ABSTRACT

SCHOENBAUM, ELIZABETH A. Genotypic Characterization of *Phytophthora cinnamomi* from Ornamental Crops in North Carolina. (Under the direction of Dr. D. Michael Benson and Dr. Ignazio Carbone.)

Forty-two *Phytophthora cinnamomi* isolates from *Camellia* spp., *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. were characterized for mating type, mefenoxam fungicide sensitivity, and aggressiveness on *Rhododendron* 'Hino de Giri'. Isolates collected from *Camellia* spp. were of the A1 mating type, while isolates from the other host plants were A2. All isolates were sensitive to mefenoxam at 100 ppm and all but one was sensitive at 1 ppm. Isolates from *Rhododendron* spp. scored higher average foliar disease and root rot ratings, while A1 isolates from *Camellia* spp. had the lowest average foliar disease and root rot ratings. The population sample of 42 isolates was also examined for DNA sequence polymorphisms in two nuclear loci, beta-tubulin (Btu) and a portion of the intergenic spacer (IGS) region of the nuclear rDNA repeat, and one mitochondrial DNA locus, cytochrome c oxidase subunit 1 (COX 1). Six base substitutions were found among the 42 isolates with a multi-locus data set. Isolates grouped into four haplotypes. Haplotype grouping corresponded to isolate mating type, plant host, and heterozygosity in the Btu locus. Our inferred multilocus rooted gene genealogy revealed a putative ancestral lineage representing the most frequently sampled haplotype in the population. This haplotype contained A2 isolates collected from *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp.. Isolates of the A1 mating type diverged more recently in the genealogy. There is an increase in heterozygosity at the Btu locus that coincides with the appearance of the A1 mating type. These findings increase our understanding of the population structure of *P. cinnamomi*.

Genotypic Characterization of *Phytophthora cinnamomi* from Ornamental Crops in North Carolina

by
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in partial fulfillment of the
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Plant Pathology

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DEDICATION

For my family and friends. For Jeff.

BIOGRAPHY

Elizabeth Schoenbaum received her Bachelor of Science degree in Horticultural Science with a minor in Agricultural Business Management from North Carolina State University. She graduated summa cum laude.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

GENERAL INTRODUCTION

Pathogen introduction. *Phytophthora cinnamomi* is a soilborne member of the Oomycota phylum. Members of this phylum produce mycelium and absorb nutrients, like true fungi, but Oomycota are not classified as Fungi due to several differences. Oomycota are diploid and produce oospores. True fungi do not produce oospores and are haploid or dikaryotic. Oomycota have cell walls composed of beta glucans and cellulose. True fungi have cell walls containing chitin. Both groups have members that produce motile zoospores, but only Oomycota have two kinds of flagella (whiplash and tinsel flagellum). Another difference occurs in the mitochondrial cristae morphology. Oomycota have tubular cristae while Fungi have flattened cristae. Oomycota are placed within the Stramenopiles divisions, along with other organisms possessing tubular cristae as well as tripartite tubular hairs.

Phytophthora cinnamomi was first described by Rands in 1922 after being isolated from Sumatran cinnamon trees. This pathogen has now been found throughout the world and is known to infect over 2,000 species of plants, many with economic importance such as *Abies fraseri* (Fraser fir), *Eucalyptus* spp., *Persea americana* (avocado), *Pinus* spp. (pine), *Quercus* spp. (oak), and species in the Ericaceae family (*Rhododendron* spp., etc.) (Zentmyer 1980; Hardham 2005). Due to wide distribution and host range, *P. cinnamomi* causes damage within a range of economic spheres from native forests to ornamental crops to forestry plantations.

Morphological characteristics and growth. *P. cinnamomi* forms noncaducous, nonpapillate, terminally-borne, ovoid to elongate, obpyriform sporangia (Erwin and Ribeiro 1996). Sporangia size and shape may vary (Erwin and Ribeiro 1996). Sporangia are not formed in pure culture but are produced in nonsterile soil extract or in weak mineral solutions (Chen Zentmyer solution) (Zentmyer 1980; Erwin and Ribeiro 1996). Zoospores may be released from sporangia under optimum temperature, pH, and soil matric potential (Benson 1984). Chlamydospores of the pathogen are often borne in clusters of three to ten, terminally or intercalary in the mycelium (Erwin and

Ribeiro 1996). They are globose, thin-walled, range in size from 31 to 50 µm, and are produced in culture (Erwin and Ribeiro 1996). *P. cinnamomi* is heterothallic, requiring A1 and A2 mating types to form oospores (Galindo-A. and Zentmyer 1964). Of the two mating types, A2 occurs most frequently (Zentmyer 1980). Self-fertilization by a single mating type may occur in response to volatiles from *Trichoderma viride*, colony aging, or other agents like avocado root extract (Brasier 1992). Some A2 isolates appeared to be self-fertile in planta, producing oospores in the roots of container grown *Acacia pulchella* although the presence of *Trichoderma* was not determined (Jayasekera et al 2007). Antheridia are 19 x 17 µm, amphigynous, and may be bicellular (Erwin and Ribeiro 1996). Oogonia are 21 to 58 µm in diameter, nearly transparent to yellow brown, round, smooth-walled, and may have a tapered base (Erwin and Ribeiro 1996). Oospores are round, nearly transparent to yellow brown, and 19 to 45 µm in diameter depending on media (Erwin and Ribeiro 1996). Cultures of *P. cinnamomi* have coralloid hyphae and abundant hyphal swellings, distinguishing them from other *Phytophthora* species (Erwin and Ribeiro 1996). Optimal growth of *P. cinnamomi* occurs at 24 to 28° C (Erwin and Ribeiro 1996). The maximum temperature range for growth is 32 to 34° C and the minimum is 5 to 6° C (Erwin and Ribeiro 1996). Morphological characteristics have been used to identify *P. cinnamomi*. This approach is limited by the morphological plasticity (Cooke et al. 2000; Hardham 2005).

Infection symptoms and characteristics. Plants infected with *Phytophthora cinnamomi* typically exhibit rotting of feeder roots (Zentmyer 1980). Lesions may spread to larger roots and may cause stem cankers (Zentmyer 1980). As the root rot progresses, plant foliage may wilt, become chlorotic, and die back (Zentmyer 1980; Erwin and Ribeiro 1996). Infected plants can collapse suddenly and die, gradually decline, or may remain symptom-free for several years (Zentmyer 1980; Erwin and Ribeiro 1996). Moisture and temperature may play a role in the rate of decline. In cool, damp conditions symptom-free infected plants are more likely to occur (Erwin and Ribeiro 1996).

Control of *Phytophthora cinnamomi*. Movement of symptom-free infected plants is one means of dispersal for *P. cinnamomi*. Water, soil, and contaminated vehicles, animals, equipment, and people can also disperse pathogen propagules (Erwin and Ribeiro 1996). This is of particular concern in a nursery environment where common industry practices, like the reuse of irrigation water, may

distribute propagules shed by symptom-free but infected plants. A study by MacDonald et al in 1994 found *Phytophthora cinnamomi* in irrigation-pond effluent collected from California container nurseries. Other studies have also found *Phytophthora* spp. in recirculated irrigation water used by ornamental plant nurseries (Bush et al 2003; Themann et al 2002). Therefore, it is important for container nurseries to obtain clean planting stock and to follow sanitary nursery practices, like the filtration of recirculated irrigation water. In a recent study, Ufer et al (2008) demonstrated the efficacy of slow sand filtration and lava grain filtration for the removal of *Phytophthora* spp. from recirculated irrigation water. Both filtration systems eliminated *Phytophthora* from the irrigation water. Filtered water must be stored in reservoirs that are sealed off from soil to prevent recontamination of water.

Chemical controls are also used by ornamental plant nurseries to control *Phytophthora*. Two fungicides, mefenoxam and fosetyl-AI or potassium phosphonate, are recommended for the chemical control of *P. cinnamomi* (North Carolina Agricultural Chemicals Manual, 2008). This study is concerned with the use of mefenoxam as a control. Mefenoxam resistance has been reported in some *Phytophthora* spp. in ornamental plants within North Carolina (Hwang and Benson 2005). However, mefenoxam resistance has not been reported in *P. cinnamomi*. Duan et al (2008) screened 51 isolates of *P. cinnamomi* for mefenoxam resistance and found all to be sensitive.

Aggressiveness. Previous aggressiveness studies with *P. cinnamomi* have shown variation in aggressiveness among isolates on different host plants. Zentmyer and Guillemet (1981) found that an A2 isolate of *P. cinnamomi* from avocado (*Persea americana*) was capable of causing disease on avocado but not camellia (*Camellia* spp.), while an A1 isolate from camellia was pathogenic on avocado and camellia. Unfortunately, Zentmyer and Guillemet's study only examined two isolates. Weste (1975) also found a difference in the level of aggressiveness of A2 and A1 isolates of *P. cinnamomi* on *Nothofagus cunninghamii*. Isolates of the two mating types were pathogenic on *N. cunninghamii*, but plants inoculated with A2 isolates, collected from *Isopogon ceratophyllus*, had a 60% mortality rate while those inoculated with A1 isolates, collected from *Tristania conferta*, had a 100% mortality rate (Weste 1975).

More recent studies by Dudzinski et al (1993), Robin and Desprez-Loustau (1998) and Huberli et al (2001) found variation in aggressiveness was not tied to host specificity.

Dudzinski et al (1993) used multiple criteria to measure aggressiveness: onset of disease symptoms, number of days until plant death, dry weight of top growth and root volume. *Eucalyptus marginata* seedlings were inoculated with 42 isolates collected within Australia from different hosts (including *E. marginata*). Aggressiveness was not linked to isolate host origin and did not correlate with mating type.

Robin and Desprez-Loustau (1998) measured lesion length on chestnut, eucalyptus, oak, and pine seedlings to rate the aggressiveness of 48 isolates collected primarily within France from 16 hosts (including chestnut, eucalyptus, oak, and pine). Some individual isolates consistently showed higher or lower levels of aggressiveness across hosts. This variation in aggressiveness occurred without any correspondence to origin host plant, isolate mating type or age of isolate in culture.

Huberli et al (2001) examined the aggressiveness of 73 Australian isolates from 2 hosts (*E. marginata* and *Corymbia calophylla*) on plants of *E. marginata* and *C. calophylla*. Lesion length and plant survival were used to determine aggressiveness. Aggressiveness was not found to be host specific.

In another study, Podger (1989) also found no relationship between aggressiveness and isolate isotype, mating type, climate of origin, or the taxonomic family of host plant. Interestingly, both Podger and Weste (1975) included the same A1 isolate [A12 (I.M.I. 158786)] in host specificity studies. Weste (1975) found this isolate to be more aggressive than A2 isolates on *N. cunninghamii*, but Podger (1989) did not find a similar pattern of aggressiveness in this isolate or between A1 and A2 isolates of *P. cinnamomi* when testing for aggressiveness on 21 host species.

