Susceptibility of Sand Pine to Phytophthora cinnamomi

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ABSTRACT

Phytophthora cinnamomi is reported as a new, virulent pathogen of sand pine. Ocala and Choctawhatchee races of sand pine seedlings with and without ectomycorrhizae were planted in soils with ca. 27 propagules of *P. cinnamomi*/g of soil. Ectomycorrhizae were synthesized by *Pisolithus tinctorius*. Noninoculated mycorrhizal and nonmycorrhizal seedlings of both races showed statistically significant increment in dry weight of foliage and roots and in number of new lateral roots; seedlings inoculated with *Phytophthora cinnamomi* showed nonsignificant increment in foliar and root dry weight and significant decrease in number of new lateral roots. Mortality of mycorrhizal and nonmycorrhizal seedlings was much higher for the Choctawhatchee race than for similarly treated Ocala race seedlings. High mortality rates were attributed to invasion of nonmycorrhizal roots by *P. cinnamomi*; ectomycorrhizae were not penetrated by the pathogen. This proof of pathogenicity of *P. cinnamomi* and its association with areas where sand pine mortality was caused by *Clitocybe tabescens* suggest its involvement in the etiology of a sand pine-root disease complex.

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Additional key words: biological control, feeder root disease.

Poor survival of transplanted seedlings and high mortality of growing stock have been reported in plantations of sand pine (*Pinus clausa* [Chapm.] Vasey) in northern Florida (1). *Clitocybe tabescens* (Fr.) Bres. kills sand pine in natural stands in Florida (8); recently, Ross (9) isolated *C. tabescens* consistently from dead and dying sand pine in several plantations in Florida and Georgia.

Preliminary pathogenicity tests with *C. tabescens* on 1-year-old seedlings of both races of sand pine, conducted in the greenhouse prior to the present study, were abandoned because of massive feeder root necrosis and mortality unrelated to *C. tabescens*. Isolations from diseased root tissue and the supporting soil medium using the apple technique (2) and Kerr's modified soil dilution technique (4) yielded high quantities of *Phytophthora cinnamomi* Rands. Soil propagule counts averaged 27/g of air-dried soil, with some as large as 70/g. The large populations of *P. cinnamomi* apparently resulted from high susceptibility of the hosts. The original infestation resulted from either incomplete fumigation of the soil at the beginning of the study, or flooding of the pots with surface water runoff following heavy rains while they were in an outdoor lath house during the summer months.

Because sand pine has not been reported as a host of *P. cinnamomi*, studies were designed to determine the susceptibility of the Ocala and Choctawhatchee races of sand pine, and to determine whether ectomycorrhizae were capable of reducing losses to this pathogen. Soil sampling and isolation studies were also undertaken to determine whether *P. cinnamomi* could be found under natural conditions where both races of sand pine were planted or grew naturally.

MATERIALS AND METHODS.—Seed of Ocala and Choctawhatchee races were surface-sterilized in 30% H_2O_2 after soaking 48 hr at 5 C in 1% H_2O_2. The seed were planted in 2.5 X 8-cm paper tubes containing triple-steamed soil which was either infested with 3-month-old mass inoculum of *Pisolithus tinctorius* (Pers.) Coker & Couch (isolate 49) or autoclaved inoculum of *P. tinctorius*. This technique has been reported in detail (5). The inoculum and soil was mixed at a 1:3 ratio. The seedlings were maintained in a special growth room, designed to exclude airborne spores of microorganisms, for 14 weeks, watered automatically, and supplemented after 12 weeks with Melin-Norkrans salts (5). After 14 weeks, when ectomycorrhizae had formed on those seedlings inoculated with *P. tinctorius*, individual seedlings were ready for transplanting. All seedlings received a 50-ml application of 12-8-6 liquid fertilizer 1 week after transplanting.

The inoculum of *Phytophthora cinnamomi* (isolate 49) was prepared according to Marx & Bryan (5), grown for 16 days, then mixed with triple-steamed soil to provide the desired number of propagules/g of soil. One hundred 15-cm clay pots were prepared with the inoculum of *P. cinnamomi*, and 100 were prepared with sterilized inoculum of *P. cinnamomi*. A nonsterile soil leachate (6) was added to all pots to promote sporangial production by *P. cinnamomi*. After incubation for 48 hr, the infested soil and sterilized inoculum-soil mixture were sampled to determine the propagules of *P. cinnamomi*/g of air-dried soil. The mixtures were held for 10 days before the mycorrhizal and nonmycorrhizal seedlings were transplanted, one seedling/pot, into the infested and noninfested soil and placed outside the special growth room for the duration of the study. One hundred seedlings of each sand pine race were used: 50 with the mycorrhizal symbiont and 50 without, and 25 of each of these

