PHYTOPHTHORA CINNAMOMI INFECTION IN SAND PINE SEEDLINGS IN FLORIDA NURSERIES AND EFFECTS ON OUTPLANT SURVIVAL

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ABSTRACT

Soil samples from seedbeds of Choctawhatchee sand pine in seven bare-root forest nurseries in Florida were analyzed for the presence of Phytophthora cinnamomi during 1981 and 1982 using lupine baiting and soil plating techniques. The pathogen was detected in only one nursery in 1981. Seedlings (2-0) from P. cinnamomi-infested seedbeds, and which displayed no above-ground symptoms of root disease, exhibited twice the mortality rate of companion seedlings from seedbeds in which the pathogen was not detected during the first 4 months following outplanting on a P. cinnamomi-free soil in central Florida. Surviving seedlings from P. cinnamomi-infested seedbeds were significantly smaller, after 16 months in the field, than surviving seedlings from seedbeds in which P. cinnamomi was not detected. P. cinnamomi was isolated from roots of dead and dying seedlings throughout the course of the 16 month post-outplant monitoring period.

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Sand pine (Pinus clausa (Chapm.) Vasey) is an indigenous species in Florida occurring on droughty, sandhill sites characterized by deep, ($\geq$ 2m), infertile, acidic sands. Two distinct varieties of the species which have evolved in two geographically separate areas of the state are recognized; Ocala sand pine (P. clausa var. clausa Ward) (OSP) in central peninsular Florida, and Choctawhatchee sand pine (P. clausa var. immuginata Ward) (CSP) in the western panhandle area. Commercial interest in sand pine has been increasing due to the species' ability to perform well on sites where other southern pines grow poorly, if at all.

Sand pine root disease (SPRD) is a complex site-related disease which represents a threat to the successful management of sand pine. It is caused by one or more root pathogenic fungi acting alone or possibly in successional and/or synergistic combinations (Barnard et al. 1985, Ross 1973, Ross and Marx 1972). Recent estimates of losses to SPRD in Florida are placed at between $1.5 and $2.5 million annually. This figure accounts for mortality losses of trees $\geq$ 10cm dbh only, and does not include 1) losses resulting from reduced growth of diseased trees, 2) reestablishment costs for plantations replanted following precommercial stand liquidation, or 3) losses in high value nurseries and seed orchards (Barnard et al. 1982).

Phytophthora cinnamomi Rands, the pathogen implicated in the littleleaf disease syndrome of shortleaf (P. echinata Mill.) and loblolly (P. taeda L.) pines, is the pathogen most frequently associated with root disease in young sand pine plantations (Barnard et al. 1985). P. cinnamomi is also a well known root pathogen of both hardwood and coniferous species in forest nurseries in the United States (Kuhlman and Smith 1975). In Florida, P. cinnamomi infections have resulted in the loss of some 500,000 sand pine seedlings in 1978 and 1979 in one commercial forest nursery due to seedling mortality and/or related quarantine of infected nursery stock (Barnard 1980 a,b). To date, P. cinnamomi has not been found in association with root disease of sand pine in natural stands (Barnard et al. 1985, Ross 1973, Ross and Marx 1972).

The introduced status of P. cinnamomi in the United States (Zentmyer 1977, 1980), the presence of the pathogen in at least one sand pine-producing forest nursery in Florida (Barnard 1980 a,b), its widespread occurrence in young sand pine plantations suffering from root disease (Barnard et al. 1985), its lack of an aerial dissemination mechanism (i.e., no airborne spores), and its apparent absence in natural sand pine stands (Barnard et al. 1985, Ross 1973, Ross and Marx 1972) have provided substantial grounds for concern regarding the introduction of a potentially dangerous pathogen into plantations via infected nursery stock. Accordingly, we conducted studies to 1) identify forest nurseries in Florida with resident populations of P. cinnamomi, 2) determine the relative incidence, distribution, and impact of the pathogen on sand pine within those nurseries, and 3) evaluate the post-outplanting impact of P. cinnamomi introduced into a pathogen free site via infected nursery stock.

