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Foliar susceptibility of eastern oak species to *Phytophthora* infection

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Summary

Seven different *Phytophthora* species were used to test the foliar susceptibility of the common eastern US oak species and understory plants to *Phytophthora* infection. The *Phytophthora* species employed were *Phytophthora cambivora*, *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora europaea*, *Phytophthora quercetorum*, *Phytophthora quercina*-like and *Phytophthora* sp1. Inoculation of detached-leaves with agar plugs containing mycelia of *Phytophthora* provided an estimate of their relative susceptibility. Lesions were always greater when foliage was wounded and young. On deciduous plants, lesion sizes were considerably reduced with the increasing foliar age, although with evergreen plants lesion sizes remained similar regardless of foliar age when more aggressive isolates were tested. Infections seldom resulted when foliage was not wounded. With young and mature foliage, *P. citricola* usually produced the largest lesions. Young foliage of *Quercus rubra* was the most susceptible to infection followed by *Castanea dentata* for both wounded and non-wounded inoculations. Mature foliage of *Hamamelis virginiana*, *Kalmia latifolia* and *Quercus alba* were the most susceptible to wound and non-wound inoculations.

1 Introduction

Phytophthora species have been usually associated with root and crown rot and stem lesions of woody plants. However, in temperate forests, their ability to infect foliage of woody hosts is not well known. Until recently, our knowledge of foliar infections was limited to a few *Phytophthora* species such as *Phytophthora citricola* Sawada, *Phytophthora citrophthora* (R.E. Smith & E.H. Smith) Leonian, *Phytophthora cactorum* (Leb. & Cohn) Schröeter or *Phytophthora ilicis* Buddenhagen & Young that can cause leaf and twig blights (Erwin and Ribeiro 1996). In Europe and the USA, surveys of *Phytophthora ramorum* Werres, DeCook & Man in't Veld directed attention to foliar lesions caused by *Phytophthora* infection. These surveys targeted ornamental plants as well as forest trees and demonstrated that a variety of other *Phytophthora* species besides *P. ramorum* were associated with necroses of foliage and twigs. These included *Phytophthora nemorosa* Hansen & Reeser, *Phytophthora pseudosyringae* Jung & Delatour, *Phytophthora foliarum* Donahoo & Lamour and *Phytophthora kernoviae* Brasier, Beales & Kirk (Hansen et al. 2003; Brasier et al. 2005; Donahoo et al. 2006; Wickland and Rizzo 2006). In addition to *P. ramorum*, 13 species of *Phytophthora* were found associated with foliar blights of ornamentals in California; *P. citricola* and *Phytophthora syringae* (Klebahn) Klebahn were the most frequently encountered (Yakabe et al. 2007). In New Zealand, *Phytophthora captiosa* Dick & Dobbie and *Phytophthora fallax* Dobbie & Dick were associated with leaf spots, petiole infection and twig and small branch infection of *Eucalyptus* trees (Dick et al. 2006). Other new associations have included *Phytophthora hibernalis* Carne and

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Phytophthora hedraiandra de Cock & Man in't Veld causing leaf spots and twig blights of Rhododendrons and Viburnum, respectively (Schwingle et al. 2006; Álvarez et al. 2007; Moralejo et al. 2007).

The demonstrated associations of *Phytophthora* species with foliar infections raised concerns about the susceptibility of different plant species to *Phytophthora* spp. Because of the quarantine restrictions, testing a variety of native plants for susceptibility to exotic *Phytophthora* species cannot be performed at most research institutions because of the necessity of using expensive bio-security containment facilities. Thus, we tested seven species of *Phytophthora*, commonly recovered from oak forests in the eastern USA (Balci et al. 2007), for their ability to infect foliage of common oak species in the eastern USA and their use to assess foliar susceptibility to *Phytophthora* infection.

