Distribution and recovery of *Phytophthora cinnamomi* in soils of mixed hardwood-pine forests of the south-eastern USA [†]

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Abstract

Inconsistent recovery of *Phytophthora cinnamomi* Rands from forest soils has been documented in climates with seasonally wet and dry periods. *Phytophthora cinnamomi* can be recovered when soils are moist or wet but can be difficult to recover from dry soil. Recovery may be complicated further by the physical location of *P. cinnamomi* in soil. Our objectives were: (1) to investigate factors that might affect recovery of *P. cinnamomi* from dry soil—i.e. length of time remoistened soil was stored, storage temperature, and presence of host tissue; and (2) to determine the spatial distribution of this organism in forest soil. Recovery of *P. cinnamomi* from soil samples that had been dried and then remoistened was very rare (1/90 samples); therefore, additional studies are needed to better understand the factors that affect recovery of *P. cinnamomi* from, and the viability of propagules present in, dry soil. Spatial distribution of *P. cinnamomi* was examined using three grids at each of three forest sites. Horizontal distribution was determined at 30-cm intervals along the soil surface of each grid. *Phytophthora cinnamomi* was found in soil samples in seven of the nine grids and was recovered in 14 to 97% of the samples from those grids. Vertical distribution at standard depths (0, 6, 23, 40, 57, and 74 cm) was studied in 13 soil cores collected at the three forest sites. *Phytophthora cinnamomi* was present in 85% of vertical cores, occurred more frequently near the soil surface than at any other depth, was detected up to 74 cm below the surface, and often was not contiguous in a core.

Keywords: pathogen distribution; pathogen recovery; *Phytophthora cinnamomi*.

Introduction

Since its introduction to North America over 150 years ago, *Phytophthora cinnamomi* Rands has become widespread in forests of the south-eastern United States of America (USA) and elsewhere around the country (Campbell 1951; Campbell et al., 1963; Crandall et al., 1945; Meadows et al., 2011; Roth, 1954; Wood & Tainter, 2002; Zentmyer, 1980). It has the potential to cause disease on over 1000 plant species—many of which are native to this region (Erwin & Ribeiro, 1996; Jordan & Tainter, 1996; Meadows et al., 2011; Nagle et al., 2010; Spainhour et al., 2001; Zentmyer, 1980). For example, *P. cinnamomi* causes root rot (also known as ink disease) on American chestnut (*Castanea dentata* [Marsh.] Borkh.) and had nearly eliminated this dominant tree species from forests of the southern Appalachian Mountains by the

early 1900s (Crandall et al., 1945; Zentmyer, 1980). Littleleaf disease on shortleaf (*Pinus echinata* Mill.) and loblolly (*Pinus taeda* L.) pines also is caused by *Phytophthora cinnamomi* (Copeland, 1952; Roth, 1954); these pines are native to this region and also are planted for commercial value (Roth 1954). Recently, *P. cinnamomi* has been found in hardwood forest soils of the mid-Atlantic and north central regions of the USA (Balci et al., 2007) and has been associated with decline of white oak in Ohio, USA (Balci et al., 2009; Nagle et al., 2010).

Reliable recovery of *Phytophthora cinnamomi* from soil is critical to understanding pathogen distribution and spread. Research in Australia—where *P. cinnamomi* has caused severe dieback of jarrah (*Eucalyptus marginata* Donn ex Sm.) forests (Podger, 1972; Weste, 1974)—demonstrated that recovery of *P. cinnamomi* was best during periods of high precipitation and that dry soil conditions drastically reduced, if not eliminated, recovery (Collins et al., 2001; Kuhlman, 1964; Marks et al., 1975; McDougall et al., 2002; Shea et al., 1980; Shearer & Shea, 1987; Weste & Ruppin, 1975; 1977; Weste & Vithanage, 1979). Recent studies in our laboratory corroborate these findings: *P. cinnamomi* was not recovered during periods of drought, which usually occur during the warmest months of the year, from soils in forests of the southern Appalachian Mountain region known to be infested with the pathogen (Meadows & Jeffers, unpublished). Therefore, it seems likely that propagules of *P. cinnamomi* in soil (presumably chlamydospores) become dormant or die during seasonally dry conditions. Our first objective in this study was to examine several factors that might stimulate dormant propagules of *P. cinnamomi* to germinate and allow recovery from naturally infested dry soil—i.e. length of time remoistened soil was stored, temperature at which remoistened soil was stored, and presence of host tissue in remoistened soil samples.