METHODS

Nursery survey. Choctawhatchee sand pine seedbeds in all seven bare-root forest nurseries in Florida were sampled in 1981 and 1982 and analyzed for the presence of P. cinnamomi. Each seedbed was divided into 30-m sections and soil cores (2.5 cm diam x 10-15 cm deep) were extracted at approximately 1-m intervals throughout each section. All cores (ca. 30-35) from within each section were bagged together in plastic bags and transported on ice to the laboratory for processing. Soil core extractors were flame-sterilized with 95% ethanol between each 30-m seedbed section. Table 1 summarizes the total number of samples collected in each nursery over the 2-year sampling period.

Table 1. Choctawhatchee sand pine production and seedbed soil sampling statistics for Florida forest nurseries 1981-82.

Nursery	Year	Total Production[a]	No. Seedbeds	Total No. Soil Samples[b]
Buckeye Cellulose Corporation	1981	6.00	102	612
	1982	3.61	105	459
Champion International Corporation	1981	1.50	19	114
	1982	4.91	89	491
Fla. Div. Forestry Chiefland	1981	6.25	50	295
	1982	3.42	52	308
Fla. Div. Forestry Munson	1981	1.00	21	105
	1982	0.54	21	73
Forest & Lakes Plantation	1981	0.76	13	65
	1982	0.47	9	45
Gilman Paper Co.	1981	1.00	49	97
	1982	0.50	25	50
St. Joe Paper Co.	1981	2.40	45	270
	1982	7.39	96	654

[a]Estimated millions of seedlings.

[b]Approximately 1 composite of 33 punch tube units per 30-m bed length.

After thorough mixing, a 450 cc subsample of each composite soil sample was placed in a plastic cup, planted with 4-6 blue lupine (Lupinus angustifolius L. 'Rancher') germlings (Kirby and Grand 1975), and placed on a greenhouse bench. These lupine baiting cups were adjusted as necessary after

1 wk to provide a standardized 4 plants per cup. All cups were watered to near saturation three times a week and closely monitored for a period of 4-5 months. All dead and dying lupine seedlings were plated directly onto an Oomycete-selective medium (PARP) (Kannwischer and Mitchell 1981) and examined after 3 days for the presence of P. cinnamomi. At the end of the greenhouse incubation period, soil from all cups which failed to yield P. cinnamomi via lupine baiting was reassessed by direct soil plating. Five grams of soil from each cup was suspended in 0.1% water agar and plated directly onto 2 plates of PARP at a rate of approximately 0.1 g soil per plate. Three days later soil was washed from the plate surfaces and plates were examined for colonies of P. cinnamomi.

Outplant study. In April 1982, twelve seedbeds in a portion of the Florida Division of Forestry's Munson Nursery with a recent history of P. cinnamomi occurrence were sown with CSP seed. Seedlings were grown and maintained for two growing seasons under standard nursery practices with the exception of 2-3 "top prunings" with a rotary mower late in the 2nd season to maintain seedlings at a convenient size for outplanting. In January of 1984 seedlings were hand lifted for outplanting. Approximately 1500 above-ground-asymptomatic seedlings (i.e., seedlings with green needles, exhibiting no above-ground symptoms of root disease) were lifted from portions of the seedbeds in which neighboring seedlings exhibited chlorosis and mortality typical of seedlings with advanced infections of P. cinnamomi (i.e., P. cinnamomi-infested seedbeds). Similarly, an additional 1500 seedlings were lifted from portions of the seedbeds which were apparently free of the pathogen (i.e., no seedlings symptomatic of infection present). Fifty seedlings were randomly selected from each treatment for dry weight and root collar diameter measurements and root isolations. Seedlings were placed in plastic bags, labelled and transported on ice to the laboratory or field.

