Research

Detection of *Phytophthora cinnamomi* in Forest Soils by PCR on DNA Extracted from Leaf Disc Baits

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Abstract

Phytophthora cinnamomi Rands causes root rot in a number of important forest tree species around the world, including American chestnut (*Castanea dentata*) and shortleaf pine (*Pinus echinata*). Conventional methods for detecting *P. cinnamomi* in forest soils may require too much time and space to permit widescale and long-term screening of the large sample numbers required for landscape-scale distribution analysis. This project compared conventional detection methods (baiting with full rhododendron leaves or leaf discs and subsequent culturing on selective media) with a molecular detection method using DNA extracted from leaf baits. These methods were comparable, and the DNA-based method was correlated with culture-based methods. In a field-validation screening using the leaf bait polymerase chain reaction method, *P. cinnamomi* was found across a range of topographic conditions, including dry ridge-top sites and moist lowland sites. Soil texture analysis supports the traditional association of *P. cinnamomi* with finer-textured soils. Further large-scale surveys are necessary to elucidate landscape-scale distribution patterns in eastern U.S. forests.

Phytophthora cinnamomi poses significant threats to forest tree species in the eastern United States, especially inhibiting restoration of American chestnut and shortleaf pine. This study describes an assay for rapid screening of forest soils, utilizing *P. cinnamomi*-specific polymerase chain reaction (PCR) amplification of DNA extracted from leaf disc baits, reducing time and space required for screening forest soils for *P. cinnamomi*. In addition, this study characterizes distribution across a gradient of soil and topographic variables, providing preliminary insights into distribution patterns of *P. cinnamomi* in an eastern U.S. forest.

A Dangerous and Clandestine Pathogen

P. cinnamomi Rands is an oomycete of global significance that originated in Southeast Asia but has been introduced around the world (Arentz and Simpson 1986; Ko et al. 1978). *P. cinnamomi* is a generalist pathogen with a broad host range, but it is perhaps most well known as the causal agent of declines in jarrah (*Eucalyptus marginata*) and other forest types in west and southeast Australia (Podger 1972; Shearer and Dillon 1995, 1996), cork oak (*Quercus suber*) and holm oak (*Q. ilex*) woodlands in Mediterranean Europe (Moreira et al. 1999; Robin et al. 1998; Scanu et al. 2013), and chestnut species (*Castanea dentata* and *C. sativa*) in North America and Europe (Day 1938; Milburn and Gravatt 1932), as well as avocado (*Persea americana*) and macadamia (*Macadamia* spp.) (Akinsanmi et al. 2016; Pagliaccia et al. 2013; Wager 1942).

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The earliest records of *P. cinnamomi*-related disease in the United States were reports of American chestnut decline in the southeast in the early to mid-1800s (Corsa 1896). Subsequently, the devastating chestnut blight fungus (*Cryphonectria parasitica*) was introduced to the eastern United States and rapidly killed back chestnuts throughout the range, functionally eliminating this dominant canopy species (Anagnostakis 2001). Although a significant research effort was dedicated to investigating disease patterns of chestnut blight, *P. cinnamomi* continued to advance on the landscape with relatively little monitoring. After decades of breeding aimed at introgressing chestnut blight resistance from Chinese chestnut (*Castanea mollissima*) into American chestnut (Diskin et al. 2006), early plantings of varieties with improved blight resistance experienced high mortality caused by *P. cinnamomi* (Jacobs 2007; Rhoades et al. 2003). This spurred a resurgence of interest in *P. cinnamomi* and its associated disease in American chestnut. Current research efforts are focused on improving American chestnut genetic resistance to *P. cinnamomi* (Olukolu et al. 2012; Zhebentyayeva et al. 2013); however, improved understanding of distribution patterns of *P. cinnamomi* on the landscape is also critical to an informed restoration effort (Jacobs 2007; Sena et al. 2018).

Although several studies have demonstrated that *P. cinnamomi* is widespread in the eastern United States, relatively little is known about how *P. cinnamomi* distribution is constrained by environmental factors on the landscape scale in Appalachia. Campbell and Hendrix (1967) found that *P. cinnamomi* was widely distributed in forest soils in the southern Appalachians (isolating it from 5 of 43 samples in mountain hardwood/conifer stands and from 14 of 31 samples in coastal pine stands) but did not identify spatial patterns of distribution. Similarly, Sharpe (2017) isolated *P. cinnamomi* from 34% of soil samples (120 of 353) collected from forest plots in North Carolina, Tennessee, Virginia, and South Carolina, and Pinchot et al. (2017) detected *P. cinnamomi* in 100% of soil samples