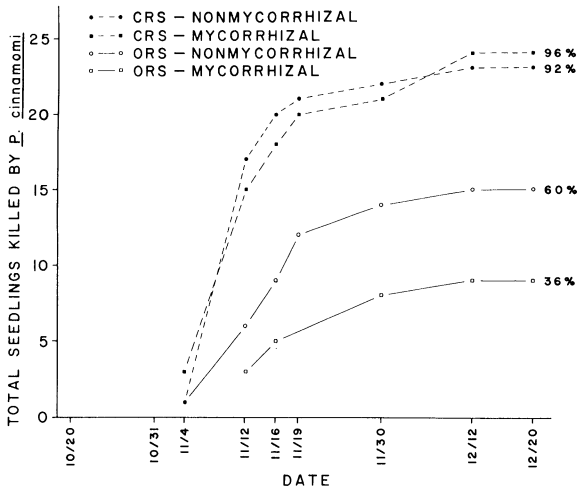

Fig. 1. Mortality of mycorrhizal and nonmycorrhizal seedlings of the Ocala (ORS) and Choctawhatchee (CRS) races of sand pine inoculated with *Phytophthora cinnamomi*.

were planted in soil with *P. cinnamomi* and 25 in soil free from the pathogen.

Measurements of seedling height, foliar dry weight, root dry weight, new lateral roots, and percentage mycorrhizae were made at the time of transplanting, at 1 month, and at 2 months later. To avoid sacrificing treatment seedlings, 10 additional plants of each treatment were used for measurements at the time of transplanting. Five live seedlings of each treatment were sacrificed after 1 month for measurement; all remaining seedlings were measured after 2 months. Lateral roots were considered newly formed if the number of laterals exceeded the number determined at initiation of the pathogenicity phase of the study. Data were analyzed by analysis of variance, except that differences in mortality rates of mycorrhizal and nonmycorrhizal seedlings were analyzed by Chi-square.

Selected samples from all treatment groups were prepared for histological examination by standard killing, dehydration, and embedding techniques. Prepared sections (12 μ) were stained with safranin and fast green (6).

Five soil samples were collected at each of eight locations in Florida and Georgia in 17 natural and planted stands of both sand pine races. The samples were collected in areas where trees had been killed by *C. tabescens* and in disease-free areas. Each sample consisted of 10 soil cores taken to a 20-cm depth within 0.25 hectares. Soils were assayed for *P. cinnamomi* with the modified Kerr's soil dilution plate technique (4).

RESULTS.—Mortality in mycorrhizal and nonmycorrhizal Choctawhatchee race seedlings (CRS) and Ocala race seedlings (ORS) inoculated with *P. cinnamomi* occurred after 2 weeks in pathogenicity studies established in the greenhouse (Fig. 1). After 2 months, 96% mycorrhizal and 92% nonmycorrhizal CRS were killed. ORS were not killed as rapidly or in as great a number as CRS; 60% of the nonmycorrhizal and 36% of the mycorrhizal ORS were killed (Fig. 1). The difference in mortality of mycorrhizal and nonmycorrhizal CRS was not significant; however, the difference in similarly treated ORS was significant ($P = .02$). The inoculum level in soil infested with *P. cinnamomi* was adjusted to 27 propagules/g of air-dried soil before seedlings were transplanted. The average inoculum level increased from 27 propagules/g of air-dried soil to 78 propagules/g, or ca. 190%. Differences were nonsignificant in inoculum level increase in soils supporting mycorrhizal and nonmycorrhizal seedlings.

When transplanted into the 15-cm clay pots outside the growth room, ca. 25% of the roots of all seedlings inoculated with *Pisolithus tinctorius* were ectomycorrhizal. Two months later, roots of CRS not inoculated with *Phytophthora cinnamomi* were 48% mycorrhizal, whereas roots of seedlings inoculated with the pathogen were 32% mycorrhizal; roots of similarly treated ORS were 53% mycorrhizal. Differences within and between races were not significant.

Seedling height among the different treatments did not vary more than 1.5 cm. Seedlings showing top growth produced lateral shoots which significantly increased foliar weight, with little increase in height.

Mycorrhizal and nonmycorrhizal CRS not inoculated with *P. cinnamomi* formed significantly (.01) more new lateral roots and increases in foliar and root dry weight than inoculated seedlings during the 2-month period; similar increases were recorded for ORS. Seedlings of both races inoculated with *P. cinnamomi* gained only slightly in foliar and root dry weight, but exhibited significant decreases (.01) in the number of new lateral roots as compared to noninoculated seedlings (Fig. 2).