In the laboratory, five root pieces representing a) the lower tap root, b) the root collar, and c) lateral and feeder roots from each of the 100 randomly selected seedlings (50 per treatment) were plated directly onto PARP. Culture plates were examined after 3 days for the presence of P. cinnamomi.

A longleaf pine (P. palustris Mill.) - turkey oak (Quercus laevis Walt.) site, located on the Austin Cary Memorial Forest near Gainesville, FL and characterized by deep sandy soils (Tavares Series), was selected for the outplanting site. Several months prior to actual outplanting, a 0.8-h portion of the site was cleared of existing vegetation with a bulldozer. In addition, six soil samples, each consisting of 10 randomly collected soil cores (2.0 cm diam x 25.0 cm deep), were collected from the site. Each composite soil sample was thoroughly mixed and 15 0.5-cc subsamples of each were placed in 1.0 cm diam. wells in plates of PARP, moistened with a few drops of sterile deionized H_2O and incubated at room temperature. After 3 days, well peripheries were examined for developing colonies of P. cinnamomi.

Outplant plots were established in January 1984, within 48 hours after lifting. Seedlings from both treatments were planted on a 1-m spacing in 10 separate alternating rows of ca. 30 trees each in two replicated blocks. On each of eight dates between March 29, 1984 and April 24, 1985, survival counts were made and all dead and dying seedlings were hand lifted, placed in separate plastic bags and transported on ice to the laboratory. The presence or absence of P. cinnamomi was determined for each dead and dying seedling by

directly plating selected root fragments on PARP and observing resulting cultures after 3 days.

RESULTS

Nursery survey. Using the soil plating and lupine baiting techniques, P. cinnamomi was recovered from only one nursery (Florida Division of Forestry, Munson Nursery) in 1981. In this nursery only 3 of the 295 30-m seedbed sections yielded the pathogen; both by lupine baiting and subsequent plating of soil from lupine baiting cups. In 1982, P. cinnamomi was not recovered from any nursery using these techniques, despite the fact that sampled seedbeds in the Munson nursery produced P. cinnamomi-infected seedlings within a matter of weeks following soil core extraction.

Outplant study. Differences in root collar diameters and seedling dry weights between seedlings from the two treatments were insignificant ($P \leq 0.05$) at the time of lifting/outplanting (Table 2). However, over half of the seedlings from P. cinnamomi-infested seedbeds yielded the pathogen upon isolation whereas the organism was completely absent in cultures from seedlings lifted from apparently noninfested seedbeds.

Table 2. Comparative seedling characteristics for 2-0 Choctawhatchee sand pine at time of lifting/outplanting[a]

Treatment[b]	Root Collar Dia. (mm)	Root Dry Wt. (g)	Top Dry Wt. (g)	Total Dry Wt. (g)	P. cinnamomi[c] (%)
I	5.3	1.2	7.0	8.2	56.0
II	5.0	1.0	5.8	6.8	0.0

[a]Means and percentages based on measurements of 50 sample seedlings per treatment. Root collar diameters and dry weights within column did not differ significantly ($P \leq 0.05$) between treatments based on standard ANOVA.

[b]I = Seedlings from P. cinnamomi-infested seedbeds.
II = Seedlings from seedbeds in which P. cinnamomi was not detected.

[c]% seedlings from which P. cinnamomi was isolated.

P. cinnamomi was not recovered from soil samples taken from the outplanting site prior to establishment of the test planting.

