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Root recovery rates for *Phytophthora cinnamomi* and rate of symptom development from root rot on *Abies fraseri* trees over 7 years

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Summary

Phytophthora root rot on *Abies fraseri* trees was monitored from 2001 to 2007 within the disease front of a 12-year-old Virginia plantation where trees had been dying of the disease since 1994. After a slow increase in early foliage symptom development from July 2001 to September 2002, the frequency of *A. fraseri* trees with early symptoms accelerated for about 15 months. While the slow increase occurred during a 18.7% lower than normal rainfall period and the acceleration occurred during a 31.2% higher than normal rainfall period, the percentage of trees with early symptoms continued to increase during the mid-winter months (December–February) when the estimated mean minimum daily soil temperature (25 cm depth) was unfavourable (<10°C) to *Phytophthora cinnamomi* pathogenic activity. The time required for trees to progress from early foliage symptoms to completely dead foliage, from November 2000 to October 2007, was highly variable, ranging from 4 to 35 months. Root recovery rates for *P. cinnamomi*, assayed on a selective medium, were 6.4 times greater for symptomatic foliage trees than for asymptomatic foliage trees in this deep, silt-loam soil. Following an atypical cold period in February 2007, when the mean minimum daily soil temperature was 0.8°C, symptomatic roots yielded only a low level of germinable propagules of *P. cinnamomi*. Further, during an atypical midsummer in 2007 (June–August), when the soil water potential was at or below −9 bars for 68 of 92 days, symptomatic roots yielded no germinable propagules of *P. cinnamomi*. Addition of thiophanate-methyl to the selective medium aided *P. cinnamomi* isolation by inhibiting many undesired pythiaceous colonies growing from symptomatic roots.

1 Introduction

Phytophthora cinnamomi Rands is an important root rot, canker and decline pathogen of forest trees worldwide, severely affecting species such as *Quercus suber* (cork oak) and *Castanea sativa* (European chestnut) in Europe (Fernández-Escobar et al. 1999; Moreira et al. 2000; Luque et al. 2002; Turchetti and Maresi 2006), and *Eucalyptus* spp. and other species in Australia (Weste and Ruppin 1977; Weste 1983; Tippett et al. 1989). In North America, it had caused, before chestnut blight, extensive destruction of *Castanea dentata* (American chestnut) in heavy soils at lower altitudes and, in more recent times, fine root necrosis on *Pinus echinata* at low altitudes in Virginia and other southeastern states (Crandall et al. 1945; Campbell et al. 1953). It has not been found to be destructive to American chestnut at high altitudes. In low-altitude pine forests in the southeastern Piedmont region, the pathogen was commonly found in soils. In hardwood and conifer forest soils of the Appalachian Mountains, *P. cinnamomi* was found less commonly and its presence was not associated with forest tree disease (Campbell and Hendrix 1967). Shallow, high-clay or other fine-textured soils with poor drainage and nutrient content commonly were associated with *P. cinnamomi* and fine root disease on *P. echinata* at low altitude (<300 m) in the Piedmont (Campbell et al. 1953, 1963).

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Abies fraseri (Fraser fir) is indigenous to high-altitude mountain forest sites in the Southern Appalachians region of the Appalachian Mountains and is not affected by *P. cinnamomi* in these sites. At mostly moderate altitudes (600–1000 m) in the Appalachian Mountains, *A. fraseri* is grown in small to large plantations derived from nursery seedling transplants. *Abies fraseri* plantation sites in the Appalachian Mountains at moderate altitude are variable in physiographic location, soil texture and soil parent material, but detailed information on the physical and nutrient characteristics of soils in these plantations is needed. In forest nursery or plantation sites, *A. fraseri* is highly susceptible to Phytophthora root rot caused by *P. cinnamom*i (Kuhlman and Hendrix 1963). *Pythium* spp. are also associated with diseased roots of *A. fraseri* but have not been identified as primary disease agents. Infected *A. fraseri* seedling transplants from the nursery may introduce *P. cinnamomi* into a plantation site. The incidence of Phytophthora root rot in an *A. fraseri* plantation can be low or high (Benson and Grand 2000), as on other susceptible host species, when environmental conditions of temperature, moisture, soil properties and inoculum are either unfavourable or favourable to disease development. Previously, no studies on the course of disease development on individual *A. fraseri* trees over close intervals of time in a plantation with active disease has been undertaken. Such information may be useful to determining more precisely the timing of symptom development in relation to physical and other environmental factors and assessing any possible tolerance of individual trees to the disease.

Quantitative assay of *P. cinnamomi* in soil has been done with baiting methods and with direct plating of soil on dilution plates using selective media (Tsao 1983). While assays of soil population density are helpful in inoculum potential studies dealing with a population of trees, they do not give an estimate of root infection level on individual symptomatic or asymptomatic trees. Recovery of a pathogen from colonization sites in roots of a host tree, assayed by plating a large length of roots on a selective medium, may be sensitive and give useful information on whether the root system of the tree is infected as well as an estimate of the activity level of the pathogen in the roots. This approach has been used for genera of root-infecting fungi such as *Cylindrocladium*, *Pythium* and *Fusarium* (Hendrix et al. 1965; Tomimatsu and Griffin 1982; James and Perez 1999), but has not yet been used to quantitatively assay *P. cinnamomi* in *A. fraseri* roots or other tree roots.