Molecular markers. Various molecular markers have been examined as means of identifying and characterizing the genetic structure of *P. cinnamomi*. Isozymes (Linde et al 1997; Old et al 1988), microsatellite polymorphisms (Dobrowolski et al 2003), single nucleotide polymorphisms (SNP) (Lee and Taylor 1992), amplified fragment length polymorphisms (AFLP) (Duan et al 2008),

and restriction fragment length polymorphisms (RFLP) (Linde et al 1999) have been used to look for nucleic acid sequence differences.

Isozyme analysis identified several multilocus genotypes of *P. cinnamomi* were found in Papua New Guinea isolates, but only three multilocus genotypes were found for isolates from Australia (Old et al 1988), one for the A1 mating type and two for A2. Genotypes found were common to isolates from around the world, indicating clonal asexual spread (Dobrowolski et al. 2003).

Pathogen variation in South Africa was likewise limited. Linde et al (1997) looked at the isozyme analysis for 135 South African *P. cinnamomi* isolates collected from 1977 to 1986 and 1991 to 1993. There was little heterozygosity and low genetic variation within the population (Linde et al 1997). Between *P. cinnamomi* isolate mating types, A1 and A2, there was high genetic distance, while there was low genetic distance between isolate collection groups, 1977-1986 and 1991-1993, and between isolate collection location groups, the Cape region and the Mpumalanga region (Linde et al 1997). These findings indicate *P. cinnamomi* is a clonally spreading organism.

Microsatellites also indicate worldwide clonal lineages and the predominance of asexual reproduction in *P. cinnamomi*. Analysis of Australian isolates for microsatellite polymorphisms found three genotype groups, one for the A1 mating type and two for A2 (Dobrowolski et al. 2003). Further sampling showed that these groups were widely distributed geographically (Dobrowolski et al. 2003).

Work by Linde et al (1999) using RFLP analysis of South African and Australian isolates found low levels of genetic distance (D_m = 0.003) between the two geographic areas. Mating type RFLP genotypes were shared between the two countries.

An AFLP analysis of a population of South Carolina isolates of *P. cinnamomi* from 23 host plant genera, including rhododendron, camellia, holly, and juniper, also report genetic dissimilarity between A1 and A2 isolates (Duan et al 2008). An examination of 200 informative loci in 49 A2 isolates and two A1 isolates revealed a higher number of polymorphisms in the A1 isolates; while A2 isolates had reduced genetic variation (Duan et al 2008).

PCR amplification and sequencing has been used to identify interspecific differences within *Phytophthora*. Nucleotide sequence polymorphisms in amplified regions have been discovered in the internal transcribed spacer (ITS) of the rRNA gene (Lee and Taylor 1992) and the *Lpv* putative storage protein genes (Kong et al 2003). The ITS region of rDNA is conserved within *P. cinnamomi* but polymorphisms were used by Lee and Taylor (1992) to distinguish five *Phytophthora* species: *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. megakarya*, and *P. palmivora*. This region is useful for examining the systematics of *P. cinnamomi* and other *Phytophthora* spp. and for new species delimitation (Cooke and Duncan 1997; Cooke et al 2000; Ristaino et al 1998). Polymorphisms in the *Lpv* putative storage protein genes also have been used to distinguish *P. cinnamomi* from other *Phytophthora* species (Kong et al 2003).

Limited molecular systematics has been done with other *Phytophthora* species or with *Phytophthora* as a genus. Interspecific variation but little intraspecific variation in the mitochondrial gene cytochrome oxidase I (COX I) has been reported in *Phytophthora* (Martin and Tooley 2003) although *P. cinnamomi* was not included. Intraspecific variation was found in COX I between European and American isolates of *P. ramorum* (Kroon et al. 2004). The intergenic spacer (IGS 2) region of the nuclear rDNA repeat was sequenced for a sample set of *P. medicaginis* and several other *Phytophthora*, including one *P. cinnamomi* isolate (Liew et al. 1998). Reduced intraspecific variation was reported within *P. medicaginis* and there was high sequence similarity between *P. medicaginis*, *P. trifolii*, and *P. megasperma* (Liew et al. 1998).

Overall, there is a scarcity of intraspecific molecular markers for population genetic analyses of *P. cinnamomi*. Past work using isozymes, microsatellites, RFLPs, and AFLPs has shown a lack of variation in *P. cinnamomi* populations. Based on isozyme (Old et al 1988) and microsatellite analysis (Dobrowolski et al 2003), *P. cinnamomi* appears to be represented worldwide by three clonal lineages. However, population analysis of *P. cinnamomi* using SNPs could reveal further genetic differentiation within *P. cinnamomi*. Examining nucleic or mitochondrial DNA could reveal additional lineages within *P. cinnamomi*. In *P. ramorum* there are three distinct lineages based on concordance in AFLP, microsatellite, and mitochondrial sequence markers (Ivors et al 2004; Ivors et al 2006; Martin 2008).

A multilocus analysis of *P. cinnamomi* using mitochondrial and nuclear DNA sequence may reveal distinct lineages of *P. cinnamomi* restricted by host, geographic, or ecological niche or provide evidence of genetic differentiation within mating type. Further identification and characterization of distinct lineages would allow for genealogical analysis as well as epidemiological work, providing a tool for tracking pathogen spread, persistence, and identifying paths of introduction and migration.

Phylogenetic inference and coalescent analysis. Population structure can be inferred from DNA sequences using phylogenetic and coalescent analyses (Carbone and Kohn 2004). Individuals with shared nucleotide differences at locus are grouped together into haplotypes. Haplotypes can then be analyzed using statistical models to infer population structure, including population subdivision along host or mating type, from the patterns of genetic diversity (Carbone and Kohn 2004; Hudson 2000). These inferences are strengthened when they are supported by sequences from multiple loci. Both mitochondrial and nuclear loci should be examined due to their differing modes of inheritance within *Phytophthora*. In *Phytophthora infestans* mitochondrial DNA is exclusively maternal, with no recombination, while nuclear DNA is inherited from both paternal and maternal parents (Gomez-Alpizar et al 2007; Kroon et al 2004). Haplotypes based on sequence polymorphisms can be coalesced into lineages sharing a common ancestor using a stochastic model known as the n-coalescent (Kingman 1982a; Kingman 1982b; Kingman 1982c; Rosenberg and Nordborg 2002).

From the coalescent analysis we can reconstruct the ancestral history of a population (Carbone and Kohn 2004). By performing multiple simulations of the coalescent process, we can infer the genealogy with the highest root probability (Carbone and Kohn 2004). The coalescent is powerful because i) it can assign polarity in the sample without the need for an outgroup, and ii) it can calculate the probability of mutations associated with different phenotypic categories using maximum likelihood estimates of the data (Carbone and Kohn 2004). Populations that are panmictic or subdivided, with or without recombination, can be modeled using the coalescent (Rosenberg and Nordborg 2002). As long as parents of extant lineages are selected randomly and the majority of mutations are selectively neutral, the coalescent model can determine the most likely ancestral lineage (Rosenberg and Nordborg 2002). For a more detailed explanation of evolutionary and coalescent

methods used in fungal population genetics, see the recent review article by Carbone and Kohn (2004).

Summary. The objective of my research is to develop molecular markers that can be used to examine intraspecific variation of *P. cinnamomi* and to characterize the population structure of *P. cinnamomi* in ornamental plant nurseries across North Carolina, California, Oregon, South Carolina, and Virginia. Given the extensive clonality reported in this species we further examined whether molecular variation was associated with mating type, sensitivity to the fungicide mefenoxam, and aggressiveness on ornamental crops. Polymorphisms were identified in two nuclear loci: beta-tubulin (Btu) and a portion of the intergenic spacer (IGS) region of the nuclear rDNA repeat, and one mitochondrial DNA locus: cytochrome c oxidase subunit 1 (COX 1). DNA sequence variation was used to test for host and geographic differentiation, and to examine the evolution of heterozygosity and mating type within a genealogical and coalescent framework.

Chapter 2

INTRODUCTION

Phytophthora cinnamomi was first described by Rands in 1922 after being isolated from Sumatran cinnamon trees (Hardham 2005). This pathogen has now been found throughout the world and is known to infect over 2,000 species of plants, many with economic importance such as *Abies fraseri* (Fraser fir), *Eucalyptus* spp., *Persea americana* (avocado), *Pinus* spp. (pine), *Quercus* spp. (oak), and species in the Ericaceae family (*Rhododendron* spp., etc.) (Zentmyer 1980; Hardham 2005). Due to wide distribution and host range, *P. cinnamomi* causes damage within a range of economic spheres from native forests to ornamental crops to forestry plantations.

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The objective of this research is to develop molecular markers that can be used to examine intraspecific variation of *P. cinnamomi* and to characterize the population structure of *P. cinnamomi* in ornamental plant nurseries from North Carolina, California, Oregon, South Carolina, and Virginia. Given the extensive clonality reported in this species we further examined whether molecular variation was associated with mating type, sensitivity to the fungicide mefenoxam, and aggressiveness on ornamental crops. Polymorphisms were identified in two nuclear loci: beta-tubulin (Btu) and a portion of the intergenic spacer (IGS) region of the nuclear rDNA repeat, and one mitochondrial DNA locus: cytochrome c oxidase subunit 1 (COX 1). DNA sequence variation was used to test for host

and geographic differentiation, and to examine the evolution of heterozygosity and mating type within a genealogical and coalescent framework.

MATERIALS AND METHODS

Isolation and storage. Isolates of *P. cinnamomi* were collected from six ornamental plant nurseries across North Carolina. Collections were made from symptomatic *Camellia* spp. (n = 6), *Ilex* spp. (n = 5), *Juniperus* spp. (n = 7), and *Rhododendron* spp. (n = 6). Host plants were container grown in a soilless pine bark based medium. Additional isolates from California, Oregon, South Carolina, and Virginia were also included in the population sample (Table 1). *P. cinnamomi* isolates were obtained via direct isolation from infected plant roots. Soil cores were collected arbitrarily from symptomatic plants at nurseries visited. Trowels were sprayed liberally with 70% ethanol and wiped down with cheesecloth before collecting a feeder root and soil core from each plant. One to two cores were collected per plant. Cores were placed in a self-sealing bag, one bag per plant, labeled with the host plant species, cultivar (if available), nursery, and date. Bags were placed in insulated, ice-filled chests. Chests were transported to the lab where the ice was removed and the chests used to hold bagged samples at room temperature until processing. Processing occurred within one to four days of collecting.