In the first 4 months after outplanting, seedlings from P. cinnamomi-infested seedbeds exhibited twice the mortality rate of seedlings taken from seedbeds in which the pathogen was not detected (Fig. 1-A). Mortality rates

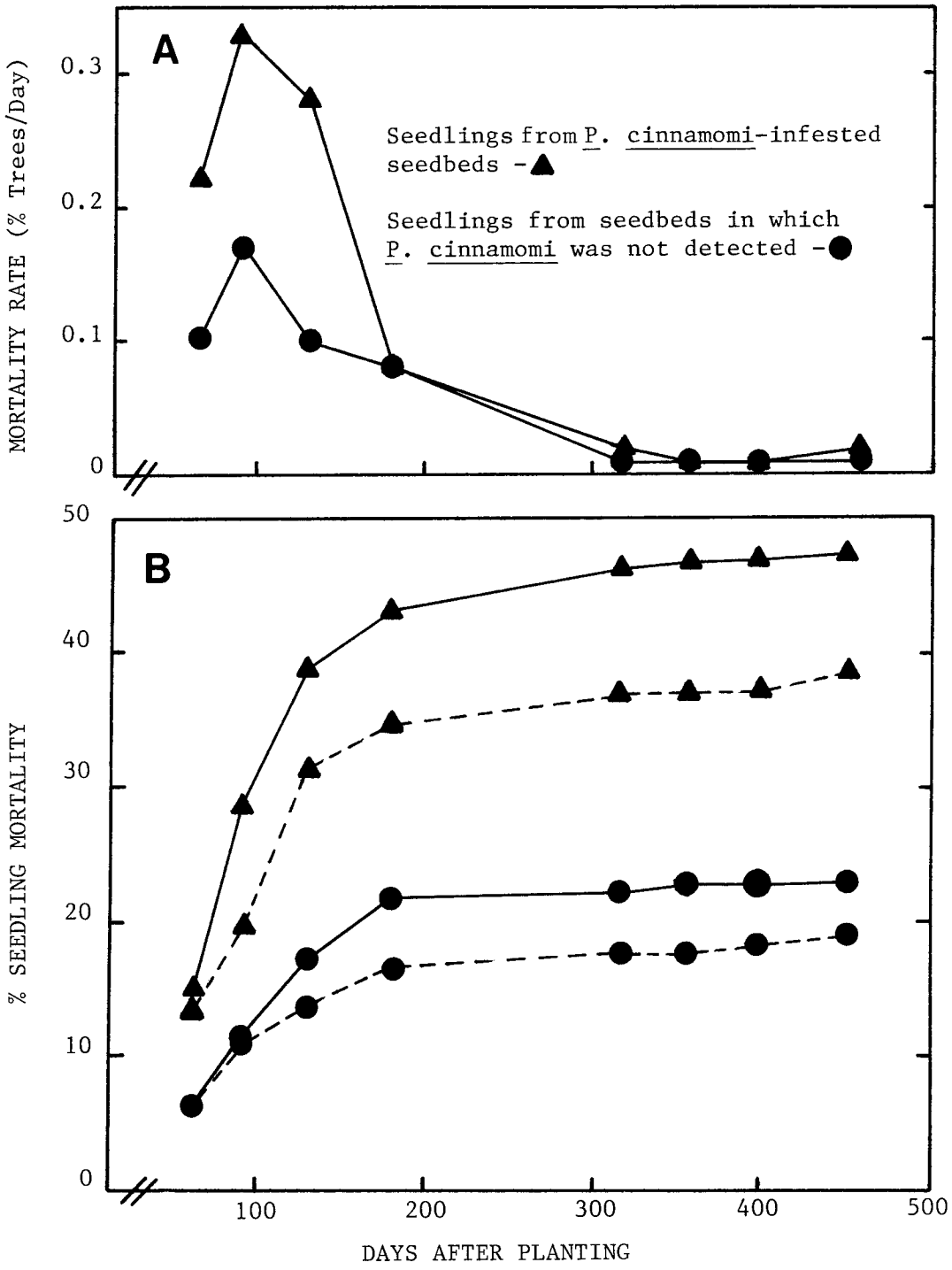

Figure 1. --Influence of nursery-initiated Phytophthora cinnamomi infections on post-outplant mortality of 2-0 Choctawhatchee sand pine. A) Seedling mortality rates. (Treatment differences at the first three observation dates are significant at $P \leq 0.01$ using Pearson's conditional X^2 test.) B) Cumulative seedling mortality in blocks 1 (——) and 2 (---) for seedlings from P. cinnamomi-infested seedbeds (▲) and seedbeds in which the pathogen was not detected (●).