collected from an American chestnut study site in southeastern Kentucky. Generally, disease caused by *P. cinnamomi* is associated with moist, poorly drained soils (Dawson and Weste 1985; Keith et al. 2012; Vannini et al. 2010); however, *P. cinnamomi* has been successfully isolated from drier ridge-top soils in some cases (Shea and Dell 1981). *P. cinnamomi* is capable of surviving periods of sustained drought by producing structures such as chlamydospores (Kuhlman 1964; Old et al. 1984; Weste and Vithanage 1979). Chlamydospores are globose structures that can form within root tissue but also on the surface of roots and other organic matter, and they can be thick- or thin-walled. Thick-walled chlamydospores inside root tissue are thought to be the most resistant to drought and other inclement conditions (McCarren et al. 2005). In addition, *P. cinnamomi* was recently discovered in asymptomatic herbaceous understory plants in Australia, suggesting that its relationships with host plants are more complicated than previously understood (Crone et al. 2013a, b). At broad landscape scales, *P. cinnamomi* is limited by freezing temperatures (Burgess et al. 2017); in the eastern United States, *P. cinnamomi* has been detected in forest soils as far north as Ohio and Pennsylvania, U.S. Department of Agriculture hardiness zone 6 (McConnell and Balci 2014).

At relatively small spatial scales, *P. cinnamomi* is randomly distributed, with variable detection even within a 1 m^2 area (Meadows and Jeffers 2011; Pryce et al. 2002). Thus, rigorous subsampling is required to reduce risk of false negative detection results. However, in addition to improved sampling design, some studies suggest a need for improved detection sensitivity. Conventionally, *P. cinnamomi* is detected from soils by flooding soil with sterile water, baiting with susceptible plant material (e.g., rhododendron leaf), culturing infected bait material on selective media (agar amended with antibiotics and fungicides), and identifying *P. cinnamomi* by morphological characteristics and/or DNA sequence (Jeffers and Martin 1986). However, some studies have demonstrated that baiting and culturing can be insensitive, returning a high frequency of false negative results (Hüberli et al. 2000; McDougall et al. 2002). Finally, conventional methods require large volumes of soil (e.g., ≈300 ml) and may be inconvenient for large-scale screening efforts or for screening of sites that are not readily accessible by road. With the development of molecular microbiology, improved methods have been established for screening potentially infected plant material. Conventional baiting and culturing detection methods are time consuming and may be insensitive, and DNA-based detection methods may be both more rapid and more sensitive. Several PCR methods have been developed for use in detecting *P. cinnamomi* from infected plant material (Kunadiya et al. 2017). Somewhat less attention has been paid to developing PCR-based methods for screening soils; however, a recent European study successfully implemented a nested touchdown PCR assay for rapid detection of *P. cinnamomi* from DNA extracted directly from chestnut plantation soils, with detection frequencies comparable to standard baiting and culturing (Langrell et al. 2011). DNA-based methods may also permit high-throughput screening of large numbers of samples by reducing the soil sample volume (as well as time and space) required for screening.

This project was initiated to compare detection assays for use in high-throughput screening of small-volume soil samples. Specifically, conventional baiting and culturing methods (one with full leaf baits and one with leaf disc baits, both followed by isolation on medium containing pimaricin, ampicillin, rifampicin, pentachloronitrobenzene [PARP]) were compared with a modified baiting method (baiting with leaf discs, followed by DNA extraction and screening with *P. cinnamomi*-specific PCR) and a soil-DNA method (soil DNA extraction followed by screening with *P. cinnamomi*-specific PCR). To our knowledge, this is the first study screening DNA extracted from incubated leaf baits for detection of *P. cinnamomi* from soils. This method-comparison analysis was conducted using soils collected from a series of plots representing a gradient of soil and topographic variables in an eastern Kentucky watershed, providing insight into *P. cinnamomi* landscape distribution patterns.

Soil Sampling and *P. cinnamomi* Detection Assays

Soil samples were collected from continuous forest inventory (CFI) plots in the 1,500 ha Clemons Fork watershed, University of Kentucky Robinson Forest, Breathitt County, Kentucky, in October to November 2016. CFI plots were selected for this analysis to make use of an extensive existing dataset (plots are surveyed periodically for a suite of topographic and vegetative parameters); the specific 47 plots surveyed were chosen to maximize accessibility (e.g., proximity to roads/trails) and minimize sampling time required. Samples were collected using a sampling spade (sterilized with 70% ethanol between plots) to a depth of ≈10 cm when possible (ridge-top and side-slope soils were frequently rocky, shallow, or both). Six subsamples were collected within a ≈1 m^2 area at plot center, combined in the field, and stored in plastic sampling bags at 4°C until processed (1 to 2 weeks).