Roots were washed in tap water, cut into small segments (5-8 mm long), and then plated onto *Phytophthora* selective V8 media (PARPH-V8) (Shew 1983; Ferguson and Jeffers 1999). Plates of roots were incubated in the dark at room temperature and examined daily for 7 days for growth of *P. cinnamomi*. Agar plugs containing possible *P. cinnamomi* mycelia were transferred to corn meal agar plates. These plates were incubated for 7 to 14 days in the dark at room temperature and examined for coral-like hyphal structure and swellings distinctive of *P. cinnamomi* morphology (Erwin and Ribeiro 1996).

After morphological identification isolates of *P. cinnamomi* were transferred to water agar and incubated for 7 to 14 days in the dark at 25° C. A single hyphal tip and accompanying agar block was transferred to corn meal agar. Plates were incubated in the dark at 25° C and then transferred as plugs to both corn meal agar slants and tubes of sterile deionized water for long-term storage.

ITS identification. All *P. cinnamomi* isolates were positively identified as *P. cinnamomi* by ITS sequencing prior to examining other loci for molecular sequence variation. To do this, we amplified the ITS using primers ITS1 and ITS2 (White et al 1990). Each isolate DNA sequence was compared to GenBank nucleotide sequences using BLASTN to verify species identification. An unrooted ITS species phylogeny was also inferred using unweighted parsimony and PAUP* 4.0 (Swofford 1998) and viewed with TreeView (Page 1996) implemented in SNAP Workbench (Bowden et al 2008; Price and Carbone 2005) for collected isolates plus ITS sequence data accessed in GenBank for *P. cinnamomi* (accession #s DQ988172, DQ988173), *P. cactorum* (accession #s EU106586, EU106588, EU106589), *P. drechsleri* (accession #s AY659464, AY659462, AY659459), *P. cryptogea* (accession #s EU000144, EU000139, EU000127), *P. megasperma* (accession #s DQ821182, DQ512954, DQ512950), *P. cambivora* (accession #s EU000089, EU000094, EU0000145), *P. palmivora* (accession #s DQ987922, DQ987921, DQ987920), and *P. infestans* (accession #s AF266779, AF228084, AF228083).

Results from BLASTN searches showed that all *P. cinnamomi* isolates, with the exception of IC368, collected from *Juniperus* spp. in Johnston County, NC, are conspecific and share a recent common ancestor with *P. cambivora* (Fig. 1). Although IC368 shows 99% DNA sequence similarity (2 SNPs) with *P. cinnamomi* it is more divergent than *P. cinnamomi* is to *P. cambivora* (99.5% sequence identity; 1 SNP) and was therefore excluded from our sample.

Mating type. Mating type was determined for all isolates. Isolates were paired with two A1 isolates, IC934 and IC935 (both from California and collected off *Camellia* spp.), and two A2 isolates, IC391 (collected in North Carolina from Azalea 'Mother's Day') and IC940 (collected in South Carolina from *Ilex crenata*), of *P. cinnamomi* and with themselves. Plugs taken from the actively growing edge of hyphal-tipped isolates and tester isolates were placed mycelial side down approximately 2 cm apart from one another on filtered carrot juice agar (Linde et al 2001) amended with 5mg cholesterol suspended in 5ml deionized water added after the medium cooled to 50° C. Pairings were incubated for 2 weeks in the dark at room temperature and then examined for the production of oospores to determine mating type (Chang et al 1974). All pairings were repeated once.

Fungicide sensitivity. Fungicide sensitivity was determined by comparing isolate growth rate in the presence and absence of the fungicide mefenoxam (Subdue MAXX; Syngenta, Greensboro, NC). Isolates were grown in the dark at room temperature on corn meal agar plates amended with three concentrations of mefenoxam: 0, 1, or 100 ppm mefenoxam active ingredient. Formulated Subdue MAXX (22% mefenoxam) was used for dilutions. Two replicate plates were done per isolate per concentration. Plugs of isolates measuring 0.5cm in diameter were transferred to the center of each plate. Colony diameter was measured after the isolate at 0 ppm mefenoxam grew to the edge of the plate. Colony diameter measurements did not include the initial plug. Isolates were scored as sensitive (growth on amended plates was less than 30% of control), intermediate sensitivity (growth between 30 and 90% of control), and resistant (growth greater than 90% of control). Percentage was derived by dividing the average diameter of the colony on amended plates by the average diameter of the colony on the control plates (Lamour et al 2003).

Aggressiveness tests. A sample of isolates from the population were tested for aggressiveness on azalea plants under both greenhouse and nursery conditions. Rice grain inoculum was prepared by culturing a selected isolate on 25g of long grain rice with 18ml of water in a 250ml flask that had been autoclaved 40 min. on two consecutive days (Holmes and Benson 1994). Cultures were allowed to grow for 14 days prior to use.

Azaleas (*Rhododendron* 'Hino de Giri') were propagated from cuttings in July 2006 and grown in the greenhouse until transplanting on 10 July 2007 into a medium of eight parts pine bark to one part sand with 3 kg dolomitic limestone/m^3 in 2.5 L plastic pots. Slow release fertilizer (4.8 g/pot) was surface applied at transplanting and again on 19 September 2007. Plants were placed under 55% shade cloth on ground fabric with daily sprinkler irrigation (1.9 cm/day) and allowed to establish at the Horticultural Field Lab, North Carolina State University, Raleigh until inoculation on 31 July 2007.

Plants were inoculated by placing two rice grains colonized with a single isolate into each of three holes approx. 2-cm deep in the medium located midway between pot rim and plant stem. The

medium was pushed back around the rice grains after placement. Test design was random complete block with five replications.

The aggressiveness test was repeated in a greenhouse test conducted at Method Road Greenhouse Complex, North Carolina State University, Raleigh. Rice grain inoculum was prepared as stated above. Azaleas (*Rhododendron* 'Hino de Giri') were propagated from cuttings in July 2007 and grown in 50 mm clay pots until transplanted on 21 February 2008 into 100 mm plastic pots containing Fafard 4p mix (Conrad Fafard, Inc., Agawam, MA) and slow release fertilizer (4 g/pot). Pots were watered daily with in-pot drip irrigation. Plants were inoculated on 28 February 2008 with one grain of colonized rice into each of three holes using the inoculation method described above. Test design was random complete block with five replications.

Plant symptoms were rated every two weeks for the nursery and every week in the greenhouse test after the initial onset of symptoms. Foliar disease rating scale was 1=no disease, 2=slight disease, 3=moderate to severe disease, 4=dead plant. Root rot and top weight were recorded at test end on 15 November 2007 for the nursery test and 1 May 2008 for the greenhouse test. Root rot rating scale was 1=healthy, 2=fine roots necrotic, 3=coarse roots necrotic, 4=rot of crown, 5=dead plant.

Statistical analysis. Statistical analysis of fungicide and aggressiveness data was performed using a Waller-Duncan *k*-ratio *t* test within SAS 9.1.3 SP4 for Windows (SAS Institute, Inc., Cary, NC).

DNA extraction. Mycelium for DNA extraction from each isolate was produced by transferring five agar plugs of a given isolate in Petri plates containing 20 ml sterile pea broth. Plates were incubated in the dark at room temperature for 3 to 5 days until mycelial mats reached a size three-fourths as large as the plate surface. Agar plugs were removed and the mycelial mats harvested. Mats were thoroughly rinsed using sterile double-distilled water, lyophilized, and then ground by mortar and pestle with liquid nitrogen. DNA was extracted using Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions.

Primer design. Primers were designed using Primer3 software (Rozen and Skaletsky 2000). Primers for amplifying Btu [BTUF1 (CCATCCTCATGGACCTGGA) and BTUR1 (GAGCGAAACCGATCATGAAG)] were designed from *P. cinnamomi* sequence (GenBank accession # EU080454). Primers for amplifying the IGS were designed from *P. cinnamomi* sequence [IGF1 (AGGGTAGGCTGACTTGTAATTT) GenBank accession # EF661646] and *Sclerotinia sclerotiorum* sequence [NS1R (GAGACAAGCATATGACTAC) Carbone and Kohn 1999)]. Primers for COX1 [NCXF (CGAGCTCCCGGTTTAAGTTT) and NCXR (CAAAACTACTAATAGCATTCCATCCA)] were designed from *P. cinnamomi* sequence (GenBank accession # AY564169).

PCR amplification. For each PCR amplification, the standard reaction mix was 3 µl double-distilled water, 2 µl 10x Buffer, 2 µl 10x dNTPs, 1 µl forward primer at 10uM concentration, 1 µl reverse primer at 10µM concentration, 1 µl Redtaq, and 10 µl of isolate DNA. This mix was amended for select isolates with the addition of 1 µl BSA for amplification.

PCR cycling was performed using an Eppendorf Mastercycler thermocycler (Eppendorf North America, Westbury, NY). The standard PCR thermal program was a single cycle at 94° C for two minutes, followed by 30 cycles of 94° C for 30 seconds, a temperature tailored to the primer for 30 seconds (BTUF1 and BTUR1: 58° C; IGF1 and NS1R: 56° C; NCXF and NCXR: 58° C), and 72° C for one minute. The standard PCR cycle was modified if needed to successfully amplify all isolates. The resulting amplified products were purified using the QIAquick PCR Purification Kit or the Denville IsoPure Kit (Denville Scientific, Inc, Metuchen, NJ), following manufacturer's instructions.

Sequencing. Purified PCR products were sequenced using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were purified using a 96-well plate (Edge Biosystems Performa DTR Version 3; Edge Biosystems, Gaithersburg, MD) according to manufacturer's instructions. Sequencing was performed by the North Carolina State University Genome Sciences Laboratory (Raleigh, NC) using a 373xl DNA Analyzer (Applied Biosystems, Foster City, CA).

Cloning. Heterozygous sites were identified through visual inspection of chromatograms for the presence of multiple peaks. Alternate alleles for heterozygous sites were obtained by direct cloning of PCR templates. Cloning was done using the TOPO TA Cloning Kit for Sequencing (Invitrogen Corporation, Carlsbad, CA) and ten clones for each heterozygous isolate were sequenced according to manufacturer's instructions.

Population genetic analysis. Sequences for each locus were aligned and trimmed using Sequencher Version 4.5 (Gene Codes Corporation, Ann Arbor, MI). Alignments were exported as NEXUS files and imported into SNAP Workbench (Bowden et al 2008; Price and Carbone 2005). Sequences were collapsed into haplotypes using SNAP Map (Aylor et al 2006) and each locus was subjected to population genetic analysis. This involved testing for i) neutrality and departures in population-size constancy using Tajima's *D* (Tajima 1989), Fu and Li's *D** and *F** (Fu and Li 1993), and Fu's *Fs* (Tajima 1989; Fu and Li 1993; Fu 1997), ii) host or geographic subdivision using Hudson's test statistics (Hudson et al 1992; Hudson 2000), and iii) isolation using the MDIV program (Nielsen and Wakeley 2001). If there was little genetic variation we i) combined alignments from all loci using SNAP Combine (Aylor et al 2006), ii) examined the data for recombination using the compatibility method implemented by SNAP Clade (Bowden et al 2008), and iii) identified the largest nonrecombining partition using CladeEx (Bowden et al 2008). This was followed by selecting an appropriate coalescent model, assuming either panmixia or subdivision as implemented in Genetree version 9.0 (Griffiths and Tavare 1994), to reconstruct the ancestral history of *P. cinnamomi* and infer the oldest lineage. Finally, we superimposed phenotypic information (mating type, heterozygosity) on the rooted gene genealogy to examine the relative ages of A1 and A2 mating types and the evolution of heterozygosity.