Histological examination of representative nonmycorrhizal roots from ORS and CRS showed penetration of the cortical cells by *P. cinnamomi*. After penetration, hyphae of the pathogen ramified throughout the cortical and vascular tissue and killed the root. Intracellular vesicle formation characteristic of *P. cinnamomi* infection was observed frequently (6, 7). Mycorrhizal roots not inoculated with *P. cinnamomi* had mantle and Hartig net formation characteristic of *Pisolithus tinctorius* (5). Mycorrhizal seedlings of both races were not penetrated directly by *Phytophthora cinnamomi*, but the nonmycorrhizal roots on the same seedling were penetrated directly. Hyphae of *P. cinnamomi* found in the mycorrhizae were restricted to the vascular tissue, suggesting that primary infection took place through the nonmycorrhizal roots and the pathogen grew into the mycorrhizae through the vascular system.

After 2 months' exposure in the greenhouse, there was no colonization of the nonmycorrhizal roots by naturally occurring mycorrhizal fungi.

Soil samples from natural and planted stands of sand pine yielded low propagule counts of *P. cinnamomi*, but the fungus was associated with areas on which decline symptoms or mortality in sand pine was observed. Recovery of *P. cinnamomi* ranged from

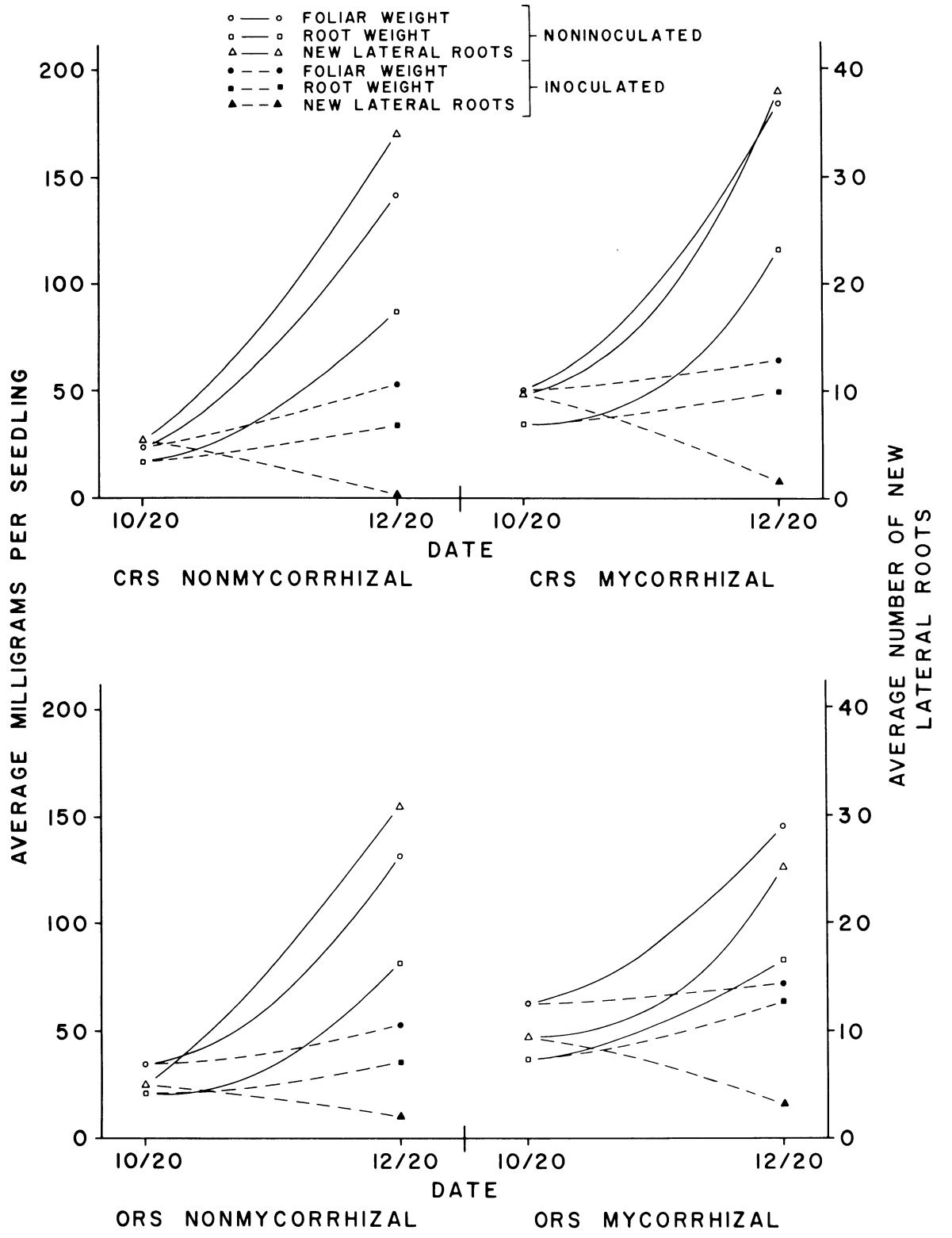

Fig. 2. Dry weight of foliage and roots and number of new lateral roots on Choctawhatchee (CRS, top) and Ocala (ORS, bottom) race sand pine inoculated with *Phytophthora cinnamomi* and on noninoculated seedlings.

0.6 to 5.2 propagules/g of air-dried soil in a total of five sand pine plantations near Niceville, Clarksville, and Yulee, Fla., and Darien, Ga. Soil samples from four of the seven plantations in which mortality from *C. tabescens* was observed had *P. cinnamomi* present. Plantations in which soils had highest *P. cinnamomi* (3.0-5.2 propagules/g) also had highest mortality rates associated with *C. tabescens*. *Phytophthora cinnamomi* was found in one Choctawhatchee race plantation where mortality from *C. tabescens* was not present. This plantation was selected for sampling because a portion was poorly drained and some trees showed decline symptoms. This was the only Choctawhatchee plantation in which *P. cinnamomi* was found. *Phytophthora cinnamomi* was not found in any of the natural stands of sand pine.

DISCUSSION.—*P. cinnamomi* is a virulent pathogen of sand pine seedlings; both mycorrhizal and nonmycorrhizal seedlings of the Choctawhatchee and Ocala races were attacked readily. This appears to nullify the root-protecting role of mycorrhizae (6, 7); however, the seedlings used in this study were only 25% mycorrhizal when inoculated, leaving a number of new lateral and feeder roots unprotected. The symbiont continued to synthesize new mycorrhizae throughout the 2-month study, as