Characterization of a Binucleate *Rhizoctonia* Species Causing Foliar Blight of Loblolly Pine

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ABSTRACT

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A previously unreported foliar blight of loblolly pine (*Pinus taeda*), caused by a binucleate *Rhizoctonia solani*-like fungus in the *Ceratobasidium* anastomosis group 3 (CAG-3), is described. Isolates of binucleate *Rhizoctonia* sp. causing loblolly pine foliar blight anastomosed with binucleate isolates from blighted seedlings of longleaf pine (*P. palustris*).

Rhizoctonia spp. are recognized as causal agents of disease in southern pine nurseries (3). Seedling blight of longleaf pine (*Pinus palustris* Mill.) has become a serious problem during the past decade (1,6). The causal agent has been identified as a binucleate *Rhizoctonia solani*-like organism in the *Ceratobasidium* anastomosis group 3 (CAG-3) (4). A foliar blight of loblolly pine (*P. taeda* L.) was recently observed in a few forest nurseries in the southeastern United States. The disease was first observed in the Virginia Division of Forestry nursery near New Kent, Virginia, in the fall of 1986 and has since been observed in forest nurseries in South Carolina and Florida.

Affected foliage of blighted loblolly pine seedlings turns gray and is covered with a fine mycelial webbing similar to that observed with Rhizoctonia web blight of woody ornamentals (5). Infected foliage detaches from the stem, often by fascicle abscission, which is also similar to that seen with web blight of woody ornamentals. *Rhizoctonia* spp. can be consistently isolated from mycelial webbing and from symptomatic needles. Loblolly pine seedlings may be entirely defoliated, although even partial defoliation renders them unmarketable. The stem and buds of loblolly pine do not appear to be infected by the fungus.

The disease generally becomes severe late in the growing season (September–November) after crown closure in the seedbeds. Infection begins in the lower portion of the crown and proceeds up the stem, but symptoms do not become noticeable until they appear on the uppermost needles of the seedlings. The disease then spreads from plant to plant, forming disease foci. General observations indicate that disease occurrence is sporadic, both temporally and spatially, within forest nurseries reporting the problem, i.e., five to 10 foci of disease have been observed in a single nursery bed, while adjacent beds remain unaffected. To date, no attempt has been made to quantify losses or to track incidence of this disease on a regional basis.

This is the first report of a foliar blight caused by *Rhizoctonia* sp. on loblolly pine seedlings. The objective of this study was to characterize the *Rhizoctonia* sp. causing late-season foliar blight of loblolly pine seedlings in forest nurseries.

MATERIALS AND METHODS

Loblolly pine seedlings, symptomatic of foliar blight, were received from the Virginia Division of Forestry nursery near New Kent, Virginia, in September 1988. The fungus was cultured by placing a small portion of mycelium from webbing surrounding affected foliage in petri dishes containing acidified (5 ml L^{-1} 50% lactic acid) potato-dextrose agar (APDA). The fungus was also cultured from symptomatic needles. Infected needles were removed from the seedlings, surface-disinfested for 5 min in a solution of 0.5% NaOCl, 1 ml L^{-1} lactic acid, and 1 ml L^{-1} Tween 20 surfactant, and placed in petri dishes containing APDA. Fungi were subcultured by transferring hyphal tips to ensure that axenic cultures were obtained and were identified as binucleate *Rhizoctonia* sp. by microscopic examination of hyphal characteristics and by nuclear staining (2).