RESULTS

Population sample. The distribution of the 42 isolates of *P. cinnamomi* grouped by host is shown in Table 1. All isolates from *Camellia* spp. were A1 and all isolates from the roots of *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. were A2 (Table 1). Oospores were infrequently observed in the majority of pairings. Results from BLASTN searches using ITS showed that all *P. cinnamomi* isolates are conspecific (Fig 1).

Fungicide sensitivity. All isolates of *P. cinnamomi* were sensitive at 100 ppm mefenoxam, as no growth was observed on plates at 100 ppm after isolates of *P. cinnamomi* had reached the edge of unamended plates. Forty-one of the forty-two isolates in this study were sensitive at 1 ppm mefenoxam (Table 2). One isolate, IC385 collected from the roots of *Ilex* spp., was intermediately sensitive (growth 30% of control) at 1ppm mefenoxam (Table 2). Actual growth of sensitive isolates at 1 ppm mefenoxam ranged from 0 to 2.4 cm compared to 8.0 cm at 0 ppm.

Aggressiveness tests – foliage disease ratings. Isolates IC382 and IC391, collected from the roots of *Rhododendron* spp., resulted in the most severe foliage disease ratings of azaleas in both the nursery and greenhouse tests (Tables 3 and 4). In the nursery, IC391 resulted in the highest foliage disease rating, 3.4, and IC382 resulted in the second highest foliage disease rating, 2.8 after 107 days at the end of the experiment (Table 3). Within 29 days of inoculation, azaleas in the nursery test inoculated with IC382 and IC391 from *Rhododendron* were symptomatic whereas symptoms were not apparent on azaleas inoculated with the other isolates of *P. cinnamomi* (Table 3). Symptoms also appeared the most rapidly in plants inoculated with isolates IC382 and IC391 that caused the most severe foliage disease ratings by the end of the experiment. In the greenhouse test, azaleas inoculated with IC382 or IC391 also had the highest ratings at the end of the experiment, although disease was not as severe in the greenhouse as in the nursery (Table 4). Within 14 days after inoculation in the greenhouse test, azaleas inoculated with isolate IC391 were symptomatic (Table 4). An isolate collected from the roots of *Camellia* spp., IC139, resulted in the lowest foliage disease rating (1; no

disease) in both the nursery and greenhouse tests (Tables 3 and 4). Non-inoculated azalea did not develop foliar symptoms of disease in either the greenhouse or nursery tests (Tables 3 and 4).

Aggressiveness tests – root rot ratings. Root rot ratings at harvest were the most severe in azaleas inoculated with isolates from *Rhododendron* spp. and were significantly different ($P \leq 0.05$) than the other ratings for azaleas inoculated with isolates from other hosts (Table 5). Isolates of *P. cinnamomi* collected from the roots of azalea caused the most severe root rot in azalea in the nursery test. Root rot ratings were 3.6 and 3.4 for isolates IC391 and IC382, respectively (Table 5). In the greenhouse test, these same isolates killed the azalea test plants (Table 5). Root rot ratings were least severe in azaleas inoculated with isolate IC139, an isolate collected from the roots of *Camellia* spp.. In both the nursery and greenhouse tests, isolate IC139 did not cause root rot of azalea (Table 5). The root rot rating for non-inoculated control plants was a 1 in both field and greenhouse experiments (Table 5).

Aggressiveness tests – plant growth. Fresh top weights at harvest on 15 November 2007 in the nursery test were greatest for plants inoculated with isolates of *P. cinnamomi* collected from the roots of *Camellia* spp. IC139 and IC931. In the nursery test, isolates IC139 and IC931 appeared to cause little disease as top weights of azaleas were not different than the top weights of the non-inoculated control (Table 5). A similar result was found in the greenhouse test (Table 5). The lowest top weights of azalea in both tests were for plants inoculated with the *Rhododendron* spp. isolates IC391 and IC382 (27.2 and 26.2 respectively) (Table 5). Azalea inoculated with the *Rhododendron* spp. isolates, IC391 and IC382, had the lowest top weights (1.0 g) at harvest in the greenhouse test (Table 5).

Sequence polymorphisms. A 627 bp segment of the IGS was sequenced for the entire sample. One sequence polymorphism and two haplotypes were observed in the IGS (Table 6). A 489 bp segment of the Btu was sequenced for the population sample revealing two SNPs and three haplotypes (Table 6). The sample was sequenced for a 718 bp portion of COX1. Three SNPs and four haplotypes were inferred from COX1 (Table 6). A combined multilocus alignment of the three loci, IGS, Btu, and COX1, yielded a total of six SNPs and four haplotypes (Table 6).

Compatibility, recombination and neutrality tests. Compatibility analysis showed no phylogenetic conflict in the single locus or combined data sets and therefore no evidence of recombination. Four different neutrality tests were examined for the combined DNA sequence alignment: Tajima's *D*, Fu and Li's *D**, Fu and Li's *F**, and Fu's F_s. These models of neutral evolution assume population size to be constant, with no migration and no recombination. Fu's tests examine mutations occurring in the genealogy at both internal and external nodes and can be informative in detecting population growth and subdivision; however, the tests differ in their strength of detection. For example, population growth and genetic hitchhiking are best detected by Tajima's *D* and Fu's F_s while Fu and Li's *D** and *F** are better for detecting population subdivision or background selection (Fu and Li 1993; Fu 1997). Nonsignificant test results can also be informative. For example, negative *D* and F_s indicates possibly a selective sweep or population growth while positive values for these tests point to population subdivision or balancing selection.

Neutrality tests did not reject selective neutrality. Tajima's *D* was 1.324 ($P = 0.926$), Fu and Li's *D** was 0.485 ($P = 0.664$), Fu and Li's *F** was 0.821 ($P = 0.803$) and Fu's F_s was 1.576 ($P = 0.809$). A *P*-value of 0.05 or less would indicate a significant deviation from neutral processes. Although neutrality tests were non-significant, all four tests were positive which is consistent with population subdivision. (Fu and Li 1993; Fu 1997)

Population subdivision. Several nonparametric methods were used to test for genetic differentiation among geographic and host phenotypes. The chi-squared (x^2) test tolerates unequal sample sizes and is generally useful except at very high rates of mutation (Hudson 2000; Hudson et al 1992). Hudson's H_{st} is a haplotype-based test that is best with unequal sample sizes, a large sample size, and low rate of mutation, while Hudson's K_s is a sequence-based test that is useful for small sample sizes, a high rate of mutation, and equal sample sizes (Hudson et al 1992). Hudson's nearest neighbor statistic (S_{nn}), is powerful when analyzing samples of variable size and mutation rate (Hudson 2000; Hudson et al 1992).

None of the statistical tests detected subdivision between the two geographic localities: North Carolina comprising 24 isolates and 18 isolates pooled from California, Oregon, South Carolina, and

Virginia. For all of the tests a significant *P*-value, indicating subdivision, would be equal to or less than the critical value of 0.05. The chi-squared permutation test value was 1.466 ($P = 0.747$), Hudson's H_{st} value was -0.010 ($P = 0.464$), Hudson's K_s value was 2.400 ($P = 0.464$), and Hudson's S_{nn} value was 0.463 ($P = 0.747$).

All four tests, however, indicated significant subdivision between *Camellia* spp. isolates (12 isolates) and isolates from *Ilex* spp., *Juniperus* spp., *Rhododendron* spp. (30 isolates). In each test, the *P*-value was significant, being less than the critical value of 0.05. The chi-squared permutation test value was 34.241 ($P \leq 0.001$), Hudson's H_{st} value was 0.622 ($P \leq 0.001$), Hudson's K_s value was 0.899 ($P \leq 0.001$), and Hudson's S_{nn} value was 0.905 ($P \leq 0.001$).

Significant host differentiation was also detected for Btu and COX1 that harbor more than one SNP (Table 7). All tests indicate strong subdivision between isolates from *Camellia* spp. and isolates from *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp., but no subdivision (i.e. panmixis) between *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. (Table 7). Analysis of migration between isolated host groups was analyzed using the MDIV program (Nielson and Wakeley 2001). This showed an almost nonexistent level of migration (Fig. 2). Although small, MDIV can provide an estimate of the rate of migration (M = 0.5) and divergence time since the last common host ancestor (Nielson and Wakeley 2001). In this case, it was not possible to estimate a reliable time of divergence between the two sample sets due to insufficient data; there is no peak in the time distribution likelihood surface (Fig. 2). The data provides no resolution of the ancestral host population prior to divergence.

Heterozygosity. Heterozygous sites were found in Btu and confirmed by cloning PCR products. Variable sites grouped into the same haplotypes shown in the multilocus SNP map (Table 6). All the isolates in haplotype 1 were heterozygous at sites 150 and 183; all the isolates in haplotypes 2 and 3 had an additional heterozygous site at variable position 150; isolates in haplotype 4 were heterozygous only at position 129 in the multiple sequence alignment.

Coalescent analysis. We examined the evolutionary history of *P. cinnamomi* using a coalescent model with subdivision and a backward migration matrix of symmetrical rates (M=0.5)

between the *Camellia* spp. host group and the mixed host group comprising *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. (Fig. 2). The rooted multilocus gene genealogy is shown in Figure 3. The genealogy shows three ancestral lineages. The most recent common ancestor of *P. cinnamomi* and the oldest lineage is represented by haplotype H2, which is of the A2 mating type. One A1 isolate from *Camellia* spp. (haplotype H3) defines a second distinct *P. cinnamomi* lineage. Haplotype H4 is the most recent common ancestor of a third ancestral lineage with haplotype H1 as an intermediate descendant.