2 Materials and methods

2.1 Plant material and isolates tested

The isolates used in this study are listed in Table 1 and were randomly selected among the representative isolates of each species. Isolates of *Phytophthora cinnamomi* Rands and *Phytophthora cambivora* (Petri) Buisman were of the A2 compatibility type as determined by matings with known A1 and A2 compatibility-type isolates. The *Phytophthora quercina*-like isolates matched *P. quercina* by sequence data; however, they differed from *P. quercina* culturally in oogonial features, growth pattern on PDA and much slower growth rate. Similarly, the classification of *Phytophthora* sp1 via morphological features did not warrant its identification as *Phytophthora europaea*, although sequence data was closest to *P. europaea* (Balci et al. 2007). Oak species used for foliar inoculation experiments included *Quercus alba* L., *Quercus bicolor* Willdenow, *Quercus imbricaria* Michaux, *Quercus macrocarpa* Michaux, *Quercus montana* Willdenow, *Quercus palustris* Muenchhausen, *Quercus rubra* L.; *Quercus shumardii* Buckley and *Quercus velutina* Lamarck. English oak (*Quercus robur* L.) was also included as an exotic species. In addition to the oaks, foliage of *Castanea dentata* (Marsh.) Borkh. and three common understory plant species in oak-dominated ecosystems were tested; *Hamamelis virginiana* L., *Rhododendron maximum* L. and *Kalmia latifolia* L.

2.2 Foliar inoculations

Inoculation tests were conducted using detached foliage from greenhouse-grown seedlings. When greenhouse-grown foliage was unavailable, leaves were collected from mature plants in the forest including foliage of *R. maximum*, *K. latifolia* and *H. virginiana*. Foliage used in a particular test was always sampled at one time, and special attention was paid to collect similar size leaves from the similar positions of the seedling or tree. Because susceptibility

Table 1. **Isolates used in this study**

Phytophthora	Isolate	Host	Location	ATCC No.
Phytophthora cambivora	OH 4/4	*Quercus velutina*	Ohio	MYA-4089
Phytophthora cinnamomi	WV Gr1/1	*Quercus rubra*	West Virginia	MYA-4085
Phytophthora citricola	OH 6/5	*Q. rubra*	Ohio	MYA-4087
Phytophthora europaea	WV BM 1/10	*Quercus alba*	West Virginia	MYA-4088
Phytophthora quercina-like	MN 023	*Q. rubra*	Minnesota	MYA-4090
Phytophthora sp1	WV BSC 1/3	*Q. rubra*	West Virginia	MYA-4091
Phytophthora quercetorum	MD 9/2	*Q. rubra*	Maryland	MYA-4186

All *Phytophthora* species were isolated from oak forest soils in the eastern USA.
ATCC, American Type Culture Collection.

of foliage has been reported to differ with leaf age (Denman et al. 2005b; Hansen et al. 2005), two age categories were selected: Age 1 (referred as young foliage) included leaves that were fully developed but no more than 3 months old; age 2 (referred as mature foliage) included leaves that were 3–6 months old. Inoculations were conducted in 2005 and completed in 2006. Young foliage was inoculated from March to June, and mature foliage from July to October. A modification of the inoculation method described by Buddenhagen and Young (1957) was used. On an average, a minimum of 20 detached-leaves were inoculated in each age class (young and mature) and treatment type (wound and non-wound), and each inoculation was repeated twice giving over 13 400 foliar inoculations. At each leaf age-category wound and non-wound treatments were employed. In total, two wounds of ca. 1 cm long were made perpendicular to the mid-vein and on abaxial side of foliage using a sterile scalpel, and an inoculum was placed on the wound with the mycelium facing the wound. For the non-wound treatment, discs of *Phytophthora* mycelia were simply placed on the leaf. The inoculum consisted of a 6-mm diameter agar disc containing mycelia cut from 7-day-old cultures of the *Phytophthora* species grown on V8 juice agar (V8A). Controls received sterile V8A discs. Inoculated leaves were incubated in plastic storage boxes ($55 \times 38 \times 13$ cm) with wet vermiculate covered with aluminium foil in the bottom to maintain high humidity. Boxes containing the inoculated foliage were sprayed thoroughly with sterile distilled water so that water drops accumulated all over the box and on the inoculated foliage. Foliage was incubated for 7 days at 20°C (±1°C) in darkness. After incubation, each leaf was scanned on a flatbed scanner, and the necrotic area resulting from the two inoculation points was quantified as a percentage of each total leaf area by using the software package ASSESS (Lamari 2002). When average necrotic areas were calculated, foliage without necrotic areas were also included. To confirm infection, re-isolations were made by transferring outer necrotic portions of inoculated leaves to a PARPNH selective medium (Balci et al. 2007).