subsequently leveled off and were not significantly different for the remainder of the 16 month monitoring period. Within each of the two outplanting blocks cumulative mortality of seedlings from the former treatment amounted to approximately twice that of seedlings from the latter for the duration of the monitoring period (Fig. 1-B). Slight differences in the percentages of post-outplant mortality were apparent between blocks 1 and 2, but relative treatment differences remained consistent within each block. After 16 months, survival of seedlings from P. cinnamomi-infested seedbeds was substantially less than that of seedlings from seedbeds in which the pathogen was not detected (Table 3). In addition, surviving seedlings from the former treatment were significantly smaller ($P \leq 0.05$) than those of the latter. P. cinnamomi was recovered from nearly half of the dead and dying seedlings outplanted from pathogen-infested seedbeds while less than 10% of the dead and dying seedlings from seedbeds which appeared pathogen-free yielded the organism. Recovery of P. cinnamomi from roots of outplanted test seedlings diminished considerably over time, but the organism was still recovered from some seedling roots 16 months following outplanting.

Table 3. Field performance after 16 months for 2-0 Choctawhatchee sand pine seedlings outplanted from P. cinnamomi-infested seedbeds and seedbeds in which P. cinnamomi was not detected.

Block No.	Treatment[a]	Survival (%)	Mean Height (cm)[b]	Mean Root Collar Diameter (mm)[b]	Dead/Dying Seedlings Yielding P. cinnamomi (%)
1	I	52.4	43.0	7.4	47.1
	II	77.1	47.7	8.4	9.3
2	I	61.4	49.0	9.1	41.1
	II	80.9	53.5	9.8	3.2

[a] I = Seedlings from P. cinnamomi-infested seedbeds.
II = Seedlings from seedlbeds in which P. cinnamomi was not detected.

[b] Mean heights and root collar diameters between treatments and within blocks differed significantly ($P \leq 0.05$) using standard ANOVA.

DISCUSSION

Several factors could be involved in the extremely low rate of P. cinnamomi detection during the soil survey phase of this study. First, soil fumigation before each pine crop with various formulations of methyl bromide is routinely employed by the nurseries surveyed. This may have maintained populations of the pathogen below readily detectable levels. In the Division

of Forestry's Munson Nursery, for example, heavy losses of sand pine to P. cinnamomi infections had been sustained in 1978 and 1979. In the survey years (i.e., 1981 and 1982), however, losses in this nursery were negligible, presumably due to reduced populations of the pathogen resulting from improved soil fumigation procedures. Second, failure to recover P. cinnamomi from soils in the other six nurseries surveyed may simply be an accurate reflection of the absence of the pathogen in these nurseries. There are no historical records of P. cinnamomi occurrence or infections in any of these six nurseries. Third, it is possible that our techniques had inherent deficiencies (e.g., sampling procedures, sample sizes, incubation/baiting methods, etc.). In this respect, however, it is perhaps noteworthy that our soil survey results were compatible with known historical records as far as the pathogen's occurrence in nurseries is concerned. Why our soil samples from confirmed P. cinnamomi-infested seedbeds (i.e., seedlings later developed root infections) at the Munson Nursery in 1982 failed to yield P. cinnamomi is unknown.