DISCUSSION

The A2 mating type represented 75 % of the 18 *P. cinnamomi* isolates collected for this study from *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. growing in North Carolina nurseries. The A1 mating type was only recovered from *Camellia* spp. in our survey. However, in previous surveys, the A1 mating type has also been recovered from *Juniperus* spp. and *Pinus strobus* in North Carolina (Benson, pers. commun.). In a South Carolina survey for *P. cinnamomi*, Duan et al (2008) arbitrarily selected 51 isolates collected from 1995-2000 from diseased ornamental plant submissions to the Clemson University Plant Problem Clinic. The isolates were from 23 plant genera. A1 mating type isolates represented 4 % of the isolates included in the survey. One A1 mating type isolate was from *Camellia* spp. and the other was from *Gardenia jasminoides*. The out of state isolates in our survey were composed of 33 % A1 mating type isolates, all from *Camellia* spp.. There were 12 out of state A2 mating type isolates, all from *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. hosts. Previous studies have found a similarly narrow host range for the A1 mating type (Duan et al 2008; Galindo and Zentmyer 1964). In the first report of mating types within *P. cinnamomi*, Galindo and Zentmyer (1964) found two A1 mating type isolates from a collection of 28 *P. cinnamomi* isolates. Isolates were obtained from avocado, cinchona, conifer, *Erica*, macadamia nut, pine, and rhododendron/azalea, but only two Hawaiian isolates collected from macadamia nut cankers were A1 (Galindo and Zentmyer 1964).

All 42 isolates of *P. cinnamomi* representing both A1 and A2 mating types from North Carolina nurseries as well as out of state isolates from ornamental hosts were sensitive to mefenoxam at 100 ppm with a range of sensitivity at 1ppm. Similarly, Duan et al (2008) found no mefenoxam resistance in 51 *P. cinnamomi* isolates from South Carolina collected from roots of 23 genera of ornamental plants. Although resistance to mefenoxam has not been reported in isolates of *P. cinnamomi*, resistance to this fungicide or its parent isomer metalaxyl has been well documented in other species such as *P. nicotianae* and *P. drechsleri* from ornamental hosts (Ferrin and Kabashima 1991; Hwang and Benson 2005).

The two A1 mating type isolates of *P. cinnamomi*, representing all isolates of this mating type collected from *Camellia japonica* in North Carolina nurseries, resulted in the least severe foliage disease and root rot ratings of azalea. These two isolates from *Camellia* spp. were less aggressive than other isolates tested on azalea wherein disease rating and top weight of azalea were similar to the uninoculated controls in both the nursery and greenhouse tests. The lower ratings and weights of azaleas inoculated with the A1, *Camellia japonica* isolates may be attributable to mating type or the host origin. Additional aggressiveness tests using A1 isolates from other host plants or A2 isolates from *Camellia* spp. would be informative.

Unlike the A1 mating type isolates, the aggressiveness tests with isolates of the A2 mating type from *Rhododendron* spp., *Juniperus* spp., and *Ilex* spp., did not consistently result in similar disease ratings or top weights of azalea as a mating type group. Azalea inoculated with isolates from *Rhododendron* spp. resulted in more severe disease ratings and top weights than the disease ratings and top weights for azalea inoculated with isolates collected from *Juniperus* spp. and *Ilex* spp. hosts.

Disease ratings for azalea plants inoculated with IC370, one of the two isolates from *Juniperus* spp. host, were moderately severe and top weight was intermediate. Plants inoculated with the other *Juniperus* spp. host isolate, IC374, resulted in root rot and top weights similar to IC370, but foliage ratings were sometimes less severe with isolate IC370. Plants inoculated isolates IC383 and IC384 from *Ilex* spp. had less severe foliage ratings in the nursery test that were significantly different ($P \leq 0.05$) than ratings for isolates of *P. cinnamomi* from *Rhododendron* spp. and *Juniperus* spp., and often grouped with isolates from *Camellia* spp.. In the greenhouse test, foliage ratings for plants inoculated with IC383 and IC384 were moderately severe, and similar to ratings for azalea inoculated with isolates from *Juniperus* spp.. Within both tests, root rot and top weights for azaleas treated with IC383 and IC384 were mixed, sometimes different from and sometimes similar to values for isolates from *Juniperus* spp. or *Camellia* spp. Aggressiveness tests using greater numbers of isolates from *Juniperus* and *Ilex* might clarify these mixed results.

In contrast, azalea inoculated with *Rhododendron* spp. isolates resulted in more severe foliage and root rot ratings and lower top weights. The test plant used in the aggressiveness tests was

an azalea type *Rhododendron* ('Hino de Giri') comparable to the host plants from which the *Rhododendron* spp. isolates were collected. These isolates, IC391 and IC382, resulted in the most severe foliage disease and root rot ratings and the lowest top weights on inoculated azalea. This could be indicative of host specialization. Aggressiveness tests using other host plants would provide more insight into the difference between isolates collected from *Rhododendron* spp. and the other isolates in this study.

Other aggressiveness studies have not found host specialization by *P. cinnamomi*. Huberli et al (2001) examined the aggressiveness of 73 Australian isolates from two hosts (*E. marginata* and *Corymbia calophylla*) on plants of *E. marginata* and *C. calophylla*. When lesion length and plant survival were used to determine aggressiveness no differences were found among the isolates (Huberli et al 2001). Dudzinski et al (1993) used multiple criteria to measure disease: onset of disease symptoms, number of days until plant death, dry weight of top growth and root volume. *Eucalyptus marginata* seedlings were inoculated with 42 isolates of *P. cinnamomi* collected within Australia from different hosts (including *E. marginata*). Again, disease parameters were not linked to isolate host origin nor to mating type. Robin and Desprez-Loustau (1998) measured lesion length on chestnut, eucalyptus, oak, and pine seedlings to rate the aggressiveness of 48 isolates collected primarily within France from 16 hosts (including chestnut, eucalyptus, oak, and pine). Some individual isolates consistently showed higher or lower levels of aggressiveness across hosts. This variation occurred without any correspondence to original host plant, isolate mating type or age of isolate in culture.

The paucity of genetic variation in *P. cinnamomi* has been reported in previous studies (Duan et al 2008; Dobrowolski et al 2003; Huberli et al 2001; Linde et al 1997; Linde et al 1999; Old et al 1988). Multiple marker systems have shown *P. cinnamomi* from multiple continents to have low levels of genetic variation. Isozymes, microsatellite polymorphisms, and AFLP analyses of *P. cinnamomi* isolates in Australia and South Africa have found *P. cinnamomi* groupings to be consistent with mating type (Dobrowolski et al 2003; Huberli et al 2001; Linde et al 1997; Linde et al 1999; Old et al 1988). RFLP analysis of South Carolinian isolates also found division along mating type (Duan et al 2008).

P. cinnamomi appears to be composed of clonal lineages, reproducing asexually, and with opposite mating types coming into contact infrequently. *P. cinnamomi* has a worldwide distribution, but the A2 mating type is more frequently sampled. The A1 mating type has been collected in just a handful of countries, including Australia, South Africa, Papua New Guinea, and the United States (Galindo and Zentmyer 1964; Duan et al 2008). Within the United States, North Carolina, South Carolina, Georgia, California, and Hawaii have reported A1 mating type isolates of *P. cinnamomi* (Duan et al 2008).

Our study included 12 *P. cinnamomi* isolates of the A1 mating type. These isolates were obtained from the same type of host plant, *Camellia* spp., but represented different collection locations: South Carolina, California, and three North Carolina counties. In total, five states were represented in our sample of 42 isolates of *P. cinnamomi*: North Carolina, South Carolina, Oregon, California, and Virginia. Haplotype and sequence based test statistics of the SNP analysis did not reveal subdivision among isolates by collection location. We did find support of subdivision by host, although we cannot rule out mating type as the barrier to gene flow. *Camellia* spp. originating isolates, all A1, differed from *Ilex* spp./*Juniperus* spp./ *Rhododendron* spp originating isolates, all A2.

The *P. cinnamomi* isolates we examined group by mating type in the coalescent analysis. From the coalescent, we infer four haplotypes. The most recent common ancestor of *P. cinnamomi* is haplotype H2, which is of the A2 mating type harboring multiple host species (*Ilex* spp., *Juniperus* spp., and *Rhododendron* spp.). One A1 isolate from *Camellia* spp. defines haplotype H3, but at this point in evolutionary time, all mutations are still derived exclusively from the *Ilex* spp. / *Juniperus* spp. / *Rhododendron* spp. / A2 population (indicated with the number 0 in Figure 3). Both haplotypes H2 and H3 are heterozygous at position 150 in the multiple sequence alignment. The frequency of the A1 mating type increases as the evolution moves forward. With the emergence of the new A1 mating type in the population, there is increased heterozygosity in haplotype H1, with an additional heterozygous site at position 183, and a new heterozygous site at position 129 in haplotype H4.

In conclusion, we did not find evidence of mefenoxam sensitivity and were likewise unable to associate aggressiveness characterization with molecular data. Coalescent analysis of the multi-

locus DNA sequence based alignment did reveal information about the ancestral origins of *P. cinnamomi*. Our data support the A2 mating type as the older mating type, occurring in a generalist ancestor. With the appearance of the A1 mating type, heterozygosity increases. Overall, though, within our population of *P. cinnamomi* isolates there is a low level of genetic variation characteristic of a clonally spread organism.

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Table 1. Isolate number, reference number, source, isolate species, host, collection year, collection location, and mating type of *P. cinnamomi* isolates.

Isolate No.	Reference No. / Source [a]	Isolate species	Host	Collection year	Collection location	Mating Type
IC139	IC139 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'R.L. Wheeler'	2005	Wake Co., NC	A1
IC928	IC928 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'Betty Sette'	2006	Guilford Co., NC	A1
IC929	IC929 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'Wildwood'	2006	Guilford Co., NC	A1
IC930	IC930 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'Marie Bracey'	2006	Orange Co., NC	A1
IC931	IC931 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'Morning Glow'	2006	Orange Co., NC	A1
IC932	IC932 / EAS	*Phytophthora cinnamomi*	*Camellia japonica* 'Duc d'Orleans'	2006	Orange Co., NC	A1
IC933	P2021 / MDC	*Phytophthora cinnamomi*	*Camellia* spp.	1966	California	A1
IC934	P2100 / MDC	*Phytophthora cinnamomi*	*Camellia* spp.	1969	California	A1
IC935	P2170 / MDC	*Phytophthora cinnamomi*	*Camellia* spp.	1972	California	A1
IC936	P2301 / MDC	*Phytophthora cinnamomi*	*Camellia* spp.	1976	California	A1
IC937	P2399 / MDC	*Phytophthora cinnamomi*	*Camellia* spp.	1982	California	A1
IC938	SC.02-1205 / SNJ	*Phytophthora cinnamomi*	*Camellia japonica*	2002	Greenville Co., SC	A1
IC383	IC383 / EAS	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	2005	Johnston Co., NC	A2
IC384	IC384 / EAS	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	2005	Johnston Co., NC	A2
IC385	IC385 / EAS	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	2005	Johnston Co., NC	A2
IC386	IC386 / EAS	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	2005	Johnston Co., NC	A2
IC387	IC387 / EAS	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	2005	Johnston Co., NC	A2
IC896	31B7 / CH	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	unknown	Virginia	A2
IC898	32B3 / CH	*Phytophthora cinnamomi*	*Ilex glabra* 'Shamrock'	unknown	Virginia	A2
IC939	SC.06-0840 / SNJ	*Phytophthora cinnamomi*	*Ilex* x 'Nellie R. Stevens'	2006	Pickens Co., SC	A2
IC940	SC.02-1208 / SNJ	*Phytophthora cinnamomi*	*Ilex crenata*	2002	Georgetown Co., SC	A2
IC367	IC367 / EAS	*Phytophthora cinnamomi*	*Juniperus conferta*	2005	Johnston Co., NC	A2
IC369	IC369 / EAS	*Phytophthora cinnamomi*	*Juniperus conferta*	2005	Johnston Co., NC	A2
IC370	IC370 / EAS	*Phytophthora cinnamomi*	*Juniperus procumbens* 'Nana'	2005	Johnston Co., NC	A2
IC371	IC371 / EAS	*Phytophthora cinnamomi*	*Juniperus procumbens* 'Nana'	2005	Johnston Co., NC	A2
IC372	IC372 / EAS	*Phytophthora cinnamomi*	*Juniperus procumbens* 'Nana'	2005	Johnston Co., NC	A2
IC373	IC373 / EAS	*Phytophthora cinnamomi*	*Juniperus procumbens* 'Nana'	2005	Johnston Co., NC	A2
IC374	IC374 / EAS	*Phytophthora cinnamomi*	*Juniperus procumbens* 'Nana'	2005	Johnston Co., NC	A2
IC897	32B1 / CH	*Phytophthora cinnamomi*	*Juniperus* spp.	unknown	Virginia	A2
IC941	SC.04-1338 / SNJ	*Phytophthora cinnamomi*	*Juniperus* spp.	2004	Pickens Co., SC	A2