2.3 Statistical analysis

Data sets were checked for normality and equal variance distributions and transformed when necessary. Analysis of variance was applied to the log-transformed data. Tukey–Kramer honestly significant difference (HSD) test was used to separate the means and to rank the oak species and *Phytophthora* species for their susceptibility or aggressiveness, respectively. Effect leverage test was used to test the significance of foliar age and treatment type on lesion development. Analyses of all data were performed using JMP® 5.0 software (SAS Institute, Inc., Cary, NC, USA).

3. Results

When species were susceptible, lesion development progressed rapidly during the 7-day incubation period, and lesions generally were not uniform in shape. With few exceptions, young foliage was routinely more susceptible. Symptoms on susceptible leaves included water-soaking and a light yellow-to-brown discoloration and wilting-like symptoms. On less susceptible leaves, lesions were more restricted and dark coloured.

There were always replicates among either the significant or insignificant isolate–foliage combinations that produced no lesions. No discolorations were observed on foliage of control treatments and for most non-wounded inoculations.

Because there was little variation in the size of the foliage used in each experiment, necrotic leaf areas were not corrected for leaf size. Re-isolation of the *Phytophthora* species from inoculated foliage was successful and the isolation frequency ranged from 80 to 100%, thus no data are presented regarding re-isolation frequency. *Phytophthora* never was recovered from any of the control inoculations.

3.1 Young foliage inoculations

In the absence of wounding, inoculation of young foliage generally did not result in lesions significantly different from the controls (Table 2). For the non-wounded inoculations, the largest infections occurred on *Q. rubra* and the smallest lesions that were significantly larger than the controls were produced on *Q. robur* using *P. quercetorum* Balci & Balci (Table 2). When young non-wounded foliage was ranked for its susceptibility, the largest necrotic areas occurred with *Q. rubra* followed by *C. dentata* (Table 2). Other species did not differ in susceptibility and when lesions formed, usually they were not significantly different than the controls (Table 2). Based on the lesion areas produced, *P. cinnamomi* was the most aggressive species followed by *P. cambivora* and *P. citricola* in the absence of wounding (Table 2). *Phytophthora quercina*-like and *Phytophthora* sp1 did not produce infections that were significantly different than the controls whether foliage was wound or non-wound inoculated (Table 2).

In contrast, wounding was a significant factor for infection of some species ($p < 0.001$), and the lesions that resulted routinely were greater than those observed in the non-wounded treatments or the controls (Table 2). The exceptions were when young foliage of *Q. rubra* was inoculated with *P. cambivora*, *P. cinnamomi*, *P. citricola* or *P. europaea* Hansen & Jung; for these combinations, lesion sizes were similar whether or not the foliage was wounded (Table 2). With wound inoculations, *P. citricola* usually produced the largest lesions (Table 2). This was particularly evident when the foliage of *C. dentata* and to a lesser extent when *Q. rubra* was inoculated using isolates of *P. citricola* and *P. cambivora* (Table 2). The smallest lesion sizes, which were significantly larger than the controls, were obtained using *P. cambivora* on *K. latifolia* foliage. As with the non-wound treatments, when young wounded foliage was ranked for its susceptibility, the largest necrotic areas occurred with *Q. rubra* and *C. dentata* (Table 2). Little variation in foliar susceptibility existed among the foliage of other plants during the wound inoculations (Table 2).

3.2 Mature foliage inoculations

Compared with young foliage, mature foliage was considerably less susceptible to *Phytophthora* infection whether wounded or not. In one instance, lesions that differed significantly in size from the respective controls were formed only on mature foliage of *Q. alba* when it was wounded (Table 3).

In the absence of wounding, *Phytophthora* species failed to cause lesions that differed from the controls on any of foliage except when *Q. alba*, *C. dentata* and *H. virginiana* were inoculated with *P. cambivora*, *P. cinnamomi* and *P. citricola* (Table 3). When mature foliage was ranked for susceptibility during non-wound inoculations, *H. virginiana* followed by *Q. alba* were the most susceptible species based on the necroses produced (Table 3). No variations existed in susceptibility of all the other plants as no lesions were produced that were significantly larger than the controls (Table 3). When lesions were produced, *P. citricola* was the most aggressive species and produced the largest lesions with the non-wounded treatments (Table 3). *Phytophthora europaea*, *P. quercetorum*, *P. quercina*-like and *Phytophthora* sp1 did not cause any lesions that were significantly larger than the controls for the non-wound treatments.