The present study was undertaken to determine the recovery rates for *P. cinnamomi* from roots of symptomatic and asymptomatic trees in an *A. fraseri* plantation in which Phytophthora root rot had been spreading and killing trees for several years, and to determine the course of symptom development over time on individual *A. fraseri* trees within the disease front of the plantation.

2 Materials and methods

2.1 Field evaluations

2.1.1 Plantation site, trees studied, soil properties and environmental data

The plantation was located near Blacksburg, Virginia at 700-m elevation on a north-facing slope of 8%. The plantation of several hundred trees was established by planting *A. fraseri* nursery seedling transplants in 1988 into a grass ground cover that was maintained by periodic mowing. Infrequently, during the study, some trees were removed by the plantation manager for commercial purposes. Unpaved roads bordered relatively closely on the east, west, and more distantly on the north, sides of the plantation. Phytophthora root rot was first observed in the plantation in 1994 at a location that was a measured distance of 27 m west of the disease front in November, 2000, where there was a concentration of symptomatic trees (Fig. 1). The research plot area encompassed the disease front and

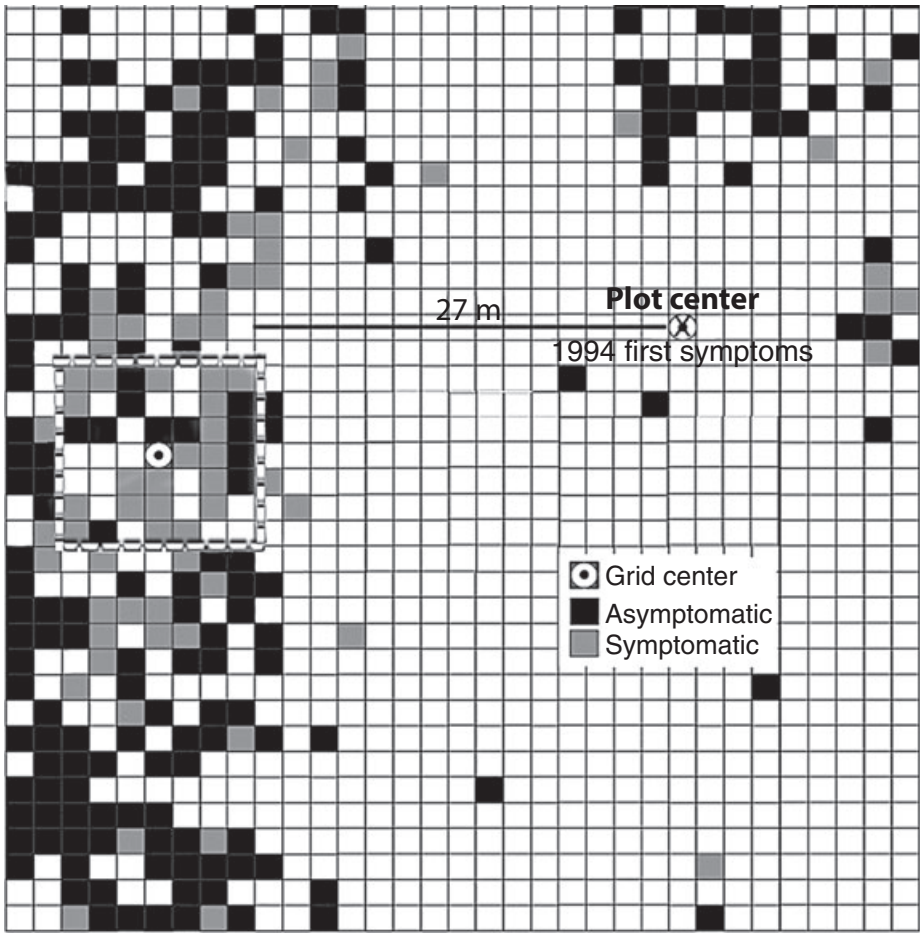

Fig. 1. Map of the main portion of the Phytophthora root rot research plot area in the *Abies fraseri* plantation showing the location of the first symptomatic trees (plot center) in 1994, the location of symptomatic trees (light shaded) in the fan-shaped disease front in late 2002, and the location of the 7×7 survey grid (highlighted by outlining) and grid centre used for monthly foliage disease assessments. The research plot is oriented south (top) to north (bottom).

surrounding trees. It contained only *A. fraseri* trees, although other conifer species were planted nearby. In the centre of the disease front, a 7×7 grid containing 32 trees (Fig. 1) was established to study the rate of foliage disease development over time.