In the lab, soils were aliquoted in appropriate volumes for comparison of detection methods (Fig. 1). For the full leaf baiting method, a 40-ml aliquot of soil was flooded with sterile water in a soil sample bag and was baited with a rhododendron leaf that had been surface sterilized by wiping with 70% ethanol. Samples were incubated in a growth chamber in the dark at 27°C for 1 week. Lesions were excised from rhododendron leaves and transferred to PARP medium (Jeffers and Martin 1986). If lesions were not present (or if fewer than three lesions were present), segments of seemingly healthy tissue were excised and transferred to PARP medium. A total of three segments were transferred to PARP medium for each full leaf bait. After 5 days, hyphal tips were transferred to water agar and incubated for 1 week at 27°C. A final transfer was made to clarified V8 agar for morphological identification.

For the leaf disc baiting method, a 40-ml aliquot of soil was flooded with sterile water in a sterile 50-ml tube and baited with leaf discs. Leaf discs (≈6 mm in diameter) were prepared by hole punching rhododendron leaves that were surface sterilized by wiping with 70% ethanol. Six leaf discs were incubated in each flooded sample in a growth chamber in the dark at 27°C for 1 week; three leaf discs were cultured as described above, and three leaf discs were frozen in 1.5-ml tubes for subsequent DNA extraction. Samples for which *P. cinnamomi* was isolated from at least one disc/piece were considered positive. Identification of isolates from leaf disc baits and full leaf baits was confirmed by *P. cinnamomi*-specific PCR as described below.

DNA was extracted from leaf disc baits (one DNA extraction per soil sample) using the Qiagen DNeasy UltraClean microbial DNA extraction kit. Leaf discs (three per sample) were transferred to a bead tube, with 300 µl of microbead solution and 50 µl of solution MD1, and vortexed at maximum speed for 20 min using a platform vortexer attachment. After vortexing, 12 µl of 1 mg/ml proteinase K was added to each sample, and samples were incubated overnight at room temperature. Supernatant was transferred to a clean 1.5-ml tube, and subsequent extraction steps were carried out according to manufacturer instructions. Amplifiable DNA was confirmed for each DNA extraction using ITS1/ITS4 primers, which amplify DNA from fungi and oomycetes (White et al. 1990). Reactions were prepared in 12.5-µl volumes, with 1 µl of genomic DNA and 11.5 µl of master mix containing 1.25 µl of 10× PCR buffer, 1.25 µl of 2 mM dNTPs, 0.75 µl of 50 mM $MgCl_2$, 0.5 µl of 10 µM primer

(each ITS1 and ITS4), 0.0625 µl of Immolase Taq polymerase (at 5 U/µl), and 7.1875 µl of water. Thermocycling conditions were 94°C for 10 min, then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, with a final extension step at 72°C for 5 min.

DNA was screened for *P. cinnamomi* using a PCR assay (primers Ycin3F, GTCCTATTCGCCTGTTGGAA; and Ycin4R, GGTTTTCTCTACATAACCATCCTATAA) targeting a 300-bp segment of the *Ypt* gene developed by Schena et al. (2008) and recommended for *P. cinnamomi* specificity by Kunadiya et al. (2017). Schena et al. (2008) tested these primers for specificity using genomic DNA from 73 isolates representing 35 species of the genus *Phytophthora* and 9 species of *Pythium*. Kunadiya et al. (2017) tested these primers against 11 species from Phytophthora clade 7 and one species from each of the other clades. Both studies reported these primers as specific to *P. cinnamomi*.

P. cinnamomi-specific PCRs (using primers Ycin3F and Ycin4R) were 25 µl containing 1.5 µl of DNA and 23.5 µl of master mix (2.5 µl of 10× PCR buffer, 2.5 µl of 2 mM dNTPs, 0.75 µl of 50 mM $MgCl_2$, 1 µl each of 10 µM primer, 1 U Immolase Taq polymerase, and 15.55 µl of water). Thermocycling conditions were 95°C for 10 min, followed by 42 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 15 s, and a final extension step of 72°C for 10 min. PCR amplicons were visualized using agarose gel electrophoresis (1.5% m/v) in 1× sodium borate buffer with GelRed nucleic acid stain (Biotium, Fremont, CA) coloaded. Samples were screened in duplicate with positive controls, *P. cinnamomi* isolate RF5 (isolated from Robinson Forest, GenBank accession number MF966152) 1.5×10^{-2} ng/PCR (found to be the limit of detection), and no-DNA template negative controls (Fig. 2).

For the soil DNA detection method, DNA was extracted from 0.25 g of soil (Qiagen PowerSoil DNA extraction kit, according to manufacturer instructions) and screened using the assay described above (one DNA extraction per soil sample).