As with the inoculation of young foliage, wounding significantly affected the infection of mature foliage ($p < 0.001$), and when lesions formed, they usually were greater in size than those observed in the non-wounded treatments or the controls (Table 3). The largest infections that differed significantly from the controls occurred on *H. virginiana* followed by *K. latifolia* and *Q. alba* (Table 3). The smallest lesions, which were significantly different than the controls, formed on leaves of *Q. alba*. When foliage were ranked for their susceptibility, *H. virginiana*, *K. latifolia* and *Q. alba* were the most susceptible. Little

Table 2. **Average necrotic leaf area (%) ± SD of various oak (*Quercus* sp.) and understory plant species (*Rhododendron*, *Hamamelis*, *Kalmia* and *Castanea*) following wound and non-wound inoculation of young foliage**

	Phytophthora cambivora		*Phytophthora cinnamomi*		*Phytophthora citricola*		*Phytophthora europaea*	
Quercus spp.	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound
Q. alba	0.9 ± 1.3 cd[1] B[2]	0.4 ± 1 ac B	1.3 ± 0.9 bc C	1 ± 1.4 a B	3.9 ± 3.9 ab D	0.6 ± 0.7 ab B	4.6 ± 9.4 a B	0.4 ± 0.8 a–c B
Q. bicolor	8.1 ± 17 a B	0.5 ± 0.7 ab B	4.7 ± 12 ab BC	1.3 ± 3.4 a B	0.3 ± 0.3 c D	0.0 ± 0.1 b B	2.9 ± 6.9 a–c B	0.3 ± 0.6 ab B
Q. imbricaria	0.1 ± 0.3 b B	0 a B	0.2 ± 0.4 b	0 a B C	0.1 ± 0.2 b D	0 a B	0.1 ± 0.2 b B	0 a B
Q. montana	4.8 ± 9.9 a B	1.4 ± 5.3 a B	0.5 ± 0.9 bc C	0.6 ± 1.1 ab B	1.4 ± 1.1 b D	0.2 ± 0.3 ab B	1.6 ± 2.6 b B	0.6 ± 1.9 ab B
Q. palustris	0.5 ± 0.7 b B	0.1 ± 0.1 b B	0.5 ± 0.8 b C	0.3 ± 0.1 ab B	1.3 ± 0.9 a D	0.6 ± 0.1 a B	0.3 ± 0.6 b B	0.0 ± 0.1 b B
Q. robur	3.7 ± 2.6 b B	0.5 ± 1.9 cd B	1.5 ± 0.8 c C	0.9 ± 1.9 ab B	6.6 ± 6.1 a CD	1 ± 0.5 a B	0.9 ± 1.2 d B	0.0 ± 0.1 d B
Q. rubra	30 ± 26 a A	29 ± 31 a A	21 ± 31 ab A	16 ± 27 ab A	16 ± 19 bc B	8.2 ± 17 cd A	13 ± 24 bc A	13 ± 21 bc A
Q. velutina	7.5 ± 12 a B	1.2 ± 3.5 a B	1.1 ± 0.5 bc C	0.6 ± 0.8 ab B	3.2 ± 2.3 b D	0.4 ± 0.7 ab B	1.1 ± 3.2 cd B	0.0 ± 0.0 ab B
Castanea dentata	7 ± 11.4 bc B	2.7 ± 5.2 bc B	14 ± 24 b AB	3.3 ± 6.5ab B	38 ± 23 a A	7.7 ± 14 a A	6.3 ± 8.7 bc AB	3.3 ± 2.2 ab B
Kalmia latifolia	0.5 ± 0.6 de B	0.0 ± 0.0 b B	4.8 ± 1.8 b BC	1.7 ± 1.6 a B	14 ± 6.5 a BC	0.0 ± 0.0 b B	1.1 ± 1.1 c B	0.0 ± 0.0 b B
Rhododendron maximum	0.6 ± 0.6 c B	0.0 ± 0.0 a B	2.7 ± 2.5 ab BC	0.2 ± 0.7 a B	3.4 ± 2.4 a D	0.0 ± 0.0 a B	0.2 ± 0.4 cd B	0.0 ± 0.0 a B
Hamamelis Virginiana	1.1 ± 0.7 bc B	0.0 ± 0.0 b B	2.1 ± 0.9 b BC	0.1 ± 0.2 b B	15.4 ± 4.5 a BC	0.8 ± 1.2 a B	0.0 ± 0.0 c B	0.0 ± 0.0 b B
	Phytophthora quercetorum		*Phytophthora quercina*-like		*Phytophthora* spl		Control	
Q. alba	1.3 ± 1.9 bc A–C	0.2 ± 0.4 bc B	0.6 ± 0.8 cd A	0.3 ± 0.9 bc A	0.4 ± 0.4 cd B	0.0 ± 0.1 c AB	0.4 ± 0.5 d	0.2 ± 0.4 bc
Q. bicolor	1.7 ± 4 bc AB	0.2 ± 0.4 ab B	0.6 ± 0.7 bc AB	0.1 ± 0.3 ab A	0.8 ± 1.1 bc B	0.4 ± 0.9 ab AB	0.5 ± 0.4 bc	0.3 ± 0.3 ab