Textural analysis of the plantation soil indicated it was a silt-loam (33% sand, 52% silt and 15% clay) and had a measured depth of >94 cm. Pressure plate analysis indicated the soil water content at −0.33 bars was 32.46%, at −0.5 bars, 25.93%, at −1.0 bars, 17.29% and at −15 bars, 11.33%. A soil-water-retention curve was drawn to determine matric water potential from per cent water content measurements. Soil water content was determined gravimetrically by drying field samples at 105°C for 24–48 h and, also, after April 2004, by a soil water monitoring station, established nearby on soil of the same texture and parent material. The sensor was installed at an angle of 30 degrees with a 15-cm range of depth and a mean depth at 23-cm. Soil temperature was monitored at a 25-cm

depth. Daily rainfall and air temperatures were monitored at the nearby Blacksburg National Weather Service station.

The soil was formed on limestone parent material and had the following chemical properties: pH (5.96); cation exchange capacity (6.7 meq/100 g); base saturation (83.2%); calcium saturation (65.6%); magnesium saturation (15.5%); potassium saturation (2.0%); organic matter (6.2%); soluble salts (77.0 mg/kg); phosphorus (34 mg/kg); potassium (53 mg/kg); calcium (881 mg/kg); magnesium (127 mg/kg); zinc (3.3 mg/kg); manganese (8.3 mg/kg); copper (1.1 mg/kg); iron (10.6 mg/kg); boron (0.4 mg/kg).

2.1.2 Foliage symptom assessment in the field

Foliage on the 32 trees in the 7 × 7 survey grid (Fig. 1), located in the centre of the disease front, was evaluated for symptoms on an approximately monthly basis (trees were evaluated for 71 of 76 months), beginning in July 2001 and ending in October 2007. Trees were rated for foliage symptoms and tagged with a metal label and given a tree number. The foliage was visually rated to the nearest 5% as either green, chlorotic, brown or defoliated. Earliest foliage symptoms were a slight chlorosis throughout the tree, brown foliage on the lowest branchlets (usually one or two), or both. The aspect (N, S, E or W) of the symptoms was recorded. Completely dead foliage trees were usually a completely brown foliage tree, a completely defoliated tree, or a mix of the two. In late 2002, all 368 *A. fraseri* trees in the research plot area (Fig. 1) of the plantation were evaluated for foliage symptom development.

2.2 Laboratory assays

2.2.1 Root recovery rate assays for Phytophthora cinnamomi

For root assays, one lateral root was excavated from an asymptomatic or a symptomatic foliage tree, selected at random, in the 7 × 7 grid (Fig. 1) or adjacent disease front to evaluate the *P. cinnamomi* recovery rate. Depth and direction of growth (N, S, E or W) of the lateral root removed was recorded as well as whether it made contact with roots of a neighbouring tree. Excavated roots were immediately taken to the laboratory, located nearby, for assay. A total of 71 trees in disease front were assayed including all 32 trees in the 7 × 7 survey grid. Nearly equal lengths of roots were assayed for both asymptomatic (38.1 m) and symptomatic (39.8 m) trees obtained from about equal numbers of trees of the two types. Symptomatic trees ranged from predominantly early to predominantly late foliage symptom development. The root assays, and foliage symptom rating of the assayed trees, were started in November 2000. Root assays were completed by June 2003. All tools were disinfested between work on individual trees and when leaving the plantation.

Roots were gently washed in water in the laboratory before dissection and plating on an agar medium selective for *P. cinnamomi*. With a sterile scalpel, washed roots were cut into segments, 1–2 cm long, and placed, with a sterile forceps, one after the other in sequence, in a Petri plate containing the selective medium. Adjacent segments from a fine root or lateral root were plated in sequence on the same Petri plate. Petri plates were incubated at room temperature and examined daily for the development of *P. cinnamomi* colonies. Extreme care was taken not to duplicate recording *P. cinnamomi* colonies that originated from both of the cut ends of adjacent root segments. Only colonies that were clearly discrete units were recorded. As *P. cinnamomi* colonies were recovered infrequently for each m of root assayed, colony interference was not common. The length of fine or lateral roots on each plate was measured with a rule. Subcultures of representative *P. cinnamomi* isolates were made on potato-dextrose agar plates.

