# Seedling Blight of Longleaf Pine Caused by a Binucleate *Rhizoctonia solani*-like Fungus

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**ABSTRACT**

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A pathogen that causes blight of longleaf pine seedlings in nurseries in Florida that was originally identified as *Rhizoctonia solani* is described in this paper as a binucleate *R. solani*-like fungus. The fungus belongs to anastomosis group 3 of *Ceratobasidium* spp. (CAG 3) on the basis of anastomosis trials with isolates representing six anastomosis groups. In greenhouse trials, longleaf pine seedlings grown in autoclaved soil infested with an isolate of the fungus showed symptoms of both preemergence and postemergence damping-off. Over a 2-yr period in one commercial forest tree nursery, losses associated with blight caused by this organism varied with seed lot age and ranged from 0.4 to 18.9%.

Interest in longleaf pine (*Pinus palustris* Mill.) management in the southeastern United States has been increasing during recent years. This interest is due partially to the low susceptibility of this species to many diseases after seedlings are established in the field. Production of longleaf pine for outplanting in Florida has been limited, however, by a severe seedling blight in forest tree nurseries. In both Florida and Mississippi, this blight has been attributed to infection by *Rhizoctonia solani* Kühn (1,6). A similar disease, in which a species of *Rhizoctonia* was associated with damping-off of longleaf pine seedlings in several forest tree nurseries in the southeastern United States, was described by Davis in 1941 (5). The specific identity and pathogenicity of the organism, however, were never established.

In Florida, symptoms appear initially as water-soaking and chlorosis of needle bases; subsequently, distal portions of these needles turn yellow and then brown. Frequently the needle bases, terminal bud, and upper taproot darken and may decay (Fig. 1). Seedlings showing early symptoms recover occasionally. Blight symptoms first appear in early May and develop throughout the summer. Typical disease foci consist of dead or missing seedlings surrounded by numerous symptomatic plants in various stages of blight development. Blight is especially severe on seedlings in sandy soils because sand accumulates around the needle bases and terminal buds. Such accumulation, sometimes referred to as "sand splash" (5), provides warm, humid conditions that apparently are conducive to seedling infection. Sand accumulation also may bring pathogen propagules into contact with susceptible host tissues.

Since the early reports of this seedling blight in Florida, detailed examination of the pathogen has revealed that it does not exhibit the multinucleate condition typical of *R. solani* (J. T. English and R. C. Ploetz, *unpublished*). Instead, a binucleate condition has been observed. We have isolated binucleate forms of *R. solani*-like fungi from diseased longleaf pine seedlings in four forest tree nurseries and two outplant sites in Florida. All isolates examined showed characteristics of *R. solani*, including right-angle

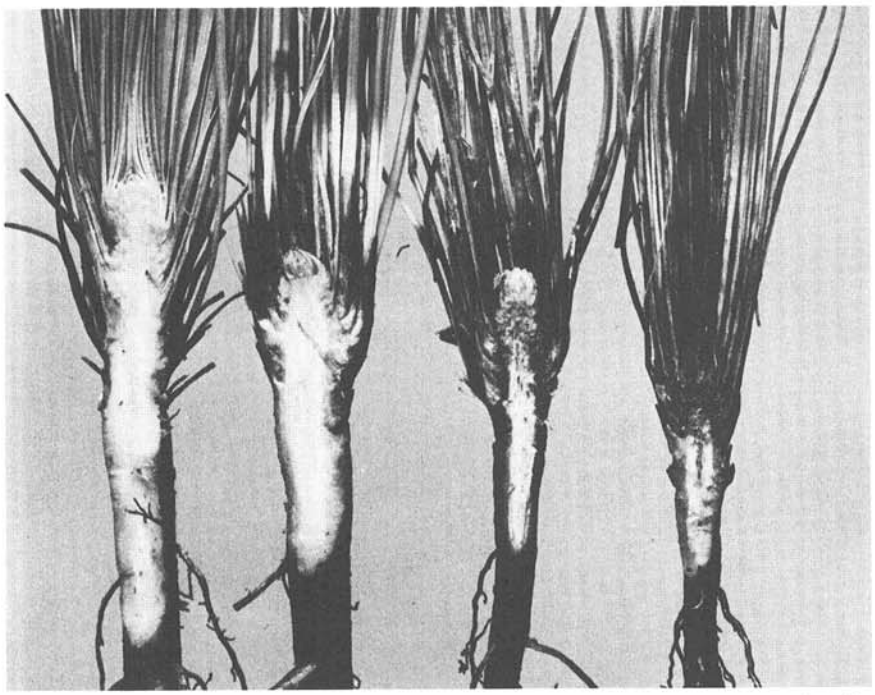

**Fig. 1.** Symptoms in longleaf pine seedlings infected with a binucleate *Rhizoctonia solani*-like fungus. (Left to right) Asymptomatic, uninfected; early necrosis of needle bases and slight discoloration of terminal bud tissues; advanced necrosis of needle bases with developing bud necrosis; and complete necrosis of terminal bud.