Table 2. ***Continued***

Quercus spp.	*Phytophthora quercetorum*		*Phytophthora quercina*-like		*Phytophthora* spl		Control	
	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound
Q. imbricaria	0.6 ± 0.2 a BC	0 a B	0.0 ± 0.0 b C	0 a A	0.1 ± 0.2 b B	0 a AB	0.0 ± 0.0 b	0 a
Q. montana	0.0 ± 0.1 c C	0.0 ± 0.0 b B	0.2 ± 0.4 c BC	0.0 ± 0.0 b A	0.1 ± 0.2 c B	0.0 ± 0.2 ab AB	0.3 ± 0.3 c	0.1 ± 0.2 ab
Q. palustris	0.3 ± 0.5 b C	0.2 ± 0.1 b B	0.3 ± 0.5 b A–C	0.0 ± 0.1 b A	0.4 ± 0.6 b B	0.0 ± 0.1 b AB	0.2 ± 0.4 b	0.1 ± 0.1 b
Q. robur	1.8 ± 1.3 c AB	0.5 ± 0.7 bc B	0.0 ± 0.1 e C	0.0 ± 0.0 d A	0.0 ± 0.0 e B	0.0 ± 0.0 d B	0.0 ± 0.1 e	0.0 ± 0.0 d
Q. rubra	2.6 ± 1.9 cd A	1.4 ± 2.4 de A	0.6 ± 0.8 de AB	0.3 ± 0.7 e A	0.8 ± 1.1 de A	0.8 ± 0.3 e A	0.1 ± 0.4 e	0.0 ± 0.1 e
Q. velutina	0.2 ± 0.3 d BC	0.0 ± 0.0 ab B	0.1 ± 0.3 d C	0.0 ± 0.0 ab A	0.2 ± 0.4 cd B	0.0 ± 0.0 ab AB	0.2 ± 0.3 d	0.0 ± 0.0 ab
C. dentata	1.1 ± 1.4 de A–C	0.0 ± 0.1 c B	0.0 ± 0.0 e C	0.0 ± 0.0 c A	0.1 ± 0.4 e B	0.1 ± 0.3 c AB	0.5 ± 1 de	0.1 ± 0.2 c
K. latifolia	0.8 ± 1.2 cd BC	0.0 ± 0.0 b B	0.0 ± 0.0 f C	0.0 ± 0.0 b A	0.1 ± 0.3 ef B	0.0 ± 0.0 b AB	0.0 ± 0.1 f	0.0 ± 0.0 b
R. maximum	1.3 ± 1.2 bc A–C	0.0 ± 0.0 a B	0.0 ± 0.0 d C	0.0 ± 0.0 a A	0.0 ± 0.0 d B	0.0 ± 0.0 a AB	0.0 ± 0.0 d	0.0 ± 0.0 a
H. virginiana	0.0 ± 0.0 c BC	0.0 ± 0.0 b B	0.1 ± 0.2 c BC	0.0 ± 0.0 b A	0.0 ± 0.0 c B	0.0 ± 0.0 b AB	0.0 ± 0.0 c	0.0 ± 0.0 b

[1]Significant differences among the *Phytophthora* species within a single oak species. Isolates with the same letter do not differ significantly from each other according to Tukey's test at p = 0.05.
[2]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 at p = 0.05.