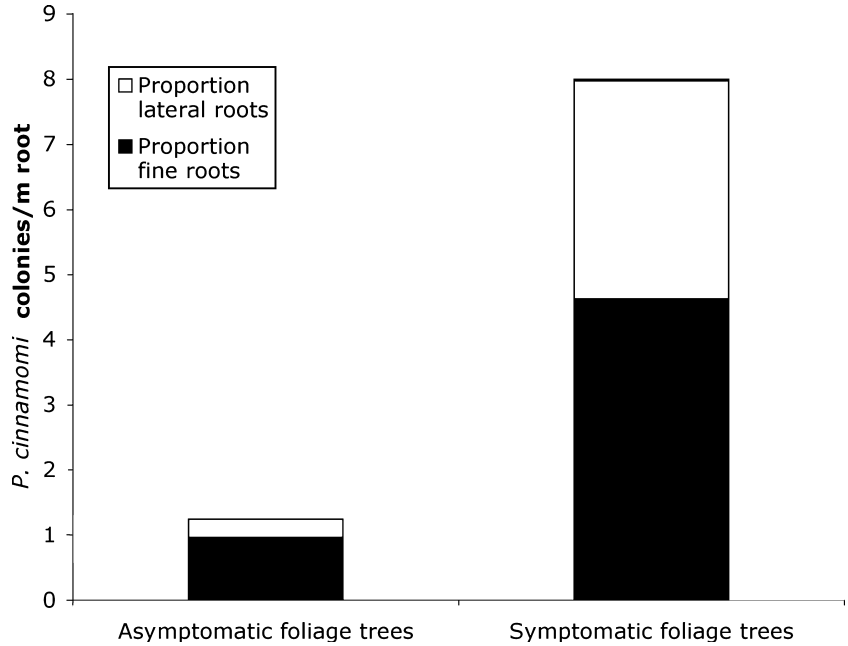

Fig. 2. Phytophthora cinnamomi recovery rates from fine and lateral roots of *Abies fraseri* trees in the disease front with asymptomatic or symptomatic foliage.

For assays done for the root-recovery study (Fig. 2), the selective medium, modified from Mitchell et al. (1986), contained the following components in 1 l of water: corn meal agar 17 g, pimaricin 10 mg, PCNB 25 mg, hymexazol 20 mg, ampicillin 250 mg, and rifampicin 10 mg. As reported by Ploetz and Parrado (1988) for avocado roots, undesired pythiaceous colonies sometimes made identification of *P. cinnamomi* colonies growing from *A. fraseri* roots difficult. After testing at different concentrations several candidate antimicrobial agents, thiophanate-methyl gave good inhibition of many of these undesired pythiaceous colonies and had little or no effect on *P. cinnamomi* at the final concentration (50 mg/l) used (0.121 ml, per l of selective medium, of the flowable fungicide, 3336F, 41.25% a.i., made by Cleary Chemical Corp., Dayton, NJ, USA). This medium was designated PH-TM medium and was used in the 2007 studies (see below).

2.2.2 2007 Studies on germinability of Phytophthora cinnamomi propagules in symptomatic roots following adverse environmental conditions

Adverse environmental conditions of soil temperature and soil water potential prevailed in the research plot in 2007 and *P. cinnamomi* propagule germinability was evaluated after these events. For these 2007 studies, smaller trees with early-stage foliage symptoms growing on the eastern edge of the disease front (that were younger than the original trees which had been removed for commercial purposes) were used. The entire root system was excavated in these studies, but as before, only fine roots and lateral roots were assayed. Following tree removal for this purpose, a steel rebar was placed in the soil at the location of the tree and a metal label affixed to the rebar. The PH-TM medium was used to evaluate *P. cinnamomi* in symptomatic roots. Root plating and incubation of Petri plates were done as indicated above.

3 Results

3.1 Recovery rates for *Phytophthora cinnamomi* from roots of asymptomatic and symptomatic foliage *Abies fraseri*

Laboratory assays of roots from asymptomatic foliage trees and symptomatic foliage trees in the 7 × 7 survey grid and adjacent disease front indicated that *P. cinnamomi* was isolated from roots of both types of trees, but that the recovery rate of *P. cinnamomi* on symptomatic trees was about 6.4 times greater than on asymptomatic trees (Fig. 2). For asymptomatic foliage trees, a high percentage of colonies of the pathogen were recovered from the fine roots. Many roots from symptomatic trees exhibited severe symptoms of root rot, including sloughing of the root cortex, but objective quantification of root rot severity was difficult to assess. Root assays indicated that the root systems of all trees in the 7 × 7 survey grid, except one, were infected with *P. cinnamomi* by 2002. The remaining tree was positive for *P. cinnamomi* infection in June 2003. Early foliage symptoms usually developed in the tree quadrant (N, S, E or W) that yielded a *P. cinnamomi* positive root sample.

3.2 Time-course of foliage symptom development on *Abies fraseri* trees

In July 2001, when the time-course study was initiated in the disease front, 23 of 32 trees (72%) in the 7 × 7 survey grid had green foliage and nine (28%) had foliage symptoms (Fig. 3). Of the latter, four had completely dead foliage symptoms. After a slow increase in early foliage symptom development from July 2001 to September 2002, the frequency of *A. fraseri* trees with early foliage symptoms accelerated for about 15 months (Fig. 3). This