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branching of hyphae with constrictions occurring at the branch bases, septum formation just beyond the points of constriction on hyphal branches, monilioid cell formation, and presence of dolipore septa. The binucleate condition of hyphal cells, however, suggests that the isolates belong within the general group of binucleate *R. solani*-like fungi, which has as a designated perfect stage a species of *Ceratobasidium* (9). Burpee et al (2) and Ogoshi et al (7,8) have described anastomosis groups among these binucleate *R. solani*-like fungi.

The purpose of this report is to establish the position of these fungal isolates from longleaf pine seedlings among binucleate *R. solani*-like organisms and to document their pathogenicity on longleaf pine. The impact of this disease on longleaf pine seedling production also is addressed.

## MATERIALS AND METHODS

**Isolate characterization.** A binucleate isolate of *Rhizoctonia* sp. (FTC 083-3583) was obtained from a symptomatic longleaf pine seedling in the Andrews State Forest Tree Nursery in Chiefland, FL. This isolate was paired with tester isolates representative of six of the seven anastomosis groups established by Burpee et al (2) to delineate binucleate *R. solani*-like fungi (CAG 1–5 and CAG 7). Tester isolates were provided by L. L. Burpee (Department of Environmental Biology, University of Guelph, Guelph, Ontario). Anastomoses between FTC 083-3583 and tester isolates were determined in a manner similar to that of Burpee et al (2). A small plug of mycelium was taken from the edge of a young culture of FTC 083-3583 growing on potato-dextrose agar (PDA) and placed at the center of a glass slide coated with water agar. Similarly, two plugs from a culture of a single tester isolate were placed at opposite ends of the slide. Slide cultures were prepared in this manner for each of the tester isolates. Slide cultures were maintained in moist chambers in the dark and examined daily with dark-field and phase-contrast microscopy for the occurrence of hyphal anastomoses. In subsequent tests, 19 isolates derived from soil within disease foci, and five additional isolates from diseased longleaf pine seedlings collected in the Andrews nursery, were examined for hyphal anastomosis with tester isolates. Isolates derived from symptomatic seedlings from three additional forest tree nurseries and two outplant sites also were tested for anastomosis with the tester isolates.

**Pathogenicity.** Trials were established to determine the ability of isolate FTC 083-3583 to infect longleaf pine seedlings both as a preemergence and postemergence damping-off pathogen. This isolate was grown on PDA in the dark for 48 hr, then four plugs were taken from the culture margin and added to a flask containing moist, autoclaved longleaf pine needle fascicles. Colonized fascicles were used as inoculum after 2 wk of incubation in the dark at 25 C. Soil was infested by placing one colonized needle fascicle on the soil surface about 1 cm from a longleaf pine seed or seedling. Recently collected longleaf pine seeds from western Florida were used in all pathogenicity trials. Before planting, seeds were soaked in water for about 10 hr to enhance germination (13).

In each trial, one longleaf pine seed was planted in each of ten 100-ml polypropylene beakers containing sandy soil obtained from the Andrews nursery. Soil had been autoclaved previously for 1.5 hr on each of two successive days. Seeds were covered with about 0.5 cm of autoclaved soil. To test the ability of the fungus to infect seedlings before emergence, soil was infested with inoculum at the time of seeding. In postemergence infection trials, soil was infested 7 days after seedling emergence. Plants were maintained in the greenhouse and watered daily for 2 wk, then all seeds or seedlings were removed from soil, surface-sterilized in 70% ethanol for 30 sec, and placed on water agar containing streptomycin sulfate (50 ppm). Fungal colonies emerging from seedlings were subcultured on PDA and 1.5% ICN water agar (ICN Pharmaceuticals, Cleveland, OH) and examined for morphological and nuclear characteristics. The number of nuclei per hyphal cell was determined by the staining technique of Burpee et al (4). All pathogenicity trials were performed twice.

**Disease impact.** Surveys of seedling mortality were performed at the end of the 1979 and 1980 growing seasons in the Andrews nursery. Each year, seeds from various seed lots of different cold storage ages had been planted the previous spring or fall according to standard nursery practice. Alternate beds within each seed lot were examined along their full lengths for seedling mortality. The numbers and sizes of patches of dead or missing seedlings were recorded. Seedling losses within these patches were estimated using average seedling densities obtained from unaffected regions of seedbeds within each seed lot. On the basis of consistent isolation of a binucleate *R. solani*-like fungus from symptomatic seedlings before and during these surveys, all mortality was considered to be caused by this organism.

## RESULTS AND DISCUSSION

**Isolate characterization.** Isolate FTC 083-3583 anastomosed only with Burpee's Bn 31 tester isolate, a member of Burpee's anastomosis group 3 (CAG 3) of *Ceratobasidium* spp. (2). The fused cells were devoid of cytoplasmic material. The points of origin of anastomosing hyphae were determined by following each component hypha back to its original agar plug. Additional isolates derived from soil and diseased plant material from the Andrews nursery, as well as isolates from other nurseries and outplant sites, also anastomosed only with isolates Bn 31 and FTC 083-3583. Therefore, the organism described previously as the causal agent of seedling blight of longleaf pine in nurseries in Florida is not *R. solani* as previously reported (1,6) but rather a binucleate *R. solani*-like fungus belonging to CAG 3. A discussion of problems associated with taxonomic placement of binucleate forms is presented by Parmeter et al (9) and Burpee et al (2).