Isolate No.	Reference No. / Source [a]	Isolate species	Host	Collection year	Collection location	Mating Type
IC942	SC.02-0403 / SNJ	*Phytophthora cinnamomi*	*Juniperus squamata* 'Blue Star'	2002	Fairfield Co., SC	A2
IC146	2312 / DMB	*Phytophthora cinnamomi*	*Rhododendron* spp.	1971	Mnt. Home, NC	A2
IC152	2386 / DMB	*Phytophthora cinnamomi*	*Rhododendron* spp.	1994	Raleigh, NC	A2
IC364	IC364 / EAS	*Phytophthora cinnamomi*	*Rhododendron* 'Girard's Crimson'	2005	Johnston Co., NC	A2
IC337	IC337 / EAS	*Phytophthora cinnamomi*	*Rhododendron* 'PJM'	2005	Wake Co., NC	A2
IC382	IC382 / EAS	*Phytophthora cinnamomi*	*Rhododendron* 'Snow'	2005	Johnston Co., NC	A2
IC391	IC391 / EAS	*Phytophthora cinnamomi*	*Rhododendron* 'Mother's Day'	2005	Vance County, NC	A2
IC151	2362 / DMB	*Phytophthora cinnamomi*	*Rhododendron* spp.	1980	Canby, Oregon	A2
IC895	29H5 / MDC	*Phytophthora cinnamomi*	*Rhododendron* 'English Roseum'	unknown	Virginia	A2
IC944	SC.05-1023 / SNJ	*Phytophthora cinnamomi*	*Rhododendron* spp.	2005	Greenville Co., SC	A2
IC945	SC.03-0778 / SNJ	*Phytophthora cinnamomi*	*Rhododendron* 'Satsuki'	2003	Charleston Co., SC	A2
IC946	SC.05-0442 / SNJ	*Phytophthora cinnamomi*	*Rhododendron* 'Lord Roberts'	2005	Abbeville Co., SC	A2

[a] Reference number and source for isolates. CH: Chuan Hong (Virginia Tech University, Virginia Beach, VA); DMB: D. Michael Benson (North Carolina State University, Raleigh, NC); EAS: Elizabeth A. Schoenbaum (North Carolina State University, Raleigh, NC); MDC: Michael D. Coffey (University of California, Riverside, CA); SNJ: Steven N. Jeffers (Clemson University, Clemson, SC)

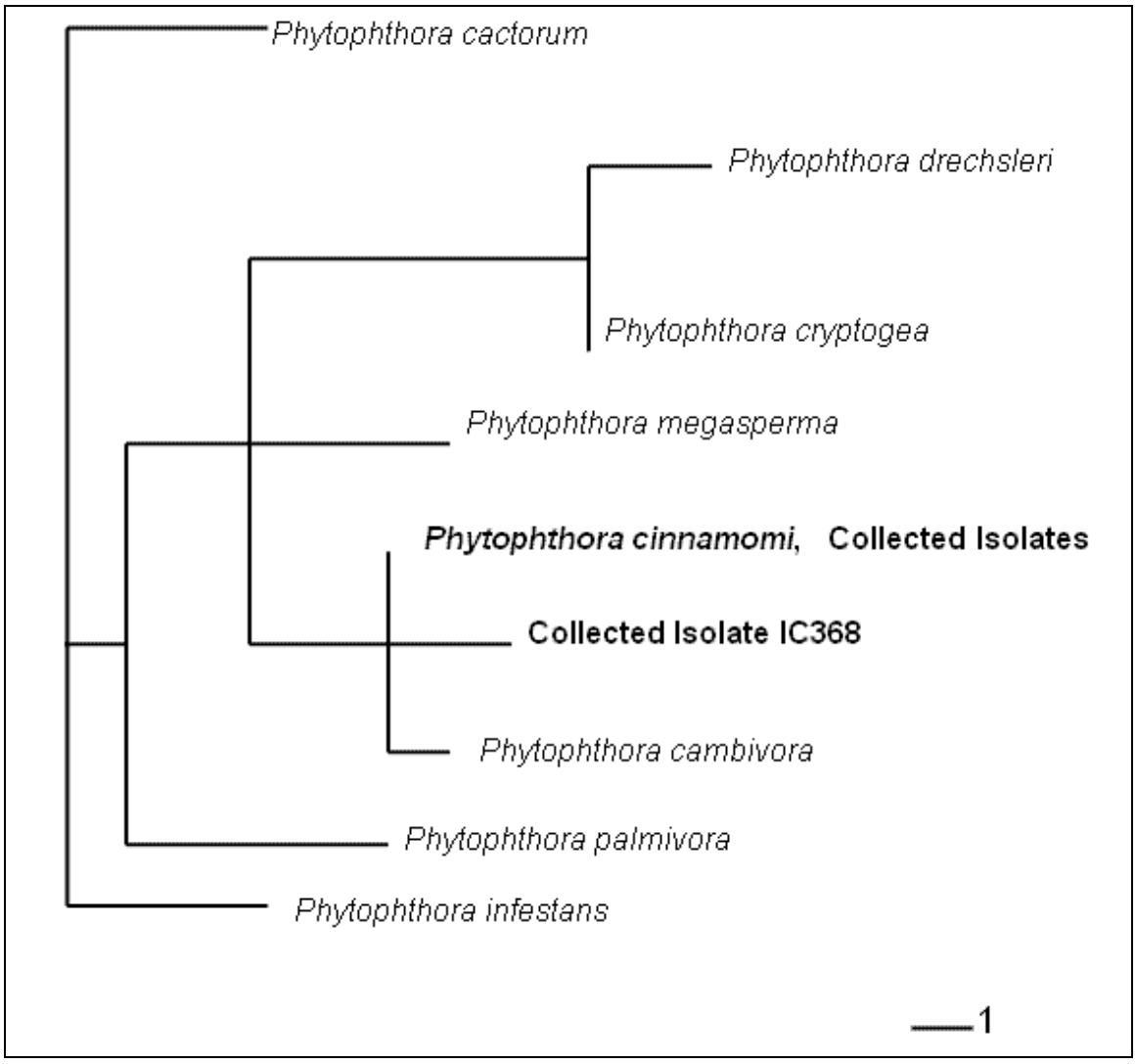

Fig. 1. Internal transcribed spacer (ITS) species phylogeny of isolates collected for this study and GenBank accessioned isolates of *P. cinnamomi* and other *Phytophthora* spp.. The figure shows the conspecificity of GenBank accessioned *P. cinnamomi* and the isolates used in this study. This phylogeny was created using Treeview (Page 1996).

Table 2. Growth of 42 isolates of *Phytophthora cinnamomi* on cornmeal agar (CMA) plates amended with 1 ppm mefenoxam.

Isolate	Mean growth at 1ppm (cm)	Percent growth at 1 ppm
IC139	0.00 f	0
IC146	0.00 f	0
IC151	0.20 ef	2.5
IC152	0.05 f	0.6
IC337	0.00 f	0
IC364	0.05 f	0.6
IC367	0.30 def	3.7
IC369	0.00 f	0
IC370	0.00 f	0
IC371	0.30 def	3.7
IC372	0.35 def	4.3
IC373	0.15 ef	1.8
IC374	1.50 b	18.7
IC382	0.65 cd	8.1
IC383	0.30 def	3.7
IC384	2.20 a	27.5
IC385	2.40 a	30
IC386	2.35 a	29.3
IC387	1.70 b	21.2
IC391	0.35 def	4.3
IC895	0.00 f	0
IC896	0.00 f	0
IC897	0.25 ef	3.1
IC898	0.00 f	0
IC928	0.00 f	0
IC929	0.00 f	0
IC930	0.00 f	0
IC931	0.00 f	0
IC932	0.00 f	0
IC933	0.00 f	0
IC934	0.25 ef	3.1
IC935	0.00 f	0
IC936	0.50 cde	6.2
IC937	0.00 f	0
IC938	0.00 f	0
IC939	1.75 b	21.8
IC940	0.00 f	0
IC941	0.75 c	9.3
IC942	0.25 ef	3.1
IC944	0.35 def	3.1
IC945	0.15 ef	1.8
IC946	0.00 f	0

[a] Values are the means of two replications. Means followed by a different letter are significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t test (k=100).

[b] Percentage was derived by dividing the mean diameter of the colony on CMA plates amended with 1 ppm mefenoxam by the average diameter of the colony on CMA plates amended with 0 ppm mefenoxam once the isolate in the unamended medium reached the edge of the 10-cm-diameter culture plate.

Table 3. Foliage disease ratings [a] in the 2007 nursery test [b] for azaleas inoculated with *Phytophthora cinnamomi* from various hosts [c d].