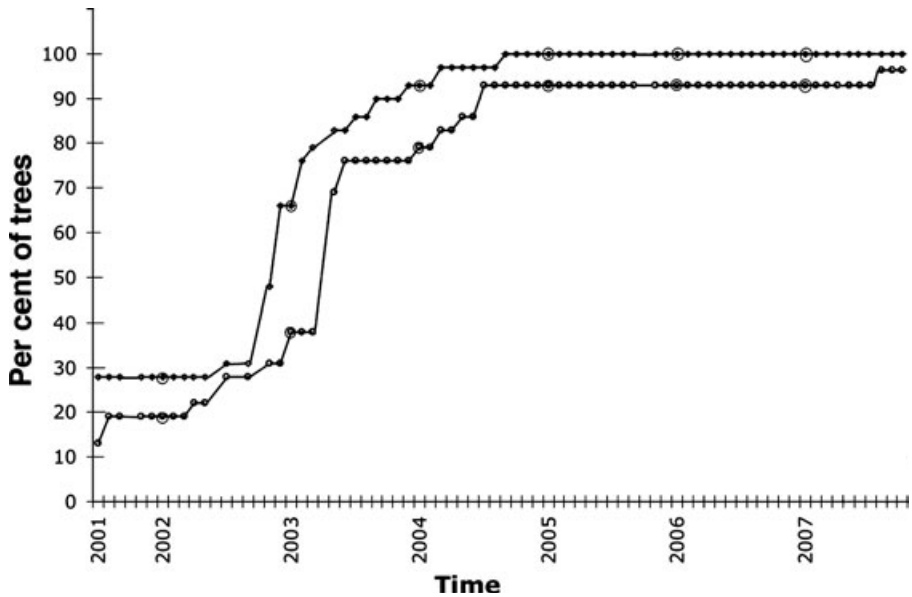

Fig. 3. Time-course development, at approximately monthly intervals (71 of 76 months were evaluated), of early foliage symptoms (upper curve) and completely dead foliage symptoms (lower curve) on *Abies fraseri* trees within a 7 × 7 grid of the disease front shown in Fig. 1. January data points for each year are marked with a large circle.

Table 1. **Time required for completely dead foliage to develop from first foliage symptoms on *Abies fraseri* trees in the Phytophthora root rot disease front.**

Tree code	Months to tree death[1] from first foliage symptoms
T51	4
T15, T48	5
T52, T53, T54, T59	6
T 46, T49	7
T55	9
T60	11
T3	15
T47	16
T12, T63, T57	17
T16	18
T9	24
T62	35

[1]Tree foliage completely brown or defoliated.

15-month period included the winter months of December, January and February 2002–2003. By September 2004, 100% of the trees in the 7×7 survey grid were symptomatic. The time-course of the development of trees with advanced symptoms (completely dead foliage) followed behind the curve for early foliage symptoms, generally, until July 2004, when the curve did not change for many months until August 2007 (Fig. 3). The time required for trees to progress from early foliage symptoms to completely dead foliage symptoms, from November 2000 to October 2007, was highly variable, ranging from 4 to 35 months (Table 1). However, for most trees the period was 9 months or less. In the research plot area (Fig. 1) overall, 19.0% of 368 trees were symptomatic in late 2002.

3.3 Environmental conditions during Phytophthora root rot development in the plantation

Rainfall and air temperature data were summarized for the 7-year study based on two 6-month periods: the dormant season (November–April) and the growing season (May–October) each year. Overall, the mean monthly rainfall (8.61 cm/month) for the 7 years was very close to the long-term mean monthly rainfall for the area (8.46 cm/month). The wettest and coolest growing season was in 2003 with 12.8 cm rainfall per month and a mean maximum air temperature of 23.4°C, respectively. This occurred during the acceleration phase of the foliage disease curve (Fig. 3). The driest and warmest growing season was in 2007 with 7.0 cm rainfall per month and a mean maximum air temperature per month of 26.4°C, respectively (see below). The coldest dormant season was 2006–2007 with a mean minimum air temperature of −3.3°C.

More insight into the association of environmental conditions, rainfall and temperature, can be gained by examining the data month by month during the slow and fast foliage disease-increase periods shown in Fig. 3. During the 15-month slow-increase period (July 2001–September 2002), the mean monthly rainfall was 6.88 cm/month or 18.7% less than the normal mean monthly rainfall. During the 15-month fast-increase period (October 2002–December 2003) the mean monthly rainfall was 11.10 cm/month or 31.2% higher than the normal mean monthly rainfall (Table 2). However, as mentioned, this fast-disease period included the winter months of 2002–2003 when mean minimum air temperatures for December–February ranged from −6.4 to −3.9°C. As this period occurred before the soil monitoring station was established, linear regression analysis (Draper and Smith 1981)

Table 2. **Rainfall amounts per month for two 15-month periods when there was either a slow or fast increase in the percentage of *Abies fraseri* trees with early foliage symptoms of Phytophthora root rot in the disease front.**

Slow-increase period		Fast-increase period	
Month	Rainfall (cm)	Month	Rainfall (cm)
July 2001	12.37	October 2002	11.53
August 2001	7.14	November 2002	12.98
September 2001	4.11	December 2002	9.68
October 2001	2.97	January 2003	3.02
November 2001	1.04	February 2003	16.00
December 2001	5.26	March 2003	7. 85
January 2002	5.44	April 2003	12.12
February 2002	1.63	May 2003	16.51
March 2002	10.87	June 2003	19.00
April 2002	6.76	July 2003	20.68
May 2002	12.85	August 2003	5. 84
June 2002	13.44	September 2003	10.24
July 2002	6.05	October 2003	4.50
August 2002	2.24	November 2003	9.83
September 2002	11.10	December 2003	6.76
Mean	6.88	Mean	11.10

Normal annual monthly rainfall mean = 8.46 cm; the 7-year monthly rainfall mean in this study = 8.61 cm.