Recovery of *Phytophthora cinnamomi* also can be affected by its physical location or spatial distribution in soil. The variable distribution of this organism in forest soils has been documented locally (Meadows et al., 2011), elsewhere in the USA (Balci et al., 2009), and in other parts of the world (Marks et al., 1973). There are some reports that *P. cinnamomi* has been recovered from deep in the soil profile and not at the soil surface (McDougall et al., 2002; Shearer & Shea, 1987; Shea et al., 1983; Weste & Law, 1973). Investigations on the physical location of *P. cinnamomi* in forest soils of the southeastern USA are limited (Roth, 1954), and this subject warrants further study. Therefore, the second objective of our study was to determine the horizontal and vertical distribution of *P. cinnamomi* in soils of three mixed hardwood-pine forests in the western regions of North Carolina (NC) and South Carolina (SC), USA.

Materials and Methods

Experiment 1: Factors affecting recovery of *Phytophthora cinnamomi* in dry forest soil

Study sites

Soil naturally infested with *Phytophthora cinnamomi* (approximately 6000 mL) was collected from each of three forest sites (Figure 1); soil was collected for the first trial from two sites in the Green River Game Lands (GRGL) near Hendersonville, NC, USA in September 2007, and soil for the second trial was collected in April 2008 from the Clemson Experimental Forest (CEF) in Clemson, SC, USA. All sites were known to be infested with *P. cinnamomi* based on previous research conducted in our laboratory. At each site, soil was collected from the uppermost 30 cm of the soil profile in areas approximately 100 m^2.
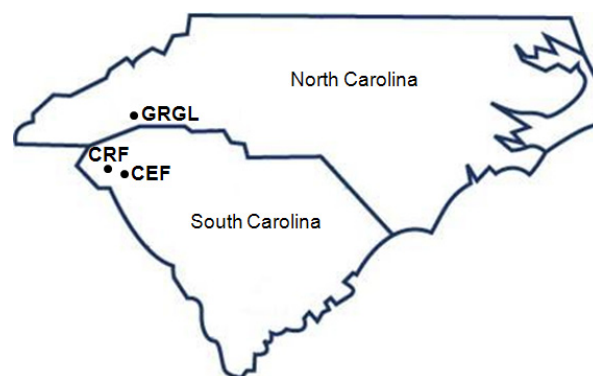

FIGURE 1: Map showing locations of the three sample sites in the western regions of North Carolina and South Carolina, USA: Clemson Experimental Forest (CEF), Chestnut Return Farm (CRF), and Green River Game Lands area (GRGL).

Sample preparation

Soil samples were returned to the laboratory, passed through a sieve (2-mm-wide mesh) to remove roots and large debris, and thoroughly mixed; therefore, there was less than 6000 mL of sieved soil remaining. Three 50-mL aliquots from each sample were assayed for the presence of *Phytophthora cinnamomi* to document initial infestation. The remaining soil was air-dried at room temperature to simulate a dry environment in the forest; soil was turned and mixed regularly to ensure uniform drying. To recover *P. cinnamomi* from dried soil, three 50-mL aliquots of dry soil were removed, moistened with 10 – 15 mL of distilled water, held for 3 days at 20 °C in the dark, and then baited (see below). This process was repeated at 7-day intervals until *P. cinnamomi* could not be recovered from an air-dried soil sample Moistening dry soil prior to baiting stimulates quiescent propagules to germinate (Jeffers & Aldwinckle, 1987).

In the first trial, the remaining dry soil from each site was divided into five 800-mL subsamples. Each subsample was moistened with approximately 200 mL of distilled water, which brought the soil to near saturation, and sealed in a plastic bag; each bag then was placed inside a second plastic bag and sealed to prevent desiccation. One subsample from each site was placed at each of five temperatures: 5, 10, 15, 20, and 25 °C. Every two weeks for 10 weeks, three 50-mL aliquots were removed from each subsample and baited for *P. cinnamomi*.

In the second trial, air-dried soil was divided into six 900-mL subsamples, and each subsample was moistened with 250 mL of distilled water. Two subsamples were placed at each of three temperatures: 5, 15, and 25 °C. To one subsample at each temperature, a wounded rhododendron (*Rhododendron catawbiense* Michx. cv. English Roseum) leaf was added to examine the influence of host tissue on pathogen recovery. Three 50-mL aliquots were removed from each soil subsample every two weeks for 12 weeks, and these were assayed for the presence of *Phytophthora cinnamomi* by baiting.