Soil Physical and Chemical Characteristics

In addition to screening for *P. cinnamomi*, soils were analyzed for the following physical and chemical parameters: pH, P, K, Ca, Mg, Zn, soil organic matter, total N, texture, and field capacity. Soil pH was measured in a 1:1 soil/water paste (Soil and Plant Analysis Council 2000). Concentrations of P, K, Ca, Mg, and Zn were measured by Mehlich III extraction and analysis by inductively coupled plasma spectrometry (Soil and Plant Analysis Council 2000; chapters 3, 6, and 7). Soil organic matter and total N were quantified by combustion using a LECO instrument (Nelson and Sommers 1982). Particle size distribution was evaluated by the micropipette method (Miller and Miller 1987), and field capacity was evaluated by the pressure plate method (Topp et al. 1993). Differences in soil physical and chemical data between samples with *P. cinnamomi* detected and

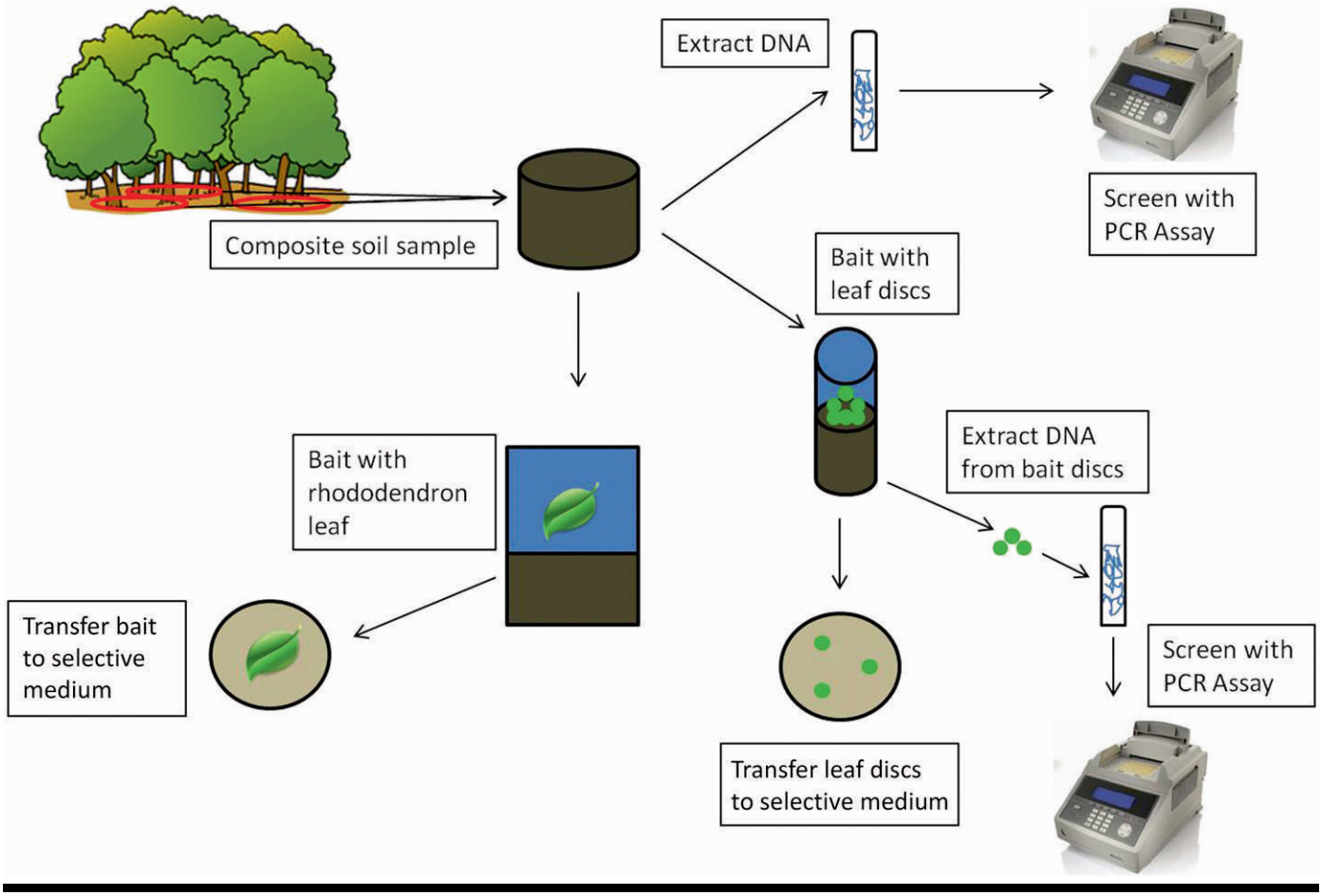

FIGURE 1

Overview of assays for detection of *Phytophthora cinnamomi* from forest soils selected for comparison in this study: DNA extraction from soil and amplification by *P. cinnamomi*-specific polymerase chain reaction (PCR), baiting with full rhododendron leaves and subsequent culturing on selective media, baiting with rhododendron leaf discs and subsequent culturing on selective media, and baiting with rhododendron leaf discs and subsequent DNA extraction and amplification by *P. cinnamomi*-specific PCR.