was used to estimate the mean minimum soil temperatures during the winter of 2002–2003. A strong relationship between mean minimum air temperature and mean minimum soil temperature (25-cm depth) was found, for November–February, over the three winters (2004–2007) for which data had been obtained. The regression equation, $Y = 0.80X - 6.89$, having an $R^2 = 0.87$, was highly significant ($p \leq 0.01$). The estimated mean minimum daily soil temperatures for the 2002–2003 winter were: November, 9.1°C; December, 3.2°C; January, 0.6°C; February, 3.7°C. Thus, the mean daily minimum January soil temperature at the 25-cm depth was near freezing and all 4 months were <10°C. The estimated mean monthly maximum soil temperatures averaged 1°C higher than the minimum values. August 2002 was the hottest and driest month in the growing season with a mean daily maximum air temperature of 28.8°C. This month was followed by a high-rainfall September (Table 2), which preceded the acceleration phase of the foliage-disease curve (Fig. 3).

3.4 Germinability rates of *Phytophthora cinnamomi* propagules from symptomatic *Abies fraseri* root systems following adverse environmental conditions in 2007

Early January 2007 was warm but late January was cold, lowering the mean daily minimum temperatures for the month, and this cold period was sustained through February when the mean minimum daily soil temperature (25 cm depth) was 0.8°C, with a daily low of 0.2°C (Fig. 4a). The upper soil horizons were frozen solid. An assay on January 19 (sample 1, Fig. 4a), before the cold period, yielded a high recovery rate of the pathogen from symptomatic roots (23.1 colonies/m root for 2.02 m of roots assayed). By mid-March, the weather was warming and an assay (sample 2, Fig. 4a) was performed on symptomatic roots. On March 15, the frequency of colonies from symptomatic roots was 90.3% lower than in January (2.24 colonies of *P. cinnamomi*/m root, for 4.01 m of roots assayed).

In mid-June, a period of very low rainfall started which continued through July and August. For 68 of 92 days in these 3 months, the soil water potential was −9 bars or lower (Fig. 4b). When symptomatic roots from the plantation were assayed on July 25 (sample 3, Fig. 4b) for germinable propagules of *P. cinnamomi*, no colonies of the pathogen were recovered from the roots when 3.40 m of symptomatic roots were assayed. A second assay gave the same results. Assays for the dry-weather pathogen, *Macrophomina phaseolina*, were also negative. The drought period continued through September and October, when the soil water potential was −9 bars or lower for 45 of the 61 days in these months.

Several trees in the plantation had increased foliage symptom severity ratings during the low soil water potentials of the 2007 summer months. For example, T62 in the 7 × 7 survey grid, first symptomatic in September 2004, had a 40% symptomatic foliage rating in May 2007, the same rating in June, a 90% symptomatic foliage rating in July, and a 100% dead foliage symptom rating in August.

4 Discussion

The results of this long-term study indicate that *P. cinnamomi* fine and lateral root infection and colonization were present in some cases many months previous to first foliage symptoms on the *A. fraseri* trees in the disease front. The roots of all trees in the 7 × 7 grid used for the time-course study were positive for the pathogen in 2001 or 2002, except for one tree in 2003, but some of these trees did not exhibit first foliage symptoms until early or late 2004. The mean root recovery rate of the pathogen on symptomatic foliage trees was 6.4 times greater than on asymptomatic foliage trees, suggesting the extent of activity of the pathogen on roots that may be needed for symptom development in the foliage. *Abies fraseri* trees with no foliage symptoms yielded considerably more colonies from the fine roots than the lateral roots, suggesting the possibility that infection of fine roots may precede that on the lateral roots. This pathogen is often difficult to recover from necrotic roots of *A. fraseri* (Benson and Grand 2000), possibly because of secondary colonization of lesions by nonpathogens. Thus, the pathogen may have been recovered mainly from the active margins of lesion areas on roots.

The development of foliage symptoms on the trees was apparently the result, in large part, of the interaction between the extent of pathogen root colonization or root rot and environmental factors, such as soil water potential and air temperature, that regulate transpiration and water deficits in the tree. The latter was documented, but root rot levels were unknown at the time foliage symptoms were first developing on the trees. Also, the rate of symptom severity change on a tree, from early foliage symptoms to completely dead foliage symptoms, was highly variable, ranging from 4 to 35 months, and individual tree reactions were variable over similar time intervals. For example, tree no. T62 was first positive for *P. cinnamomi* infection of roots in November 2002 and for first foliage symptoms in September 2004. The foliage symptom severity rating in May 2007 was 60% green and 40% defoliated. For the 32 months previous to May 2007, this tree showed only a very slow increase in the development of foliage symptoms, even over wet and dry intervals of time. However, when the long 3-month, low-rainfall and low-soil-water potential period occurred in midsummer of 2007, this tree exhibited a rapid increase in foliage disease severity rating until mid-August 2007, when the disease severity rating was 100% dead foliage. In contrast, tree no. T58, positive for *P. cinnamomi* on roots in September 2002 and first foliage symptoms in April 2003, did not progress in foliage symptom severity for the next 54 months (to the end of this study), even during the severe summer drought of 2007. These data suggest root rot did not progress on this tree during this long time interval, either as a result of root disease escape or to tolerance or resistance of the tree to the pathogen.