Qualitative recovery and detection of Phytophthora *spp.*

Phytophthora spp. were recovered from soil aliquots using a baiting bioassay—a procedure that has been validated and is used routinely in our laboratory (Ferguson & Jeffers, 1999; Meadows et al., 2011; Wood, 2002). Each aliquot of soil (50 mL) was placed in a 470-mL plastic box (Arrow Plastic Manufacturing Co., Elk Grove, IL, USA), and flooded with 100 mL of distilled water. Six camellia (*Camellia japonica* L. cv. Governor Mouton) leaf discs (5 mm in diameter) were floated on the surface of the water as baits, and the boxes were held at room temperature (22-25 °C) for 3 days. On the third day, leaf discs were blotted dry and embedded in PARPH-V8 selective medium (Ferguson & Jeffers, 1999) to detect species of *Phytophthora*. Plates were held at 20 °C for up to 14 days and examined regularly for colonies of *Phytophthora* spp. Colonies of *P. cinnamomi* were recognised easily based on typical morphological features (Erwin & Ribeiro, 1996; Zentmyer, 1980), and colonies of other species were subcultured and saved. These species were identified using both morphological and molecular characters. Identification was determined by sequencing the internal transcribed spacer (ITS) region of rDNA by the primers ITS4 and ITS6 and these sequences were compared to those catalogued in established databases (GenBank, Phytophthoradb. org, Phtyophthora-id.org) (Cooke et al., 2000; Grünwald et al., 2011; White et al., 1990). Identification was confirmed by subsequent observation of morphological features (Balci et al., 2008; Erwin & Ribeiro, 1996). The presence or absence of *P. cinnamomi* in each soil aliquot was recorded.

Experiment 2: Spatial distribution of *Phytophthora cinnamomi* in soils at three forest sites

Sample sites

Three mixed pine-hardwood forest sites were selected—one in western North Carolina, USA, and two in western South Carolina, USA—where *Phytophthora cinnamomi* was known to occur based on previous studies. The Clemson Experimental Forest (CEF) is located near Clemson, SC, USA; the Chestnut Return Farm (CRF) is located near Seneca, SC, USA; and the Green River Game Lands (GRGL) is located near Hendersonville, NC, USA (Figure 1).

Soil sample collection

To determine horizontal distribution of *Phytophthora cinnamomi* in soil, three square grids (210 cm per side) were established at each study site, providing a total of nine grids (Figure 2: A – I). Each grid contained 64 grid-points at 30-cm intervals (Figure 2), and one soil sample (2 cm in diameter and 20 cm deep) was collected with a soil sampling tube (Oakfield Apparatus, Inc., Oakfield, WI, USA) at each grid-point. Grids were established and samples were collected in March 2008 at CEF, in April and May 2008 at CRF, and in May and June 2008 at GRGL. Each soil sample (approximately 50 – 60 mL) was tested qualitatively for the presence of *P. cinnamomi* using a baiting bioassay, see below.

To determine the vertical distribution of *Phytophthora cinnamomi* in the soil profile, two or three vertical cores were collected at each forest site as close as possible to grid-points where *P. cinnamomi* was detected in the horizontal distribution study, providing a total of 13 cores (Figure 2: 1 – 13). Relatively undisturbed cores (5 cm in diameter) were collected in 17-cm increments using a soil core sampler (AMS, Inc., American Falls, ID). Cores went as deep as possible, which was down to approximately 50 to 80 cm, before hitting a layer of rock or other impenetrable material. From each increment core, a 1-cm section was removed from the middle, which produced subsamples (approximately 60 mL) from six standard depths: 0, 6, 23, 40, 57, and 74 cm. Subsamples were removed carefully from each core to prevent cross-contamination, thoroughly mixed, and assayed qualitatively to detect the presence of *P. cinnamomi* and also quantitatively to determine propagule density. In addition, pieces of roots occasionally found in some soil cores were embedded in PARPH-V8 to attempt to isolate *Phytophthora* spp.