samples with *P. cinnamomi* not detected (by any of the three assays) were assessed using a *t* test, assuming unequal variances (SAS 9.3, PROC TTEST).

Comparison of *P. cinnamomi* Detection Methods

Because artificially infested soils (known positives) and sterilized soils (known negatives) were not used as part of the method-comparison analysis, the true *P. cinnamomi* infestation status of each soil sample is not known. Thus, we report detection results from the three methods and how they compare with one another. *P. cinnamomi* detection frequency varied across detection methods; direct soil DNA extraction and amplification were ineffective (none of the 47 samples tested were identified as positive), and results from the soil DNA extraction and amplification method were not included in further analysis. Thus, only results from the full leaf bait and culture, leaf disc bait and culture, and leaf disc bait and PCR methods are presented here. Overall, of the 47 samples screened, 26 samples were negative by all three assays, and 21 samples were screened as positive by at least one assay and considered positives (Table 1). The full leaf bait and culture method and the leaf disc bait and PCR method both screened 15 plots as positive and 32 plots as negative, whereas the leaf disc bait and culture method screened only 10 plots as positive and 37 plots as negative. Samples screened as negative by one method but positive by the two other methods were considered false negative results. The full leaf bait and culture method returned two false negatives, and the leaf disc bait and culture method returned three false negatives, whereas the leaf disc bait and PCR method returned no false negatives.

Screening of DNA extracted from soil was unsuccessful at detecting *P. cinnamomi*. This is likely owing to the limited capacity of the soil DNA extraction kit, which only extracted DNA from very small soil samples (≈0.25 g). Because *P. cinnamomi* incidence is highly variable across small spatial scales, the likelihood of *P. cinnamomi* propagules being included in any given 0.25-g aliquot is low. In addition, if *P. cinnamomi* was present in the aliquot, the concentration of target DNA in the soil DNA extract might be below the detection limit of the PCR method. Development of efficient DNA extraction technology for purification of high-quality DNA from larger volumes of soil would make this method more effective. Langrell et al. (2011) reported high-quality DNA extraction from 10-g soil samples using a cetyl trimethylammonium bromide/chloroform method; however, we chose to use the commercial kit to increase throughput and maximize reproducibility. PCR inhibition is a major concern when working with environmental samples (Hedman and Radstrom 2013). To minimize this risk, we selected a DNA extraction kit (Qiagen PowerSoil) recommended for reducing PCR inhibitors in soil DNA extracts, and we confirmed presence of amplifiable DNA in each soil DNA extract using ITS1/ITS4 primers as described earlier. Despite these steps, PCR inhibition may still have been an impediment in the soil DNA method; absence of PCR inhibition should be confirmed in subsequent attempts at developing a soil DNA-based method. In addition, continued development of sensitive molecular detection methods would permit detection of lower concentrations of target DNA, also improving method sensitivity. Use of high-sensitivity nested assays with DNA extracted from large volumes of soil may provide the level of sensitivity necessary for reliable detection of low propagule concentration in soils.

P. cinnamomi detection frequency by the remaining three methods was similar, ranging from 10 to 15 positives and from 32 to 37 negatives. *P. cinnamomi* detection by amplification of DNA extracted from leaf baits returned no false negatives, whereas detection by culturing leaf disc baits returned three false negatives. DNA-based microbial detection methods have been demonstrated to be more sensitive than traditional culturing methods for detection of *P. cinnamomi* from infected plant material (Hüberli et al. 2000; Williams et al. 2009). Culturing is necessarily selective and will not successfully detect all propagules present. In contrast, DNA-amplification-based methods are capable of detecting propagules that may not be successfully cultured, including dead or stressed propagules, and are not affected by competing microbes.