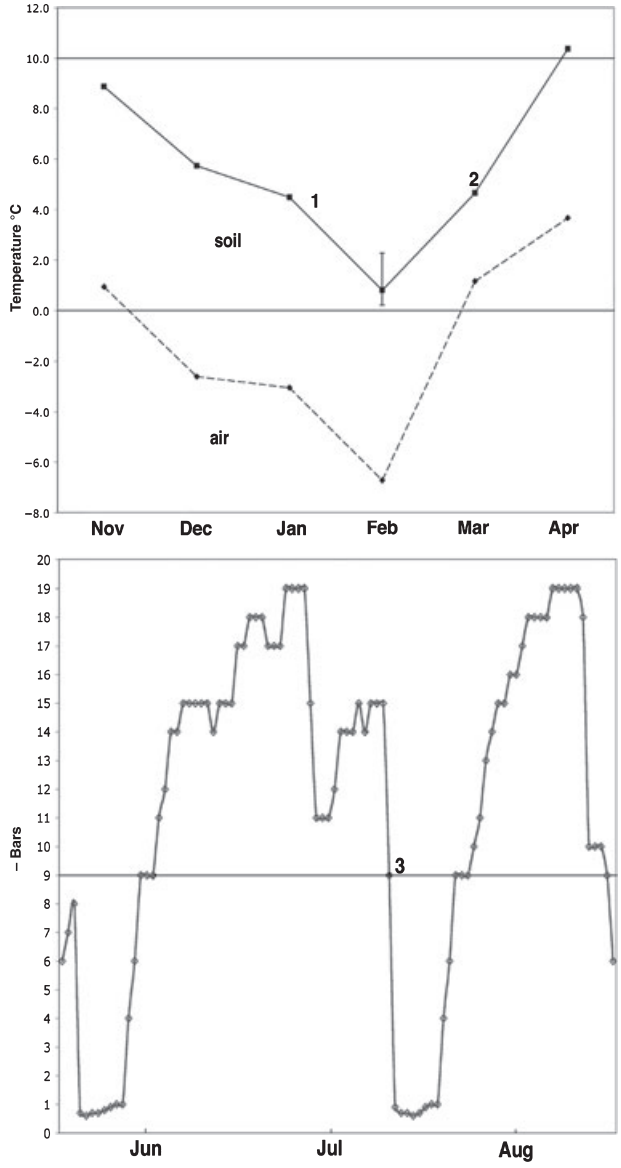

Fig. 4. (a) Top graph shows the mean minimum daily soil (25 cm depth) and air temperatures during the 2006–2007 winter. Lowest and highest soil temperatures are indicated by the vertical bar for February. The 10°C line indicates the temperature above which the pathogen is likely to be pathogenically active; the 0°C line indicates the temperature near or below which pathogen survival may be adversely affected (see text), (b) Bottom graph shows the daily soil water potentials (23 cm depth) during mid-summer, 2007. The −9 bar line indicates the water potential at or below which (to −19 bars) the pathogen is likely not to be pathogenically active (see text). The numerals 1, 2 and 3 are the dates (January 19, March 15 and July 25, respectively) of sampling symptomatic *Abies fraseri* root systems for *Phytophthora cinnamomi*.

The rapid increase in the time-course of foliage symptom development from September 2002 to December 2003 was associated with higher than normal rainfall during that 15-month period, although events, such as possible drought stress in August 2002, in the months previous to this time may have been also important. Similar observations have been made for *P. cinnamomi*-incited diseases in the past. Quantitatively, this increased rainfall amounted to a 31.2% increase in mean monthly rainfall over the normal monthly rainfall average of 8.46 cm. In contrast, an 18.7% decrease in mean monthly rainfall, *vs*. the normal monthly rainfall average, occurred during the slow increase disease period, also 15 months long. These data suggest that moderate changes from the normal amount of monthly rainfall may be critical to high or low losses of *A. fraseri* because of Phytophthora root rot. However, research in Australia has shown that soil structural properties may be more important than high rainfall. Mean annual rainfall amounts of 9.17 cm/month (sandy soil), 5.29 cm/month (sandy soil) and 12.70 cm/month (clay loam soil) were all associated with forest sites where *P. cinnamomi* was destructive to *Eucalyptus* spp. and other forest species (Weste and Ruppin 1977). However, the pathogen was most destructive at the site with lowest monthly rainfall. These authors indicated that shallow soil, poor drainage, and low water-holding capacity were the most important factors associated with severe disease development and only the low rainfall site had these properties.