Qualitative recovery and detection of Phytophthora cinnamomi

Fresh soil samples from each grid (approximately 50 mL) and subsamples from each core (40 mL) were

assayed in the same manner and within one week of collection. Each sample or subsample was placed in a 470-mL plastic box. Samples from grids were flooded with 100 mL of distilled water, and subsamples from cores were flooded with 80 mL of distilled water. Flooded samples and subsamples were baited, as described above in Experiment 1, using three rhododendron leaf pieces and three camellia leaf discs as baits.

Quantitative recovery and detection of Phytophthora cinnamomi

A separate subsample from each soil core was subjected to a direct-plating procedure to estimate propagule density, i.e. the number of colony-forming units (CFU) per gram of soil. In the direct-plating procedure, 20 mL of soil was mixed with 50 mL of 0.3% water agar, and 1 mL of this mixture was spread onto each of five plates of PARPH-V8. To determine

the average mass of the soil, 1 mL of this mixture also was placed on each of three small aluminum weighing dishes and dishes were placed in an oven to dry; the average mass of the three dried soil aliquots was calculated. Plates were maintained at room temperature for 3 days and then soil gently was washed from the agar surface. Plates were maintained at 20 °C for an additional 11 days. Numbers of colonies on each plate were counted, and mean propagule density in each subsample was calculated.

Data analysis

Data on the presence or absence of *Phytophthora cinnamomi* in each horizontal soil sample were used in a spatial autocorrelation test (Black-black join count autocorrelation; Schabenberger & Gotway, 2005) to identify degree of aggregation.
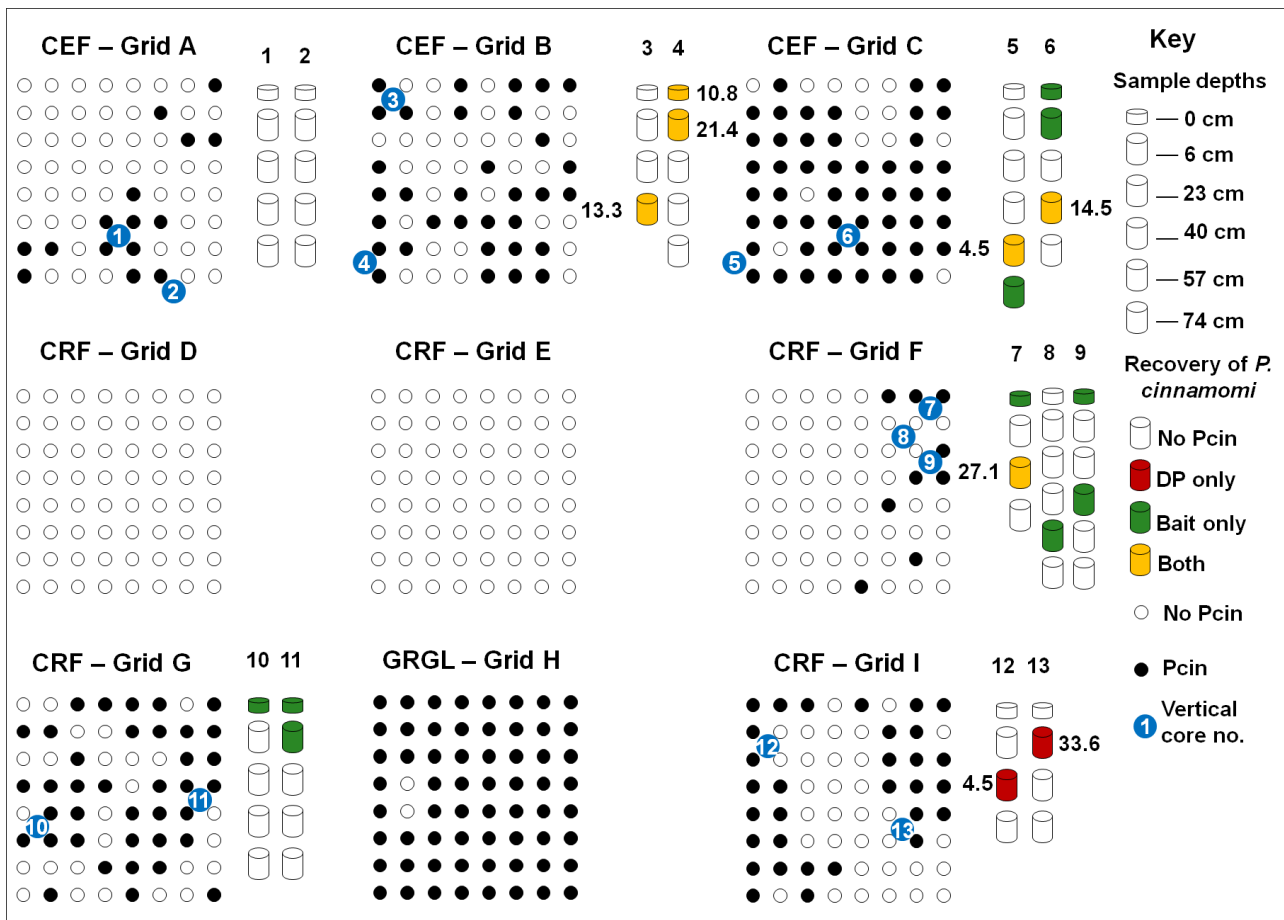