Table 3. **Average necrotic leaf area (%) ± SD of various oak (*Quercus* sp.) and understory plant species (*Rhododendron*, *Hamamelis*, *Kalmia* and *Castanea*) following wound and non-wound inoculation of mature foliage**

	Phytophthora cambivora		*Phytophthora cinnamomi*		*Phytophthora citricola*		*Phytophthora europaea*	
Quercus spp.	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound
Q. alba	1.2 ± 1.9 bc[1] AB[2]	0.8 ± 1.1 a A	0.9 ± 0.8 bc CD	0.4 ± 1 ab A	4.1 ± 3.9 a B	0.5 ± 0.8 ab B	1.8 ± 1.7 b A	0.0 ± 0.0 bc B
Q. bicolor	0 a C	0 a B	0 a E	0 a C	0 a C	0 a B	0 a D	0 a B
Q. imbricaria	0.0 ± 0.0 a C	0 a B	0.0 ± 0.0 a E	0 a C	0.0 ± 0.0 a C	0 a B	0.0 ± 0.0 a D	0 a B
Q. montana	0.0 ± 0.1 b C	0 a B	0.2 ± 0.4 ab DE	0.0 ± 0.1 a C	0.6 ± 0.8 a C	0.0 ± 0.2 a B	0.0 ± 0.0 b D	0 a B
Q. palustris	0.0 ± 0.0 a C	0 a B	0.0 ± 0.0 a E	0 a C	0.1 ± 0.3 a C	0 a B	0.0 ± 0.0 a D	0 a B
Q. robur	0.2 ± 0.3 bc C	0 a B	0.3 ± 0.5 bc E	0 a C	2.1 ± 1.1 a BC	0 a B	0.1 ± 0.4 bc D	0 a B
Q. rubra	0.7 ± 2.5 ab BC	0 a B	0.2 ± 0.3 cd DE	0 a C	1 ± 0.4 a BC	0 a B	0.0 ± 0.0 d CD	0 a B
Q. velutina	0.3 ± 0.7 ab BC	0 a B	0.1 ± 0.3 b E	0 a C	0.5 ± 1.1 a C	0 a B	0.1 ± 0.4 ab D	0 a B
Castanea dentata	0.7 ± 0.7 a–c A–C	0 ± 0.1 b B	1.3 ± 0.7 a C	0.1 ± 0.1 b BC	2 ± 2.7 a BC	0.7 ± 0.1 a B	0.9 ± 1.1 ab BC	0.0 ± 0.1 b B
Kalmia latifolia	0.0 ± 0.0 c C	0 a B	3.7 ± 2. 8 b A	0 a C	12 ± 9.6 a A	0 a B	0.2 ± 0.4 c CD	0 a B
Rhododendron maximum	0.1 ± 0.4 bc BC	0 a B	0.6 ± 0.8 b C–E	0 a BC	2.6 ± 1.2 a BC	0 a B	0.1 ± 0.3 bc D	0 a B
Hamamelis virginiana	2.1 ± 1.2 bc A	0.1 ± 0.2 b B	2.4 ± 0.7 b B	0.4 ± 0.5 b AB	12.4 ± 4.6 a A	3.3 ± 4.7 a A	1.4 ± 0.7 b–d AB	0.1 ± 0.2 b A