Most of the binucleate isolates that Burpee et al placed into CAG 3 originated from the southeastern United States (2). The isolates were collected from roots, fruits, seeds, and foliage of several plant species. Three binucleate isolates of *Rhizoctonia* sp. obtained from Florida were placed in anastomosis groups described by Burpee et al (2). One of these isolates was a member of CAG 2 and the other two were the sole members of CAG 7. All three of these isolates were collected from foliage. Ploetz (10) recently reported the recovery of CAG 3 isolates from soil and soybean roots in Florida. Ogoshi et al (8) established a separate system of anastomosis groups of binucleate *R. solani*-like fungi with isolates from Japan. Recent comparisons made by Ogoshi et al (7) between isolates from Japan and from the United States indicated that Burpee's CAG 3 and CAG 6 corresponded with Ogoshi's AG-E classification.

**Pathogenicity.** During 2 wk of incubation in a greenhouse, no seedlings emerged from infested soil in either of the two replicates of the preemergence pathogenicity trial. Seventy and 80% of longleaf pine seedlings emerged from noninfested soil in the first and second replicates, respectively. Nonemerged seedlings in infested soil were examined and appeared to be affected at several stages of seed germination. Often only decayed radicles were noted. In some cases, however, seedlings had developed to the point of hypocotyl and cotyledon emergence. In such cases, the radicles, hypocotyls, and cotyledons all were discolored and decayed. A binucleate isolate of *Rhizoctonia* sp. resembling FTC 083-3583 was recovered from 100% of these nonemerged seedlings. Emergence failures in noninfested soil were associated with apparently nonviable seeds. No known pathogens were isolated from these seeds.

During 2 wk of incubation, 100% of seedlings grown in soil infested after seedling emergence died in both replicates of the postemergence infection trial. None of the seedlings grown in noninfested soil showed symptoms. Infected plants showed blight symptoms typical of those observed in nurseries. Often mycelial

webs of *Rhizoctonia* sp. could be seen enmeshing terminal buds and needle bases at or slightly above the soil surface. This sign of the pathogen had not been seen in nurseries, presumably because buds and needle bases are often covered with sand accumulated through the actions of wind and rain. A binucleate form of *Rhizoctonia* sp. resembling FTC 083-3583 was isolated from all symptomatic seedlings in the post-emergence pathogenicity trial.

Isolates selected randomly from fungal colonies growing from diseased seedlings in both preemergence and postemergence trials were paired with isolate Bn 31 and examined for hyphal anastomoses. Hyphal anastomoses occurred in all cases.

Pathogenicity of isolates assigned to CAG 3 is not unique. Three of the CAG 3 isolates classified by Burpee et al (2) were reported by Sanders et al (12) to cause foliar blight of bentgrass. Burpee et al (3) also reported the ability of nine CAG 3 isolates to cause preemergence and postemergence damping-off of bean, pea, and tomato seedlings. Saksena and Vaartaja (11) described damping-off of pine seedlings in Saskatchewan and Manitoba caused by *R. endophyticum* Saksena & Vaartaja. This species was later found to be a binucleate *R. solani*-like fungus (9). However, Burpee et al (2) were unable to place this organism into any of their seven anastomosis groups. Whether or not our binucleate *R. solani*-like fungus is the same organism with which Davis (5) worked is unknown.

The source of inoculum for binucleate *R. solani*-like fungi within Florida nurseries is unknown. Seedbed soils generally are fumigated before each pine crop is planted. Whether inoculum occurs as residual propagules in soil after fumigation or whether it arrives in the form of windblown basidiospores, as mycelium or sclerotia in seed, or on untreated pine needle mulch is unknown. Fascicles of longleaf pine were used for inoculum production in this study because pine needle mulch is used commonly in nursery operations in Florida. The rapid colonization of needle fascicles, needles, and soil observed during this study suggest that the fungus is capable of rapid population buildup in fumigated nursery soil or on mulch regardless of the mode of dissemination.

**Disease impact.** An estimated 8.5% of the longleaf pine seedlings in the Andrews nursery were killed in 1979 by the binucleate *R. solani*-like fungus. Mortality varied dramatically with seed lot age. Percentages of mortality were 18.9, 5.5, and 0.4% for seed lots stored for 10, 2, and 0.5 yr, respectively. In 1980, mortality was estimated at 4.7% of the total longleaf pine crop; 7.9 and 1.3% of 0.5- and 1-yr-old seed lots, respectively, were lost.

Estimates of disease impact during these 2 yr are conservative. In addition to seedlings lost to mortality, numerous other seedlings showed progressive symptoms of blight development. Inclusion of these seedlings in the survey would have magnified crop loss estimates. The influence of nonlethal infection on seedling survival and growth after outplanting is unknown. Nursery losses sustained during the two survey years were not unusually high. In the years subsequent to these surveys, substantial levels of seedling blight have been observed in several Florida nurseries.

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