The association of P. cinnamomi with SPRD in plantations and not in natural stands (Barnard et al. 1985, Ross 1973, Ross and Marx 1972), coupled with a) the introduced status of P. cinnamomi in the United States (Zentmyer 1977, 1980), b) the pathogen's lack of an aerial dissemination mechanism (i.e., no airborne spores), and c) the documented occurrence of the pathogen in at least one sand pine-producing forest nursery in Florida (Barnard 1980a,b), has led to the hypothesis that root disease in young plantations could be related to P. cinnamomi infections originating in infested nursery soils. Confirmation of this hypothesis on a stand-by-stand basis in existing plantations, however, is not feasible because it is not possible to ascertain whether P. cinnamomi was indigenous to the site or introduced via infected planting stock after a plantation has been established. Such a determination can be made only by appropriate sampling before seedlings are planted in each individual stand. The high incidence of P. cinnamomi on seedlings from pathogen-infested seedbeds (Table 2) and the apparent absence of the pathogen in the soil at the outplanting site in our study lend credibility to a nursery-initiated root disease hypothesis. To our knowledge, this study provides the first empirical data confirming the potential for nursery-initiated P. cinnamomi infections to cause mortality in young Choctawhatchee sand pine plantations. In addition, this study provides the first documentation of growth reduction in young sand pine resulting from pathogenic root infections (Table 3). Previous SPRD impact assessments have included mortality alone for trees $\geq$ 10 cm dbh (Barnard et al. 1982).

The source of the P. cinnamomi recovered in the field (Table 3) from seedlings outplanted from seedbeds which were apparently pathogen-free is uncertain. Three possibilities require consideration. First, the organism could have been present in the soil on the outplanting site at levels which precluded detection via the pre-outplant soil sampling we performed. Second, some degree of cross-contamination between seedling treatments may have occurred during lifting, planting, or subsequent post-outplant sampling. Third, it is possible that P. cinnamomi was present in the apparently pathogen-free seedbeds at levels which a) caused no evidence of disease in the nursery and b) avoided detection via our 50-seedling subsample (Table 2). Of these possibilities we consider the last to be most probable for the following reasons. A 15-year-old sand pine plantation within 10 m of our test planting is exhibiting no evidence of root disease infection. This, we regard as highly unlikely if indeed P. cinnamomi were present on the site. In addition, we were

particularly careful to avoid cross-contamination during the plantation establishment and monitoring phases of this study. Finally, it is highly unlikely that P. cinnamomi, once present in a forest nursery (as is in the case of the Munson Nursery), would be completely eradicated by routine soil fumigation. Regardless of the source of P. cinnamomi in our "check" seedlings, the marked differences in both pathogen recovery (Tables 2 and 3) and seedling mortality (Figs. 1-A,B) between treatments clearly indicate a "nursery" P. cinnamomi contribution.

P. cinnamomi is an important and destructive root pathogen of sand pine (Barnard et al. 1985, Ross 1973, Ross and Marx 1972). Our data confirm the potential for and emphasize the threat of nursery-initiated infections "carried over" into young plantations. The extent to which nursery-initiated infections of P. cinnamomi have contributed to Florida's sand pine root disease scenario is unknown. However, in light of currently available information, it is distinctly possible, and perhaps even probable, that a substantial proportion of root disease problems encountered in young plantations may be linked to nursery-initiated P. cinnamomi infections. Forestry organizations producing sand pine in bare-root nurseries would be well advised to 1) evaluate seedbed soils for resident populations of P. cinnamomi, 2) prevent the movement of P. cinnamomi within and between nurseries on contaminated equipment, etc., 3) grow seedlings in nurseries or portions of nurseries which are pathogen-free or not conducive to pathogen development (e.g., avoid soils which are fine textured and poorly drained), 4) systematically evaluate all sand pine crops for sublethal and/or asymptomatic infections of P. cinnamomi, and 5) consider quarantining crops with known P. cinnamomi infections. The Florida Division of Forestry has recently suspended all sand pine production at its Munson Nursery in order to minimize the threat of transferring this dangerous pathogen to plantation sites via sand pine seedlings with sublethal and/or asymptomatic infections.

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