Table 3. ***Continued***

Quercus spp.	*Phytophthora quercetorum*		*Phytophthora quercina*-like		*Phytophthora* spl		Control	
	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound	Wound	Non-wound
Q. alba	0.9 ± 1 bc A	0.0 ± 0.0 c A	0.1 ± 0.4 d A	0.1 ± 0.3 bc A	0.7 ± 1.3 cd A	0.0 ± 0.1 bc A	0.1 ± 0.3 d	0.0 ± 0.0 c
Q. bicolor	0 a C	0 a A	0 a A	0 a A	0 a BC	0 a A	0 a	0 a
Q. imbricaria	0.0 ± 0.0 a C	0 a A	0.0 ± 0.0 a A	0 a A	0.1 ± 0.7 a BC	0 a A	0.0 ± 0.0 a	0 a
Q. montana	0.4 ± 0.8 ab AC	0 a A	0.0 ± 0.0 b A	0 a A	0.0 ± 0.0 b BC	0 a A	0.1 ± 0.3 b	0 a
Q. palustris	0.0 ± 0.0 a C	0 a A	0.0 ± 0.0 a A	0 a A	0.0 ± 0.0 a BC	0 a A	0.0 ± 0.0 a	0 a
Q. robur	0.1 ± 0.4 bc C	0 a A	0.0 ± 0.2 c A	0 a A	0.4 ± 0.6 b AB	0 a A	0.3 ± 0.5 bc	0 a
Q. rubra	0.1 ± 0.3 cd C	0 a A	0.0 ± 0.0 d A	0 a A	0.0 ± 0.0 d C	0 a A	0.0 ± 0.0 d	0 a
Q. velutina	0.3 ± 0.5 ab BC	0 a A	0 b A	0 a A	0.0 ± 0.0 b C	0 a A	0.0 ± 0.0 b	0 a
C. dentata	0.4 ± 0.5 bd BC	0.0 ± 0.1 b A	0 d A	0.0 ± 0.1 b A	0 d BC	0.0 ± 0.1 b A	0.2 ± 0.5 cd	0.0 ± 0.1 b
K. latifolia	0.7 ± 0.8 bc AB	0 a A	0.0 ± 0.0 c A	0 a A	0.0 ± 0.0 c BC	0 a A	0.2 ± 0.7 c	0 a
R. maximum	0.1 ± 0.2 bc BC	0 a A	0.0 ± 0.0 c A	0 a A	0.0 ± 0.0 bc BC	0 a A	0.0 ± 0.0 c	0 a
H. virginiana	0.0 ± 0.0 cd C	0 b A	0.0 ± 0.0 cd A	0 b A	0.0 ± 0.0 cd BC	0 b A	0.0 ± 0.0 d	0 b

[1]Significant differences among the *Phytophthora* species within a single oak species. Isolates with the same letter do not differ significantly from each other according to Tukey's test at p = 0.05.
[2]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 at p = 0.05.

variation existed among the foliage of the other tested plants (Table 3). As with inoculation of young foliage, *P. citricola* produced the largest lesions on mature foliage, and thus overall was the most aggressive isolate used in this experiment (Table 3). Furthermore, when *P. citricola* was used to inoculate wounded young or mature foliage of *K. latifolia* ($p = 0.375$), *R. maximum* ($p = 0.222$) and *H. virginiana* ($p = 0.118$), the lesions that were larger than the controls did not differ significantly in size. This was also true when *K. latifolia* ($p = 0.08$) and *H. virginiana* ($p = 0.231$) were wound inoculated with *P. cinnamomi*. No lesions were formed on *Q. bicolor*, *Q. imbricaria* and *Q. palustris* whether the foliage was wounded or not (Table 3).

4 Discussion

The seven different *Phytophthora* species that were commonly recovered from rhizosphere soils in oak ecosystems were used to assess possible foliar associations that could exist between *Phytophthora* and a variety of oak species and understory plants; relationships that had previously never been established. On very susceptible plants, many of the *Phytophthora* isolates produced foliar necroses and particularly with young foliage of *Q. rubra* and *C. dentata*. In the UK, young foliage of *C. sativa* Miller was found to be infected under field conditions by *P. ramorum* (Denman et al. 2005a; b), suggesting that young foliage of *Castanea* species maybe particularly susceptible to *Phytophthora* infection. Among the oak species tested, young foliage of *Q. rubra* was the most susceptible. In an experiment similar to this, little variation existed when several oaks were tested for their susceptibility to *P. ramorum* (Tooley and Kyde 2007). However, considerable variation existed when numerous plants native to Appalachians, other than oaks, were tested for their susceptibility to *P. ramorum* (Linderman et al. 2007). These tests included under-story, mid-story and over-story species. Considerable variation in foliar susceptibility to *P. ramorum* was also demonstrated when various Ericaceous plants were tested (Tooley et al. 2004).

In this experiment, the age of the foliage was an important factor in lesion formation. For plants with deciduous foliage, young foliage was always more susceptible. The smaller lesions produced on mature foliage, suggests that susceptibility to *Phytophthora* infection decreases as the season progresses. This finding agrees with other foliar assays, where susceptibility decreased with increasing leaf age when inoculated with *P. ramorum* (Denman et al. 2005b; Hansen et al. 2005). However, the susceptibility of the evergreen species *R. maximum* and *K. latifolia* remained the same whether young or mature leaves were wound inoculated with *P. citricola* or *P. cinnamomi.* This finding draws attention to evergreen plants as being a more susceptible host throughout the year; a feature that could contribute to their role in inoculum production and pathogen spread. *Kalmia latifolia* and to a lesser degree *R. maximum* were also susceptible to *P. ramorum* infection when not wounded and inoculated with suspension of sporangia (Tooley et al. 2004). In Europe, as well as in California, evergreen plants were shown to be the key host plants that support inoculum build-up and dissemination of *P. ramorum* and other *Phytophthora* species in the nursery trade.