FIGURE 2: Diagram of the nine grids (designated A – I), each 210 cm on a side, used to study the spatial distribution of *Phytophthora cinnamomi* in soil at three forest sites: Clemson Experimental Forest (CEF), Chestnut Return Farm (CRF), and Green River Game Lands area (GRGL). Horizontal distribution was based on soil samples collected at 64 grid-points (30 cm apart) in each grid: *P. cinnamomi* was either not recovered (No Pcin = ○) or recovered (Pcin = ●) by a baiting bioassay. Distribution in the soil profile was based on subsamples taken at six standard depths from 13 vertical cores. *Phytophthora cinnamomi* was recovered only by direct plating (DP only), only by baiting (Bait only), or by both assays (Both). Numbers next to subsamples from standard depths are mean propagule densities (CFU/g of soil). *Phytophthora cinnamomi* was not recovered in Grids D and E at CRF.

Results

Experiment 1: Factors affecting recovery of *Phytophthora cinnamomi* from dry forest soil

Phytophthora cinnamomi was recovered from all aliquots of fresh soil that were assayed before being air-dried. However, after the three soil samples had been completely air-dried, which took at least 14 days, *P. cinnamomi* was not recovered in any of the aliquots tested. *Phytophthora cinnamomi* was detected only once in both trials—from one camellia bait used to assay a soil subsample that had been stored at 25 °C for 4 weeks in the first trial. *Phytophthora cinnamomi* was not recovered from any of the other soil subsamples in either the first or second trial.

Surprisingly, *Phytophthora quercetorum* Balci & Balci, a recently described species, was recovered from dried and remoistened soil collected from the CEF site. This species was detected in samples without a wounded rhododendron leaf that had been stored at each of the following duration and temperature combinations: 2 weeks at 5 °C, 15 °C, and 25 °C; 4 weeks at 25 °C; and 10 weeks at 15 °C and in one soil sample with a wounded rhododendron leaf that had been stored for 4 weeks at 15 °C. *Phytophthora quercetorum* was not recovered from fresh CEF soil that was assayed at the start of the experiment.

Experiment 2: Spatial distribution of *Phytophthora cinnamomi* in soil at three forest sites

Phytophthora cinnamomi was recovered from soil samples in seven out of nine (78%) horizontal grids (Figure 2). The two grids from which *P. cinnamomi* was not recovered (D and E) were located at the same site (CRF). In each positive grid, *P. cinnamomi* was detected in 9 to 62 (14 to 97%) of 64 total samples. The mean number of samples per grid from which *P. cinnamomi* was recovered was 27 of 64 (42%). The means at each location were: CEF, 33/64 (52%); CRF, 3/64 (5%); and GRGL, 44/64 (69%). In the spatial autocorrelation statistical analysis, the distribution of *P. cinnamomi* in each grid was not significantly aggregated and, therefore, was random.

