party S07.02.09 Meeting, Freising, Germany, September 11–18, 2004. Ed. by Brasier, C.; Jung, T.; Osswald, W. (Published by Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK), pp. 135–138.

Holmes, K. A.; Benson, D. M., 1994: Evaluation of *Phytophthora parasitica* var. *nicotianae* for biocontrol of *Phytophthora parasitica* on *Catharanthus roseus*. Plant Dis. **78,** 193–199.

Jeffers, S. N.; Aldwinckle, H. S., 1986: Seasonal variation in extent of colonization of two apple rootstocks by five species of *Phytophthora*. Plant Dis. **70,** 941–945.

Jönsson, U., 2004: *Phytophthora* species and oak decline – can a weak competitor cause significant root damage in a nonsterilized acidic forest soil? New Phytol. **162,** 211–222.

Jönsson, U.; Jung, T.; Rosengren, U.; Nihlgard, B.; Sonesson, K., 2003: Pathogenicity of Swedish isolates of *Phytophthora quercina* to *Quercus robur* in two different soils. New Phytol. **158,** 355–364.

Jönsson, U.; Jung, T.; Sonesson, K.; Rosengren, U., 2005: Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathol. **54,** 502–511.

Jonsson-Belyazio, U.; Rosengren, U., 2006: Can *Phytophthora quercina* have a negative impact on mature pedunculate oaks under field conditions? Ann. For. Sci. **63,** 661–672.

Jordan, A. P.; Tainter, F. H., 1996: The susceptibility of southern Appalachian oaks to *Phytophthora cinnamomi*. Castanea **61,** 348–355.

Jung, T.; Blaschke, H.; Neumann, P., 1996: Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur. J. For. Path. **26,** 253–272.

Jung, T.; Blaschke, H.; Osswald, W., 2000: Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant Pathol. **49,** 706–718.

Jung, T.; Hansen, E. M.; Winton, L.; Osswald, W.; Delatour, C., 2002: Three new species of *Phytophthora* from European oak forests. Mycol. Res. **106,** 397–411.

Jung, T.; Hudler, G. W.; Jensen-Tracy, S. L.; Griffiths, H. M.; Fleischmann, F.; Osswald, W., 2005: Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. Mycologist **19,** 159–166.

Larson, M. M., 1970: Root regeneration and early growth of red oak seedlings: influence of soil temperature. For. Sci. **16,** 442–446.

Linde, C.; Kemp, G. H. J.; Wingfield, M. J., 1999: Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*. Eur. J. Plant Pathol. **105,** 231–239.

Luque, J.; Parladé, J.; Pera, J., 2002: Seasonal changes in susceptibility of *Quercus suber* to *Botryosphaeria stevensii* and *Phytophthora cinnamomi*. Plant Pathol. **51,** 338–345.

Marçais, B.; Dupuis, F.; Desprez-Loustau, M. L., 1993: Influence of water stress on susceptibility of red oak (*Quercus rubra*) to *Phytophthora cinnamomi*. Eur. J. For. Path. **23,** 295–305.

Marçais, B.; Dupuis, F.; Desprez-Loustau, M. L., 1996: Susceptibility of the *Quercus rubra* root system to *Phytophthora cinnamomi*; comparison with chestnut and other species. Eur. J. For. Path. **26,** 133–143.

Maurel, M.; Robin, C.; Capron, G.; Desprez-Loustau, M. L., 2001: Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulations, mineral

nutrition and vulnerability to water deficit of five oak and chestnut species. For. Pathol. **31,** 353–369.

McShea, J. W.; Healy, W. M., 2002: Oak Forest Ecosystems. Ecology and Management for Wildlife. Baltimore, MD, USA: The Johns Hopkins Univ. Press, p. 432, ISBN 0-8018-6745-2.

Mirchetich, S. M.; Campbell, R. N.; Matheron, M. E., 1977: *Phytophthora* trunk canker of coast live oak and cork oak trees in California. Plant Dis. Rep. **61,** 66–70.

Moreira, A. C.; Ferraz, J. F. P.; Clegg, J., 2000: The involvement of *Phytophthora cinnamomi* in cork and holm oak decline in Portugal. In: First Int. Meeting on Phytophthoras in Forest and Wildland Ecosystems, Grand Pass, OR, USA, August 30–September 3, 1999. Ed. by Hansen, E. M.; Sutton, W., pp. 132–135.

Rizzo, D. M.; Garbelotto, M.; Davidson, J. M.; Sloughter, G. W.; Koike, S. T., 2002: *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis. **86,** 205–214.

Robin, C.; Desprez-loustau, M. L., 1998: Testing variability in pathogenicity of *Phytophthora cinnamomi*. Eur. J. Plant Pathol. **104,** 465–475.

Robin, C.; Desprez-Loustau, M. L.; Delatour, C., 1992: Factors influencing the enlargement of trunk cankers of *Phytophthora cinnamomi*. Can. J. For. Res. **22,** 367–374.

Robin, C.; Dupuis, F.; Desprez-loustau, M. L., 1994: Seasonal changes in northern red oak susceptibility to *Phytophthora cinnamomi*. Plant Dis. **78,** 369–374.

Robin, C.; Desprez-Loustau, M. L.; Capron, G.; Delatour, C., 1998: First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Ann. Sc. For. **55,** 869–883.

Robin, C.; Capron, G.; Desprez-Loustau, M. L., 2001: Root infection by *Phytophthora cinnamomi* in seedlings of three oak species. Plant Pathol. **50,** 708–716.

Sánchez, M. E.; Caetano, P.; Ferraz, J.; Trapero, A., 2002: *Phytophthora* disease of *Quercus ilex* in south-western Spain. For. Pathol. **32,** 5–18.

SAS, 2002: JMP Statistics and Graphics Guide: Version 5. Cary, NC: SAS Institute, Inc., 707 pp.

Shearer, B. L.; Tippett, J. T., 1989: Jarrah Dieback: the dynamics and management of *Phytophthora cinnamomi* in the Jarrah (*Eucalyptus marginata*) forest of south-western Australia. CALM Res. Bulletin No.3, 76 pp.

Tainter, E.; O'Brien, G. J.; Hernandez, A.; Orozco, F.; Rebolledo, O., 2000: *Phytophthora cinnamomi* as a cause of oak mortality in the State of Colima, Mexico. Plant Dis. **84,** 394–398.

Tooley, P. W.; Kyde, K. L., 2007: Susceptibility of some eastern forest species to *Phytophthora ramorum*. Plant Dis. **91,** 435–438.

Venette, R. C.; Cohen, S. D., 2006: Potential climatic suitability for establishment of *Phytophthora ramorum* within the contiguous United States. For. Ecol. Manage. **231,** 18–26.

Vettraino, A. M.; Natili, G.; Anselmi, N.; Vannini, A., 2001: Recovery and pathogenicity of *Phytophthora* species associated with a resurgence of ink disease in *Castanea sativa* in Italy. Plant Pathol. **50,** 90–96.

Vettraino, A. M.; Barzanti, G. P.; Bianco, M. C.; Ragazzi, A.; Capretti, P.; Paoletti, E.; Luisi, N.; Anselmi, N.; Vannini, A., 2002: Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. For. Pathol. **32,** 19–28.

Vettraino, A. M.; Morel, O.; Perlerou, C.; Robin, C.; Diamandis, S.; Vannini, A., 2005: Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with ink disease and crown decline. Eur. J. Plant Pathol. **111,** 169–180.

Wood, A. K.; Tainter, F. H., 2002: First report of *Phytophthora cinnamomi* on *Quercus laurifolia*. Plant Dis. **86,** 441.