In most incidences, artificial wounding was required for infections to occur. In experiments using *P. ramorum*, disease incidence and severity increased when wound inoculations were made (Denman et al. 2005b). In our study, the infection rates after wounding usually were comparable and much larger in some instances to those obtained in *P. ramorum* studies (Tooley et al. 2004; Denman et al. 2005b; Tooley and Kyde 2007). However, artificial wounding was not necessary when zoospores or sporangia of *P. ramorum* was used to test the susceptibility of foliage (Tooley et al. 2004; Denman et al. 2005b; Hansen et al. 2005; Tooley and Kyde 2007). The higher infection rates obtained using zoospores as compared with agar plugs could be explained by a larger number of infective propagules available during the leaf-dip inoculations, hence more

opportunities for the organism to enter the foliar tissue. Further, infection rates increased when greater zoospore inoculum was used in detached-leaf assays (Hansen et al. 2005). However, after pathogen entry, infection and invasion of foliar tissue should be similar. Utilizing agar plugs as the inoculum source, especially with non-wound treatments, undoubtedly is a more conservative method to assess aggressiveness of a *Phytophthora* species or the susceptibility of foliage.

In this test, *P. citricola* was the most aggressive *Phytophthora* species whether young or mature foliage was inoculated. The large number of reports that associated *P. citricola* with foliar and twig blight (Erwin and Ribeiro 1996; Yakabe et al. 2007) presumably is related to its foliar aggressiveness as this study demonstrated. *Phytophthora citricola* also has been found associated with foliar lesions of rhododendron foliage near streams in forest settings (S. Oak, pers. comm.). This species is certainly among the most aggressive *Phytophthora* species worldwide and has been reported in association with hundreds of different plant species (Erwin and Ribeiro 1996). Because of its ability to infect numerous plants and different plant parts, the species might be a useful predictor of the potential invasiveness of other *Phytophthora* species such as *P. ramorum*.

The second most aggressive species were *P. cambivora* and *P. cinnamomi*. These two species have not been associated with foliar infections but effectively caused necrosis when foliage was young and wounded. Similarly, *P. europaea* caused lesions mostly on young wounded foliage in our experiment. This species was found to infect the leaves of *Umbellularia californica* (Hook. & Arn.) Nutt. (D. Rizzo, pers. comm.), an evergreen forest species in California that also harbours *P. ramorum* (Rizzo et al. 2002; Davidson et al. 2005). Our finding supports its ability to infect foliage and cause significant lesions. *Phytophthora quercetorum* has only been reported in association with oak roots (Balci et al. 2007, 2008). Its ability to cause small foliar lesions following wounding suggests that it could be a weak foliar pathogen. *Phytophthora quercina*-like and *Phytophthora* sp1 were ineffective in causing any foliar infections during the experiments, suggesting that these two *Phytophthora* species would be unlikely foliar invaders.

The organisms used in this experiment were all recovered from forest soils and for most of them a significant aerial phase has not been demonstrated. This, however, does not imply that, under different environmental conditions or with exposure to other hosts, they might not become important foliar pathogens. This maybe what has occurred in the case of *P. europaea* and *P. pseudosyringae* in California where it was found infecting the foliage of *U. californica* and some other evergreen plants but has only been recovered as a soil-borne organism in areas of the eastern USA and Europe (Balci and Halmschlager 2003; Jung et al. 2003; Balci et al. 2007).

Artificial inoculations using agar plugs provided a quick assessment of foliar susceptibility and demonstrated that differences exist among *Phytophthora* species in their ability to infect foliage. However, true susceptibility under field conditions could be considerably different, as environmental factors play an important role in the disease etiology. Care must be taken to interpret the importance of small necrotic lesions as an indication of host susceptibility. For some plant species, foliar infections may play a role in disease epidemiology, even though the plant may not be killed; this is the case with *P. ramorum* (Rizzo et al. 2002; Davidson et al. 2005). In particular, evergreen species, including those with less susceptible foliage, may be important producers of inoculum relevant for sporulation and dissemination of the pathogen while not being detrimental to the health of the plants they infect.

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