		Foliage disease rating (1-4)					
Isolate	Isolate host origin	29 Aug	12 Sept	26 Sept	10 Oct	24 Oct	7 Nov
IC391	*Rhododendron* spp.	2.0 a	3.2 a	3.0 a	3.0 a	3.0 a	3.4 a
IC382	*Rhododendron* spp.	2.4 a	2.8 a	2.0 b	2.2 b	2.2 b	2.8 b
IC383	*Ilex* spp.	1.0 b	1.0 c	1.0 c	1.0 d	1.0 d	1.2 d
IC384	*Ilex* spp.	1.0 b	1.4 bc	1.4 bc	1.2 cd	1.2 cd	1.2 d
IC370	*Juniperus* spp.	1.0 b	1.8 b	1.6 bc	1.8 bc	1.8 bc	2.0 c
IC139	*Camellia* spp.	1.0 b	1.0 c	1.0 c	1.0 d	1.0 d	1.0 d
IC931	*Camellia* spp.	1.2 b	1.4 bc	1.0 c	1.0 d	1.0 d	1.0 d
Untreated Control		1.0 b	1.0 c	1.0 c	1.0 d	1.0 d	1.0 d

[a] Foliage disease rating scale: 1=no disease, 2=slight disease, 3=moderate to severe disease, 4=dead plant.
[b] Plants in the nursery test were inoculated on 31 July and then rated every two weeks after symptoms were first observed.
[c] Values are the means of five replications.
[d] Means followed by a different letter within each test column are significantly different ($P = 0.05$) within that test according to the Waller-Duncan k-ratio t test (k=100).

Table 4. Foliage disease ratings [a] in the 2008 greenhouse test [b] for azaleas inoculated with *Phytophthora cinnamomi* from various hosts [c d].

		Foliage disease ratings (1-4)						
Isolate	Isolate host origin	13 Mar	20 Mar	27 Mar	3 Apr	10 Apr	17 Apr	24 Apr
IC391	*Rhododendron* spp.	1.8 a	2.4 a	2.8 ab	3.4 a	3.6 a	3.4 ab	3.8 a
IC382	*Rhododendron* spp.	1.2 b	2.2 ab	3.0 a	3.6 a	3.8 a	3.8 a	3.6 ab
IC383	*Ilex* spp.	1.2 b	1.8 bc	1.8 cd	2.4 bc	2.8 b	2.8 b	3.4 abc
IC384	*Ilex* spp.	1.0 b	1.4 cd	1.4 cd	2.0 cd	2.0 c	1.8 c	2.8 cd
IC374	*Juniperus* spp.	1.0 b	1.0 d	1.2 cd	2.0 cd	2.4 bc	3.0 b	3.0 bcd
IC370	*Juniperus* spp.	1.0 b	1.4 cd	2.0 bc	2.6 b	2.6 b	3.0 b	3.4 abc
IC139	*Camellia* spp.	1.0 b	1.2 d	1.2 cd	1.2 ef	1.0 d	1.0 d	1.4 e
IC931	*Camellia* spp.	1.0 b	1.4 cd	1.6 cd	1.6 de	2.0 c	2.0 c	2.6 d
Untreated Control		1.0 b	1.0 d	1.0 d	1.0 f	1.0 d	1.0 d	1.0 e

[a] Foliage disease rating scale: 1=no disease, 2=slight disease, 3=moderate to severe disease, 4=dead plant.
[b] Plants in the greenhouse test were inoculated on 28 February and then rated every week after symptoms were first observed.
[c] Values are the means of five replications.
[d] Means followed by a different letter within each test column are significantly different ($P = 0.05$) within that test according to the Waller-Duncan *k*-ratio *t* test (k=100).

Table 5. Fresh top weight and root rot rating for azaleas inoculated with *Phytophthora cinnamomi* from various hosts at harvest [a].

		Top weight (g)		Root rot rating [b]	
Treatment	Isolate host origin	Nursery test [c]	Greenhouse test [c]	Nursery test [c]	Greenhouse test [c]
IC391	*Rhododendron* spp.	27.2 b	1.4 c	3.6 a	5.0 a
IC382	*Rhododendron* spp.	26.2 b	1.4 c	3.4 a	5.0 a
IC383	*Ilex* spp.	67.8 a	3.0 bc	2.2 bc	4.2 ab
IC384	*Ilex* spp.	76.2 a	6.0 b	1.8 cd	3.2 c
IC374	*Juniperus* spp.	no data	5.0 bc	no data	3.8 bc
IC370	*Juniperus* spp.	43.6 b	5.4 bc	2.8 ab	3.8 bc
IC139	*Camellia* spp.	81.0 a	13.2 a	1.0 d	1.0 d
IC931	*Camellia* spp.	79.6 a	6.6 b	1.2 d	1.6 d
Untreated Control		85.0 a	14.6 a	1.0 d	1.0 d

[a] Plants were harvested on 15 November 2007 and 1 May 2008, 107 and 62 days after inoculation in the nursery and greenhouse test, respectively. Values are the means of five replications.
[b] Root rot rating scale: 1=healthy, 2=fine roots necrotic, 3=coarse roots necrotic, 4=rot of crown, 5=dead plant.
[c] Means followed by a different letter within each test column are significantly different ($P = 0.05$) within that test according to the Waller-Duncan *k*-ratio *t* test (k=100).

Table 6. Single nucleotide polymorphisms (SNP) map and inferred haplotypes for: intergenic spacer region (IGS), beta-tubulin (Btu), cytochrome c oxidase subunit 1 (COX1), and multi-locus alignment.

Locus	IGS [a]		Btu [b]		COX1 [c]		IGS + Btu + COX 1 [d]	
Position [e]		4		11		556		1111
		7		28		174		110016
		6		93		962		280638
								938514
Site Number [f]		1		12		123		123456
Consensus [g]		T		TT		TGC		TTTGCC
Site Type [h]		t		vt		ttt		vttttt
Character Type [i]		-		--		-i-		i--i--
Haplotype [j] (Frequency [k])	H1(39)	C	H1(12)	.C	H1(12)	..T	H1(12) [l]	.C..T.
	H2(3)	.	H2(28)	A.	H2(25)	CA.	H2(25) [m]	A.CA..
			H3(2)	..	H3(3)	.A.	H3(3) [n]	A..A.T
					H4(2)	...	H4(2) [o]	.....

[a] IGS Haplotypes:
H1: IC139, IC146, IC151, IC152, IC337, IC364, IC367, IC369, IC370, IC371, IC372, IC373, IC374, IC382, IC383, IC384, IC385, IC386, IC387, IC895, IC896, IC897, IC898, IC928, IC929, IC930, IC931, IC932, IC933, IC934, IC935, IC937, IC938, IC939, IC940, IC941, IC942, IC944, IC946
H2: IC391, IC936, IC945
[b] Btu Haplotypes:
H1: IC139, IC151, IC928, IC929, IC930, IC931, IC932, IC933, IC934, IC935, IC937, IC938
H2: IC146, IC152, IC364, IC367, IC369, IC370, IC371, IC372, IC373, IC374, IC382, IC383, IC384, IC385, IC386, IC387, IC391, IC895, IC897, IC898, IC936, IC939, IC940, IC941, IC942, IC944, IC945, IC946;
H3: IC337, IC896
[c] COX1 Haplotypes:
H1: IC139, IC151, IC928, IC929, IC930, IC931, IC932, IC933, IC934, IC935, IC937, IC938
H2: IC146, IC152, IC364, IC367, IC369, IC370, IC371, IC372, IC373, IC374, IC382, IC383, IC384, IC385, IC386, IC387, IC895, IC897, IC898, IC939, IC940, IC941, IC942, IC944, IC946
H3: IC391, IC936, IC945
H4: IC337, IC896
[d] Haplotypes for multi-locus data set:
H1: IC139, IC151, IC928, IC929, IC930, IC931, IC932, IC933, IC934, IC935, IC937, IC938
H2: IC146, IC152, IC364, IC367, IC369, IC370, IC371, IC372, IC373, IC374, IC382, IC383, IC384, IC385, IC386, IC387, IC895, IC897, IC898, IC939, IC940, IC941, IC942, IC944, IC946
H3: IC391, IC936, IC945
H4: IC337, IC896
[e] Position: indicates the variable site in the multiple sequence alignment. The position numbers are shown vertically down the column of the table.
[f] Site number: denotes the number (quantity) of variable sites.
[g] Consensus: shows the consensus haplotype sequence. Haplotype sequences that match the consensus are indicated with a dot in the single nucleotide polymorphism (SNP) map following haplotype designation and frequency.
[h] Site type: the variable site is characterized as a transition (t) or transversion (v),
[i] Character Type: the variable site is characterized as informative (i) or uninformative (-).
[j] Haplotype designation.
[k] Frequency of haplotype in sample.

[l] Isolates are heterozygous at positions 150 and 183 in the Btu locus.
[m] Isolates are heterozygous at position 150 in the Btu locus.
[n] Isolates are heterozygous at positions 150 in the Btu locus.
[o] Isolates are heterozygous at positions 129 in the Btu locus.

Table 7. Population subdivision tests for host differentiation at Btu and at COX1 loci in isolates of *Phytophthora cinnamomi* from host plants: *Camellia* spp., *Juniperus* spp., *Ilex* spp., and *Rhododendron* spp..

Btu		x^2 [a]	Hudson's H_{st} [b]	Hudson's K_s [c]	Hudson's S_{nn} [d]
Subdivision between					
Camellia spp.	*Ilex* spp.	18.333 (*P*-value: 0.000)*	0.823 (*P*-value: 0.000)*	0.185 (*P*-value: 0.000)*	0.909 (*P*-value: 0.000)*
Camellia spp.	*Juniperus* spp.	17.370 (*P*-value: 0.000)*	0.722 (*P*-value: 0.000)*	0.287 (*P*-value: 0.000)*	0.876 (*P*-value: 0.000)*
Camellia spp.	*Rhododendron* spp.	15.719 (*P*-value: 0.001)*	0.597 (*P*-value: 0.001)*	0.416 (*P*-value p: 0.001)*	0.802 (*P*-value: 0.001)*
Ilex spp.	*Rhododendron* spp.	1.172 (*P*-value: 0.482)	0.014 (*P*-value: 0.482)	0.103 (*P*-value: 0.482)	0.475 (*P*-value: 0.482)
Juniperus spp.	*Ilex* spp.	0.867 (*P*-value: 1.000)	-0.041 (*P*-value: 1.00)	0.383 (*P*-value: 1.000)	0.425 (*P*-value: 1.000)
Juniperus spp.	*Rhododendron* spp.	2.009 (*P*-value: 1.000)	0.024 (*P*-value: 0.482)	0.269 (*P*-value: 0.482)	0.500 (*P*-value: 0.482)
COX1					
Subdivision between					
Camellia spp.	*Ilex* spp.	22.000 (*P*-value: 0.000)*	0.881 (*P*-value: 0.000)*	0.185 (*P*-value: 0.000)*	0.954 (*P*-value: 0.000)*
Camellia spp.	*Juniperus* spp.	21.000 (*P*-value: 0.000)*	0.752 (*P*-value: 0.000)*	0.379 (*P*-value: 0.000)*	0.904 (*P*-value: 0.000)*
Camellia spp.	*Rhododendron* spp.	16.654 (*P*-value: 0.001)*	0.555 (*P*-value: 0.001)*	0.657 (*P*-value: 0.001)*	0.791 (*P*-value: 0.001)*
Ilex spp.	*Rhododendron* spp.	1.172 (*P*-value: 0.482)	0.014 (*P*-value: 0.482)	0.207 (*P*-value: 0.482)	0.475 (*P*-value: 0.482)
Juniperus spp.	*Ilex* spp.	2.895 (*P*-value: 0.737)	-0.005 (*P*-value: 0.382)	0.767 (*P*-value: 0.382)	0.475 (*P*-value: 0.591)
Juniperus spp.	*Rhododendron* spp.	4.491 (*P*-value: 0.199)	0.101 (*P*-value: 0.482)	0.539 (*P*-value: 0.078)	0.583 (*P*-value: 0.078)

An * indicates significant *P*-values (*P*-value ≤ 0.05) indicating subdivision.