Thirteen soil cores were collected from six of the seven grids where *Phytophthora cinnamomi* had been recovered, and it was recovered from 11 (85%) of these cores (Figure 2). Vertical cores were not collected from Grid H because this site became inaccessible before cores could be collected. The two cores from which *P. cinnamomi* was not recovered were from the same grid at the CEF site (Figure 2, Grid A). *Phytophthora cinnamomi* was recovered from up to three subsamples from the standard depths in each core (Figure 2). In all vertical cores combined, *P. cinnamomi* was recovered from 18 of 64 (29%) subsamples. The pathogen was recovered at all six of the standard depths but not always in contiguous subsamples; it was found in six subsamples at 0 cm, four subsamples at 6 cm, two subsamples at 23 cm, three subsamples at 40 cm, two subsamples at 57 cm, and one subsample at 74 cm. The proportion of vertical cores with *P. cinnamomi* present near the surface (0-6 cm; 7/13) was the same as the proportion of cores with *P. cinnamomi* present at depths below the surface (23-74 cm; 7/13). However, *P. cinnamomi* was found more frequently in subsamples near the surface than in subsamples below the surface; it was recovered from ten subsamples collected from the upper 6 cm of soil and from only one, two, or three subsamples collected at any of the other standard depths (Figure 2). Mean propagule density in subsamples collected from 0 to 6 cm (n = 3) was 21.9 CFU/g of soil and the mean propagule density in subsamples collected from 23 to 57 cm (n = 5) was 12.8 CFU/g of soil (Figure 2). Of the 18 subsamples from which *P. cinnamomi* was recovered, it was recovered by baiting in 16 (89%) and by direct plating in eight (44%); in 10 of these subsamples it was recovered only by baiting, in two subsamples it was recovered only by direct plating, and in six subsamples it was recovered by both methods. *Phytophthora cinnamomi* was not recovered from any of the root pieces assayed from the vertical cores.

Discussion and Conclusion

In this study, our attempts to recover *Phytophthora cinnamomi* from air-dried soil were not successful. Our assumption was that propagules of *P. cinnamomi* in air-dried soil were either dormant or no longer viable. The factors we manipulated (i.e. length of time remoistened soil was stored, temperature at which remoistened soil was stored, and presence of host tissue in soil samples) were not sufficient to reliably stimulate propagules (presumably chlamydospores) of *P. cinnamomi* to germinate—if they still were viable. Our results corroborate those reported by others where recovery of *P. cinnamomi* was limited or not possible from dry soil regardless of soil temperature (Collins et al., 2001; Kuhlman, 1964; Marks et al., 1975; McDougall et al., 2002; Shearer and Shea, 1987; Weste & Ruppin, 1975; 1977; Weste & Vithanage, 1979).

In our study, small pieces of fine roots may have passed through the sieve when soil was screened and, therefore, were present in the soil during our experiments. Others have reported that amino acids and organic acids from root exudates enhanced germination of chlamydospores and that chlamydospore germination was not affected in soil not

amended with root exudates (Malajczuk & McComb, 1977; Mircetich et al., 1968; Mircetich & Zentmyer, 1969; Sterne et al., 1977). However, the presence of fine roots in soil samples in our study clearly did not enhance germination and recovery of chlamydospores of *P. cinnamomi*.

Two scenarios are possible to explain our results: (1) if propagules of *Phytophthora cinnamomi* can survive air-drying, it is likely that factors other than the ones we investigated are involved in stimulating propagules so that *P. cinnamomi* can be recovered; and (2) if propagules of *P. cinnamomi* cannot survive air-drying, then we need to reconsider how this pathogen survives or persists in soils that are subject to seasonal drying. This second scenario leads one to question the role of chlamydospores in long-term survival of *P. cinnamomi*. Recently, McCarren et al. (2005) reviewed the role of chlamydospores as survival structures of *P. cinnamomi*. We agree with their conclusions that additional research is needed to elucidate the survival mechanism of *P. cinnamomi* in soil.

Although we did not reliably recover *Phytophthora cinnamomi* in air-dried soil, we did recover *P. quercetorum* from soil collected at the CEF site in Experiment 1. This is the first report of *P. quercetorum* in South Carolina and only the second report of this species in the USA (Balci et al., 2008). The fact that it was recovered from air-dried soil and not from fresh soil is not surprising; it is homothallic and likely survives unfavorable conditions as oospores that germinated once the dried soil was moistened (Jeffers & Aldwinckle, 1987). This organism was described only recently and was found in rhizosphere soils from oaks in forests of both eastern and north-central regions of the USA (Balci et al., 2008). To date, pathogenicity has not been documented for and there are no known hosts of this newly described species of *Phytophthora*.