Although sensitivity was similar between the leaf disc bait and PCR method and the full leaf bait and culture method (15 positives and 32 negatives each), the leaf disc bait and PCR method was more

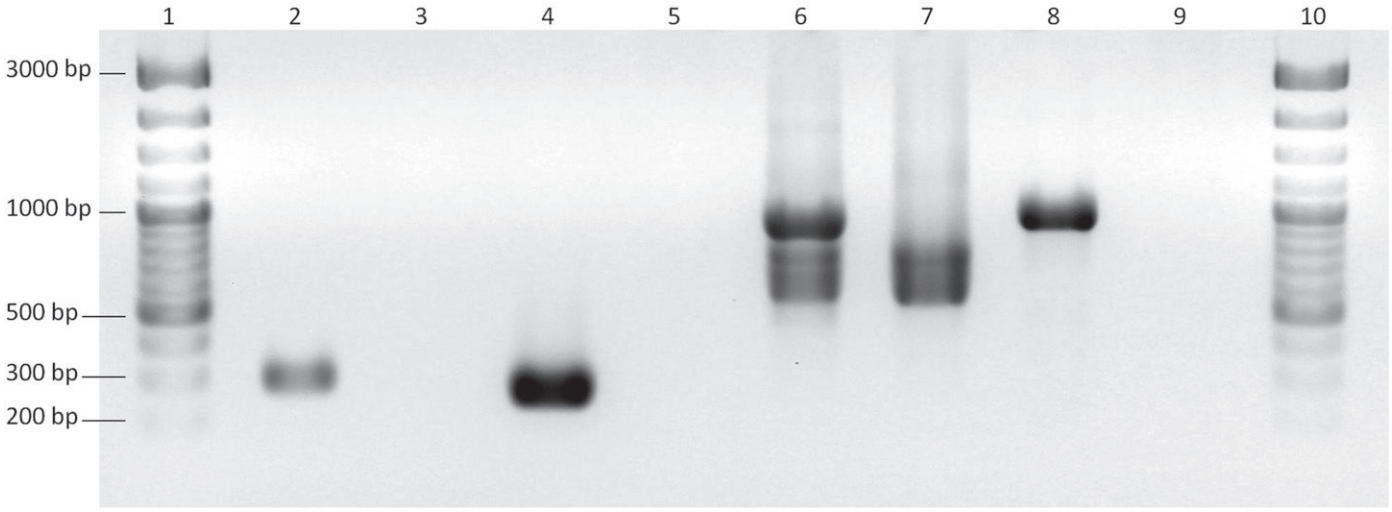

FIGURE 2

Gel (1.5% m/v agarose) image showing *Phytophthora cinnamomi*-specific PCR (lanes 2 to 5, 300-bp fragment of *Ypt* gene, using primers Ycin3F and Ycin4R) and confirmation of amplifiable DNA (lanes 6 to 9, using primers ITS1 and ITS4): marker (lanes 1 and 10), *P. cinnamomi* positive control, isolate RF5 (1.5×10^{-2} ng/PCR) for *Ypt* gene (lane 2) and internal transcribed spacer region (lane 8), no-DNA template (3 and 9), plot 360 *P. cinnamomi* positive (4 and 6), and plot 126 *P. cinnamomi* negative (5 and 7).

convenient and required less operator time, elapsed time, and lab space. Traditional baiting and culturing is relatively time constrained: cultures must be transferred to new media within a particular window of time—and likely transferred multiple times to produce isolates for identification. Each transfer step requires preparation of sterile media and sterile space and can require hours of technician time. In contrast, the bait-PCR method was more flexible: after the baiting step, bait discs were stored at –20°C until it was convenient to extract DNA and proceed with PCR. In addition, traditional baiting and culturing can require relatively large volumes of soil, with large space requirements for storage and incubation. In contrast, the bait-PCR method using 50-ml tubes required little storage and incubation space during the baiting phase (50-ml tubes could be stacked neatly in racks) and even less space after baiting (bait discs were transferred to 1.5-ml tubes). The similarity between the leaf disc bait and PCR method and the full leaf bait and culture method suggests that the space and time requirements of screening for *P. cinnamomi* can be reduced without sacrificing sensitivity. If incubation space is not limiting, and if propagule density is low, the volume of soil used for baiting can be increased to likely improve sensitivity. Subsequently, extracting DNA from baits and proceeding with PCR reduces space and time required for screening and improves flexibility.

P. cinnamomi Distribution Within the Clemons Fork Watershed

In this study, *P. cinnamomi* was detected (by one or more screening methods) in a total of 21 plots out of 47 sampled (44%). Plots in which *P. cinnamomi* was detected ranged from xeric ridgetop sites to sites located in natural drainage areas, including plots near perennial streams (Fig. 3). Although *P. cinnamomi*-associated disease is typically thought to be spatially restricted to moist, poorly drained soils (Dawson and Weste 1985; Keith et al. 2012; Vannini et al. 2010), the pathogen itself has also been recovered from dry sites with little to no observable disease symptoms (Shea and Dell 1981). Our results support the observation that *P. cinnamomi* occurs across a variety of environmental conditions; however, further research will be necessary to evaluate the impact *P. cinnamomi* has on susceptible hosts across this range of environmental conditions.