evidenced by the 100% increase in mycorrhizae, but on most seedlings, new lateral roots developed so rapidly as a result of fertilization that the fungal symbiont did not have time to colonize the new roots before they were attacked by *P. cinnamomi*. More new lateral roots were produced by CRS than ORS. This probably explains why the CRS were killed rapidly with no apparent differences in mortality between the mycorrhizal and nonmycorrhizal seedlings. Although production of new lateral roots was undoubtedly stimulated by fertilization, their number is probably a valid race difference. Our observation that hyphae of *P. cinnamomi* are restricted to the vascular tissue of the mycorrhizae also supports this reasoning. Mortality rate of ORS was not as high as CRS. In fact, some protection by the mycorrhizae in ORS was evidenced by the lesser mortality rate in the mycorrhizal seedlings. An artificial system such as that established in this greenhouse study may not be representative of field conditions.

The association of *P. cinnamomi* with areas of decline and mortality in sand pine, and proof of pathogenicity of this organism on sand pine, suggest its involvement in the etiology of sand pine root disease previously associated with *C. tabescens*. Although *P. cinnamomi* was not found in all areas where sand pine mortality occurred, the recovery rate of propagules was so low in most areas that they could have been easily missed in the sampling procedure. In many areas where sand pine has been planted, large amounts of hardwood debris were left in the soil after site preparation, and this is conducive to massive buildup of *C. tabescens* inoculum. If sand pine planted in such areas are weakened by feeder root necrosis caused by *P. cinnamomi*, they could be attacked by *C. tabescens*, resulting in the rapid mortality observed in the field (9). In sand pine, crown symptoms similar to those of littleleaf disease (3, 9) have been observed, but such trees do not persist as long as littleleaf-affected shortleaf pine because of the rapid attack by *C. tabescens*.

The recovery of *P. cinnamomi* from forest soils across north Florida and south Georgia suggests a wide distribution of this pathogen in areas where sand pine may be planted. Considering its virulence, the presence of *P. cinnamomi* may be the limiting factor in establishing plantations of sand pine outside its natural range, particularly in poorly drained sandy and heavier clay soils. The recovery rate of *P. cinnamomi* from soils in the various locations suggests that the level of infestation is too low to cause a serious disease problem. However, recovery rate of *P. cinnamomi* from nursery soil where Fraser fir was being killed and in severe littleleaf disease sites (4) was within the range found in this study.

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