The horizontal distribution of *Phytophthora cinnamomi* in forest soils was studied in grids 4.4 m² in area and in the uppermost 20 cm of the soil profile. Distribution was not significantly aggregated within individual grids although an aggregated pattern might have been evident if a larger-sized grid had been used. Although, distribution was apparently random at each site, the number of soil samples in which *P. cinnamomi* was found varied among sites—i.e. it was detected in almost all samples in some grids and in few to none of the samples in other grids. There is strong evidence that certain soil physical and chemical properties are associated with the presence of *P. cinnamomi* in forest soil (Blowes et al., 1982; Copeland, 1952; Erwin & Ribeiro, 1996; Marks et al., 1975; Nagle et al., 2010; Shearer & Shea, 1987; Weste & Ruppin, 1975), but these properties were not measured in our study. Aggregated distributions of *P. cinnamomi* in forest soil have been reported—particularly when

soils were collected around susceptible hosts in jarrah forests with dieback (Blowes et al., 1982; Marks et al., 1975; Shearer & Shea, 1987; Weste & Ruppin, 1977). In our study, susceptible hosts were present at each site—e.g. white oak (*Quercus alba* L.), rhododendron (*Rhododendron* spp.), mountain laurel (*Kalmia latifolia* L.), shortleaf pine (*Pinus echinata*), and loblolly pine (*P. taeda*)—but, disease symptoms were not observed on plants at any of these forest sites.

Phytophthora cinnamomi was recovered at all depths sampled in the soil profile down to 74 cm—the maximum depth we were able to sample—but was recovered most frequently near the soil surface (i.e. in samples collected at 0 and 6 cm). These results are consistent with those reported in jarrah dieback sites in Western Australia where *P. cinnamomi* was detected at depths down to 80 cm (Marks et al., 1975; Shea et al., 1982; 1983; Weste & Law, 1973) and, in some of these reports, propagule density was greater at 20 cm and deeper than at the soil surface (Marks et al., 1975; Weste, 1974). In our study, mean propagule density appeared greater within the top 6 cm than deeper in the soil, but more samples would have to be assayed to determine if this was a significant difference. Currently, we routinely sample soils to a depth of approximately 20 to 25 cm, which is the working length of a standard soil sampling tube, when trying to recover *Phytophthora* spp. Based on the results from the vertical cores, our routine sampling would have recovered *P. cinnamomi* in eight (73%) of the 11 cores in which it actually was present. Conversely, we would have failed to recover *P. cinnamomi* from the three cores where it was present only at depths of 40 to 74 cm. Overall, we consider a 73% success rate to be both reasonable and acceptable for routine sampling—particularly when one considers that collecting samples to depths of 60 to 80 cm in many soils can be either very challenging or impossible. Sampling to a depth of 40 cm would have recovered *P. cinnamomi* in only one additional core. However, sampling soils to depths deeper than 25 cm, when possible, may increase the chances of recovering *P. cinnamomi* in forest soils.

Interestingly, the distribution of *Phytophthora cinnamomi* in the vertical soil profile was discontinuous—i.e. it was not always recovered in contiguous subsamples—and, in some cores, was present deep in the soil profile and not at the surface. A discontinuous distribution of *P. cinnamomi* in the soil profile has been reported by others (Marks et al., 1975; Weste, 1974), but it was found associated with vertical roots of jarrah trees (Shea et al., 1982; 1983). Based on our results, it appears that *P. cinnamomi* resides in "pockets" in the soil profile; it is possible that it was associated with roots growing down through the soil, but it was not recovered from any of the root pieces we assayed.

Another outcome of this study was confirmation that, overall, the baiting assay was more effective than a direct plating assay for recovery of *Phytophthora cinnamomi* in forest soil. Of the 18 soil samples in which *P. cinnamomi* was found, it was recovered by baiting in 16 and by direct plating in only eight. This is not surprising because baiting assays a much larger volume of soil than does plating. However, direct plating of soil has the advantage of providing quantification of propagule density and, therefore, allowing direct comparisons of populations in different soils.

In summary, we were not able to recover *Phytophthora cinnamomi* from air-dried soil, which leads us to question if chlamydospores can survive this type of adverse environmental conditions. If they do survive, then other factors beyond those investigated in this study might be involved in triggering propagule germination and recovery. In addition, the widespread and random distribution of *P. cinnamomi* in soil to depths well below the surface—where the soil is not as dry as at the surface—may allow this pathogen to successfully survive in nature. More investigations are necessary to fully understand the persistence and spatial distribution of this cosmopolitan plant pathogen in forest soils.

Acknowledgements

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