The soil physical and chemical variables measured may shed some light on environmental constraints to *P. cinnamomi* distribution (Table 2). Soils in which *P. cinnamomi* was detected were characterized by lower pH, suggesting a tolerance for acidic soils. *P. cinnamomi* also tended to be detected in soils with lower cation concentrations (Ca, Mg, and Zn), which may relate to osmotic sensitivity of zoospores (Byrt et al. 1982). Finally, soil texture appeared to differ between these sample groups, with soils in

TABLE 1
Assay screening results for forest soils collected from 47 plots in Robinson Forest, Eastern Kentucky, U.S.A.[a]

Plot number	Full leaf bait and culture	Leaf disc bait and culture	Leaf disc bait and PCR
102	+	–	–
103	–	–	–
104	–	+	+
113	–	–	–
114	–	–	+
116	–	–	+
117	+	+	+
118	+	+	+
119	+	+	+
120	+	–	+
126	+	–	–
127	–	–	–
128	–	–	–
129	–	–	–
130	+	–	+
131	–	–	–
132	–	–	–
143	–	–	–
144	–	–	–
145	+	+	+
146	–	–	–
160	–	–	–
161	–	–	–
163	–	–	–
178	–	–	–
179	–	–	–
181	–	–	–
184	–	–	–
185	–	–	–
194	–	–	–
195	+	+	+
198	+	–	+
199	+	+	+
226	–	–	–
240	–	+	–
241	+	–	–
345	–	–	–
346	–	–	–
351	–	–	–
355	–	–	–
356	–	+	+
357	–	–	–
360	–	–	+
361	+	–	–
367	–	–	–
368	+	–	–
369	+	+	+
Plots positive	15	10	15
Plots negative	32	37	32
False negatives	2	3	0

[a] Positive indicates plots in which *Phytophthora cinnamomi* was detected by a given assay, and negative indicates plots in which *P. cinnamomi* was not detected by a given assay. False negatives were defined as plots screened as negative by one method but positive by the two other methods. + = *P. cinnamomi* detected; – = *P. cinnamomi* not detected; and PCR = polymerase chain reaction.