[a] Test statistic tolerating unequal sample sizes; best when mutation rates are not very high.

[b] Haplotype-based test statistic best with unequal sample sizes, a large sample size, and a low rate of mutation.

[c] Sequence-based test statistic best for small sample sizes, a high rate of mutation, and equal sample sizes..

[d] Test statistic for analyzing samples of variable size and mutation rate.

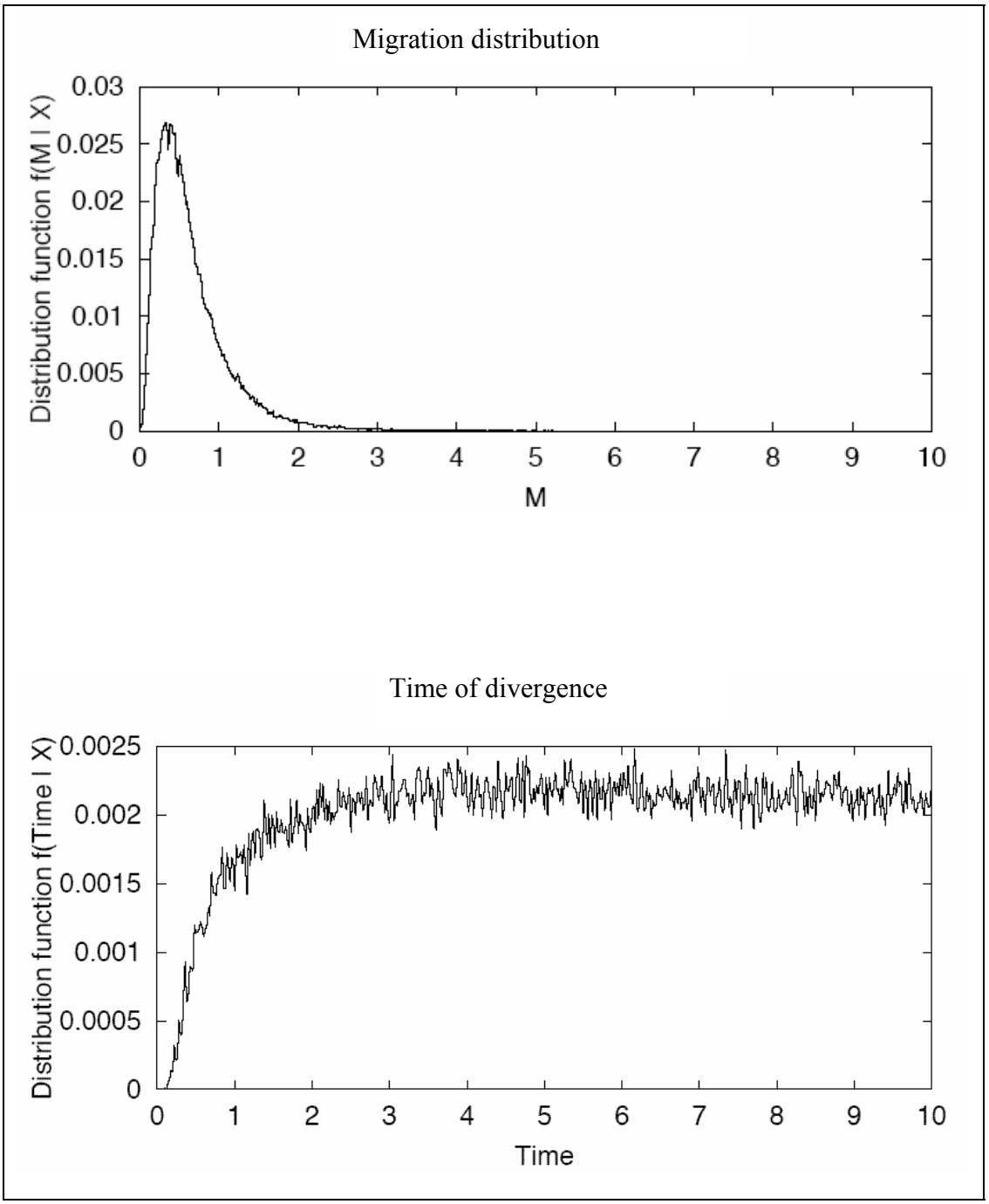

Fig. 2. Migration and divergence time posterior probability distributions between samples, using MDIV (Nielson and Wakeley 2001) for the multi-locus data set. Samples were isolates of *Phytophthora cinnamomi* from two host sets: i) *Camellia* spp. and ii) *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp.. Migration (M) or time is represented along the X axis and the Y axis depicts the likelihood function of migration [f(M|X)] or the likelihood function of time of divergence [f(Time|X)]. The data were simulated assuming an infinite sites model, 2,000,000 steps in the chain with an initial 500,000 steps before estimating the posterior distribution. Different starting random number seeds were used in ten independent replicates, all simulated under the same starting parameters and model. Each

replicate produced similar parameter estimates. MDIV results were plotted using gnuplot (Williams, T. and Kelley, C.)

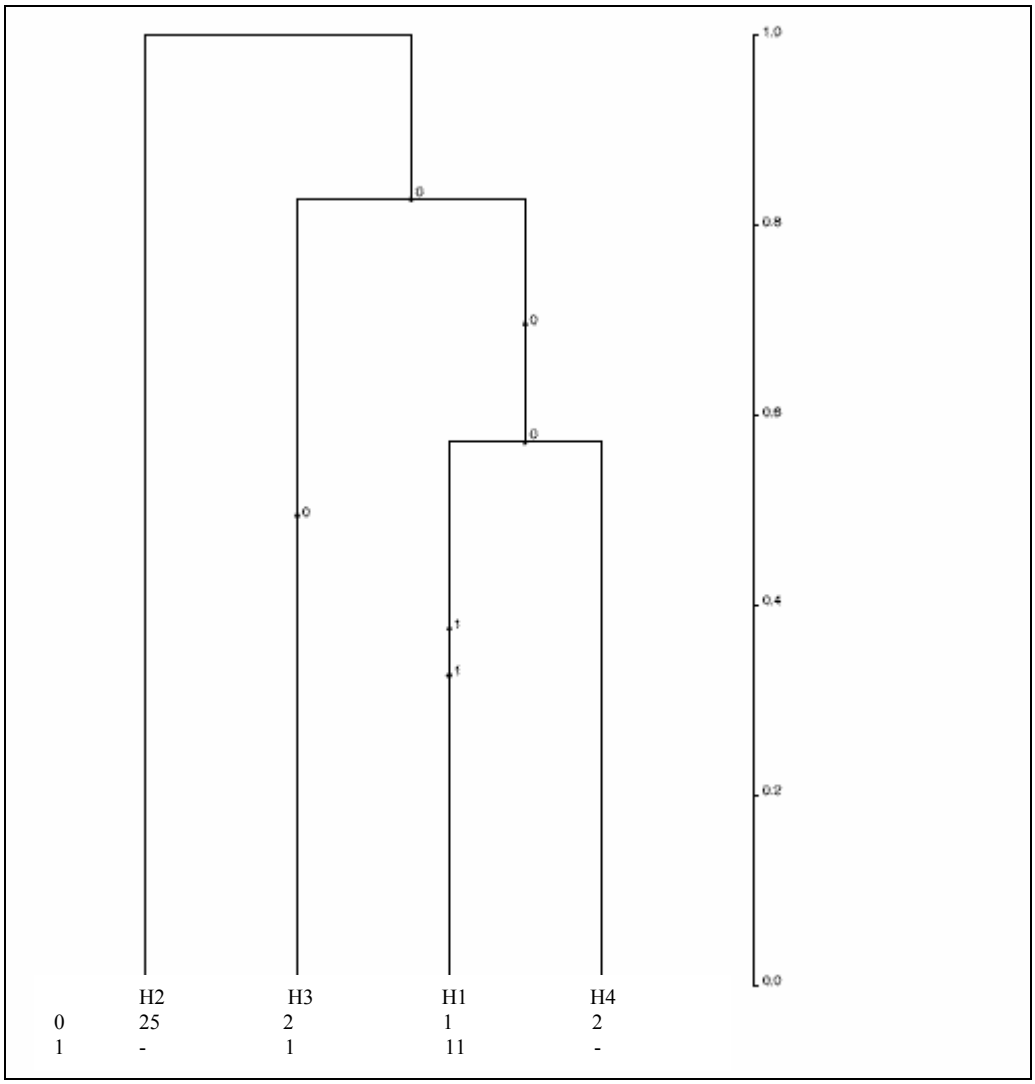

Fig. 3. Multilocus rooted gene genealogy with the highest root probability based on a symmetrical migration matrix with M = 0.5 estimated using MDIV (Nielson and Wakeley 2001) and 1,000,000 coalescent simulations using Watterson's estimate of theta = 1.59 (Watterson 1975). A 0 indicates mutations appearing in isolates from *Ilex* spp., *Juniperus* spp., and *Rhododendron* spp. (all of the A2 mating type); 1 indicates mutations appearing in isolates from *Camellia* spp. (all of the A1 mating type). The time scale on the right coalesces from present (bottom) backwards to past (top) and shows estimated time in coalescent units of effective population size.
H1: haplotype 1 is composed of 11 A1 mating type isolates from *Camellia* spp. host plants and one A2 mating type isolate from *Rhododendron* spp. host plant. Isolates are heterozygous at positions 150 and 183 in the Btu locus.
H2: haplotype 2 is composed of eight A2 mating type isolates from *Ilex* spp. host plants, ten A2 mating type isolates from *Juniperus* spp. host plants, and seven A2 mating type isolates from *Rhododendron* spp. host plant. Isolates are heterozygous at position 150 in the Btu locus.
H3: haplotype 3 is composed of one A1 mating type isolate from *Camellia* spp. host plant and two A2 mating type isolates from *Rhododendron* spp. host plants. Isolates are heterozygous at positions 150 in the Btu locus.
H4: haplotype 4 is composed of one A2 mating type isolates from *Ilex* spp. host plant and one A2 mating type isolate from *Rhododendron* spp. host plant. Isolates are heterozygous at positions 129 in the Btu locus.