The *A. fraseri* upland plantation site of the present study has a deep (>94 cm), fertile, silt-loam soil with good drainage characteristics and little pathogen-infested soil movement or erosion following rains, because of a dense-grass ground cover. The disease front had mainly spread east and slightly north (down) across the slope, following the direction of maintenance vehicle travel. Thus, the disease front had spread only about 4 m/year over 7 years, compared with 12–170 m/year, and 400 m/year in drainage water, for Australian sites (Weste 1983). Some pathogen spread was associated with root contact between infected, symptomatic roots of one *A. fraseri* tree and asymptomatic roots of a neighboring tree. While this Virginia soil, of limestone parent material, has a high percentage base saturation of calcium (65.6%), which has been associated with low disease risk to *P. cinnamomi* (Broadbent and Baker 1974; Shea and Broadbent 1983), the disease has nevertheless been destructive with almost all symptomatic trees in the research plot of the plantation being killed over time. The soil pH (5.96) and soil water content at or near field capacity (−0.33 bars = 32.5% water) following high rainfall are favourable to pathogen sporulation and lesion growth in roots, while the soil organic matter content (6.2%) is above that (2.8%) associated with a high risk to *P. cinnamomi* (Weste 1983; Tippett et al. 1989). As different *A. fraseri* plantations may have high or low disease levels (Benson and Grand 2000), further research on the properties of plantation soils is needed.

The rapid increase in foliage disease development in the time-course study encompassed the winter months of December, January and February 2002–2003. Except for a slight pause, the disease frequency curve continued to climb through the winter when the estimated mean daily minimum soil temperatures were well below 10°C, which has been generally considered to be the low temperature for *P. cinnamomi* pathogenic activity (Weste and Ruppin 1977; Weste 1983; Luque et al. 2002). This anomalous finding may be possibly due to activity of another disease agent, possibly abiotic, during winter. The possible role of *Pythium* spp. was considered but rejected for frozen soil. When the soil temperature at a 25-cm depth was near freezing, the upper soil horizons were completely frozen and winter drying injury may have occurred on trees with stress from extensive root rot and reduced root systems *vs.* root-disease-free *A. fraseri* trees, which exhibited no evidence of drying injury. Further, severe winter northwest drying winds often developed in the area where the north-facing plantation is located, exposing these normally freeze-resistant trees to severe wind blasts. Additional research is needed to test any hypothesis advanced to explain the development of foliage symptoms in winter, which were also

observed in a nearby plantation, within an *A. fraseri*-Phytophthora root rot disease area, during the winter of 2006–2007.

The *P. cinnamomi* root recovery rate in mid-March on symptomatic roots was low, following an atypical cold period in February when the mean minimum daily soil temperature was 0.8°C. This was the coldest February in the 7 years of the study. Roots for assay were collected mostly between soil depths of 5 and 25 cm. Benson (1982) found that inoculum of *P. cinnamomi* in infested root segments of *A. fraseri* was inactivated in naturally infested soil when the soil temperature dropped to 0°C or below. In this study, only one of two *A. fraseri* root isolates of *P. cinnamomi* from the plantation was able to resume colony growth at 27°C following exposure to 1°C for 3 days (G. J. Griffin, unpublished data). Low temperatures at or near 0°C were also detrimental to the survival of the root-rot pathogen, *C. parasiticum*, in soil, and survival in the field was less at a soil depth of 5 cm than at the 13 or 25 cm soil depths in late February (Graham and Griffin 1988). Soil temperatures at or below 0°C occurred most frequently in the upper 5-cm soil horizon and not at all at the 25-cm depth. Thus, low-temperature-sensitive pathogens, like *P. cinnamomi*, may be able to survive in soil at the lower soil depths.

The atypical summer of 2007 also was highly detrimental to *P. cinnamomi* activity in the research plot as the soil water potential was below −9 bars for 68 of the 92 days in June, July and August, when summer temperatures and rainfall often make these months highly favourable to pathogenic activity by *P. cinnamomi*. In addition, the drought period and low soil water potentials continued through most of September and October, with 45 of 61 days at or below −9 bars. Weste and Ruppin (1977) found that the disease potential of *P. cinnamomi* in soil was zero when the soil water potential was −9 bars or lower, but high rainfall after the drought period resulted in a rapid increase of the disease potential of the pathogen. Such extended low-soil water potentials and high temperatures may also be stressful to the normally high-altitude *A. fraseri*, predisposing it to later pathogenic activity, or to foliage symptom development, as discussed above. Other workers have indicated the importance of seasonal drought, especially on shallow, poorly drained soils, in *P. cinnamomi*-incited diseases (Campbell et al. 1953; Weste and Ruppin 1977; Moreira et al. 2000).

The 2006–2007 winter was the coldest, but the 2007 summer was the driest and hottest, during this 7-year study. While there is concern that *P. cinnamomi* may become more important as a pathogen in the future because of the effects of global warming, Benson and Grand (2000) have reported that the incidence of Phytophthora root rot had not increased on *A. fraseri* in North Carolina plantations compared with a survey done 21 years earlier. Overall, the mean monthly rainfall in this 7-year study was 8.61 cm, which is very close to the long-term monthly mean of 8.46 cm. The average annual temperature in this 7-year study (11.2°C) was higher than the normal average annual temperature (10.6°C). Longer time intervals may clarify these relationships and trends.

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