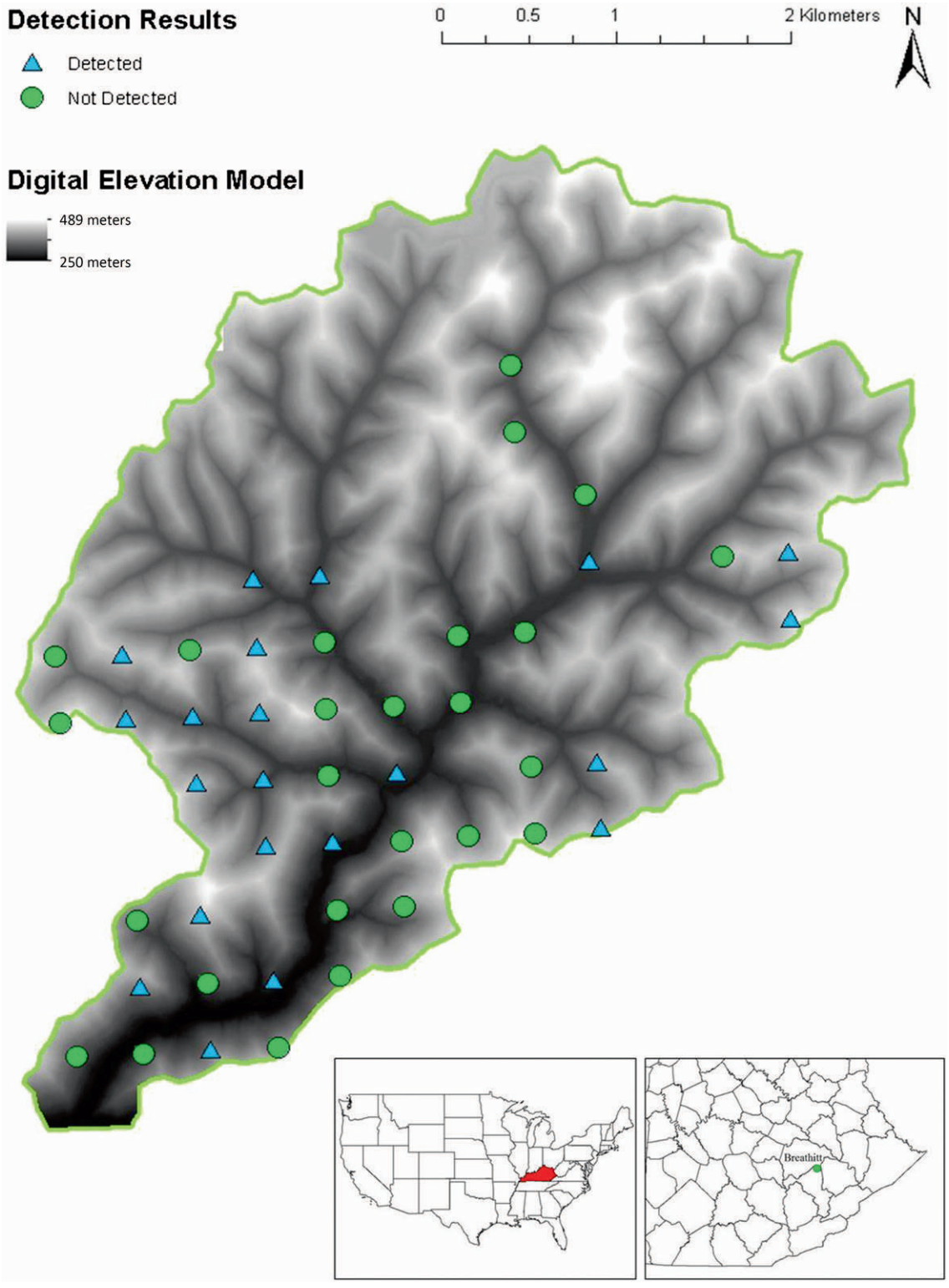

FIGURE 3

Phytophthora cinnamomi distribution map, depicting screening results for 47 samples collected within the Clemons Fork Watershed, Robinson Forest, Kentucky, U.S.A.

which *P. cinnamomi* was detected characterized by lower percent sand and higher percent silt and clay. These results are consistent with traditional associations of *P. cinnamomi* with fine-textured soils (Dawson and Weste 1985; Keith et al. 2012; Vannini et al. 2010). Broader surveys are necessary to characterize landscape-scale distribution patterns of *P. cinnamomi* in central Appalachian forest soils.

Recommendations for Practitioners

This study suggests that detection assays for *P. cinnamomi* can be further developed to reduce time and space required for screening, without sacrificing sensitivity. Specifically, our study supports the use of PCR on DNA extracted from leaf disc baits for high-throughput detection of *P. cinnamomi* from relatively small

TABLE 2
Soil physical and chemical characteristics (means ± SE) of soil samples in which *Phytophthora cinnamomi* was detected or not detected by any of three screening assays[a]

Characteristic	Detected	Not detected	*P* value
Soil pH	3.80 ± 0.12	4.31 ± 0.14	**0.01**
P (mg/kg)	10.9 ± 1.1	13.7 ± 1.2	0.09
K (mg/kg)	90.3 ± 6.7	110.6 ± 10.5	0.11
Ca (mg/kg)	320.3 ± 60.7	709.5 ± 124.0	**0.008**
Mg (mg/kg)	79.5 ± 8.0	138.6 ± 20.9	**0.01**
Zn (mg/kg)	2.4 ± 0.24	3.2 ± 0.45	**0.001**
SOM (%)	7.72 ± 0.92	7.26 ± 0.58	0.67
Total N (%)	0.215 ± 0.02	0.233 ± 0.02	0.53
% Sand	44.6 ± 3.6	59.7 ± 2.3	**0.001**
% Silt	41.8 ± 3.0	29.6 ± 1.8	**0.001**
% Clay	13.6 ± 0.82	10.8 ± 0.53	**0.007**
% Fines	55.4 ± 3.6	40.3 ± 2.3	**0.001**
Plant available water (%)	21.1 ± 1.6	16.8 ± 0.94	**0.03**
Field capacity (%)	36.7 ± 2.2	32.0 ± 1.3	0.08
Wilting point water (%)	15.6 ± 0.93	15.2 ± 0.90	0.73

[a] *P* values of *t* tests (unequal variance assumed) are shown, with $P < 0.05$ considered significant (significant *P* values shown in bold). SOM = soil organic matter.

soil samples. However, results using this method should be interpreted with caution, and acceptable thresholds for false positives and negatives should be considered before deploying this method in future experiments. This approach can be customized to meet specific investigator needs: for example, sample size used for baiting can be increased to improve sensitivity, if lab space is not limited. In addition, this study demonstrates that *P. cinnamomi* is not restricted to moist lowland soils but is also capable of survival in dry ridge-top soils. This suggests that dry ridge-top sites are not necessarily *Phytophthora*-free and may not be ideal sites for restoration of susceptible species.

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