Glasshouse-grown loblolly pine seedlings were transplanted outdoors into a wooden frame (1.2 × 1.2 × 0.3 m) containing natural soil. Seedlings were approximately 20 cm tall and were transplanted at a density of about 300 m^{-2} to simulate late-season nursery conditions. Inocula from four isolates of *Rhizoctonia* sp. were applied to the loblolly seedlings by either of two methods. With one method, each isolate of *Rhizoctonia* sp. was obtained from a different loblolly pine seedling. Sterile water (20 ml) was added to each of four petri dishes containing a 4-day-old culture of the fungus, and each was scraped with a rubber policeman to remove mycelium. The mycelial suspensions were combined in a spray bottle and brought up to a volume of 1 L. Inoculum density of the suspension was not quantified. The suspension was sprayed over the top of three rows of loblolly pine seedlings until runoff. With the other inoculation method, mycelial plugs (5 mm in diameter) from 4-day-old, APDA-grown cultures of the same four isolates used in the first inoculation method were placed directly on the foliage of loblolly pine seedlings. Each mycelial plug was pressed onto the foliage with a sterile transfer needle. Plugs were placed in the midcrown region on two sides of each seedling in each

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of three rows. Remaining, uninoculated seedlings served as a barrier to separate the two treatments. Seedlings used as controls were maintained in plastic window boxes (30 × 11 × 10 cm) placed 2 m from inoculated seedlings. Seedlings were sprinkled with tap water every evening through the 3 mo of the test to maintain high relative humidity.

In October 1989, loblolly pine seedlings symptomatic of foliar blight were received from the Westvaco Corporation nursery near Summerville, South Carolina, and from the Virginia Division of Forestry nursery near New Kent. To further characterize the fungus causing loblolly pine seedling blight and to determine its relationship to the causal agent of longleaf pine seedling blight, five isolates of *Rhizoctonia* sp. were obtained from seedlings from South Carolina and 10 isolates were obtained from seedlings from Virginia. Each isolate was obtained from a different loblolly pine seedling. The fungus was isolated from infected needles and subcultured using the procedures described previously. Three isolates of *Rhizoctonia* sp. from longleaf pine seedlings were also obtained, one each from Hauss Nursery in Atmore, Alabama; Andrews Nursery in Chiefland, Florida; and Munson Nursery in Munson, Florida.

Nuclear conditions of the 15 isolates from loblolly pine and three isolates from longleaf pine were determined using the procedure of Burpee et al (2). Nuclear conditions of the four isolates collected in 1988 and of one isolate collected in 1986 also were determined at this time. A small drop of 0.5% aniline blue in lactophenol was placed directly on the mycelium of a 4-day-old culture on 2% water agar. The stained area was covered with a 18 × 18 mm coverslip, cut from the agar, and placed on a microscope slide. The slide preparation was heated gently over a flame until the agar melted and uniform staining developed. Hyphal cells were examined microscopically at 450× and 1,000×. The nuclear condition study was replicated once.

Anastomosis groups were determined by the procedure of Parmeter et al (8). The 15 isolates from loblolly pine collected in 1989 and the three isolates from longleaf pine were paired with five tester isolates of *R. solani* Kühn. Tester isolates obtained from Don Sumner (Coastal Plain Experiment Station, Tifton, GA) were categorized by *Ceratobasidium* anastomosis grouping (CAG) and included BN-1, BN-4, BN-6, BN-9, and BN-14a (CAG-1 through CAG-5). The 15 isolates from loblolly pine and the three from longleaf pine also were paired with each other. Mycelial plugs (7 mm in diameter) from the margin of 4-day-old cultures of the paired isolates were placed approximately 5 cm apart on 2% water agar in each of two petri dishes. Plates were incubated in the dark at 25 C for 3 days prior to anastomosis evaluation.

Overlapping hyphae of paired isolates on water agar plates were stained with 0.05% aniline blue in lactophenol, covered with a microscope slide coverslip, and examined at 450× for anastomosis. To ensure that anastomoses were between paired isolates, hyphal strands were traced to their origins. Isolates were considered in the anastomosis group of a tester isolate if cell wall and cytoplasmic fusion were observed at a minimum of four locations between paired isolates. The anastomosis study was replicated once.

RESULTS AND DISCUSSION

The technique used to determine nuclear condition did not uniformly stain nuclei of the loblolly and longleaf pine isolates. Location of a hyphal cell with distinctly stained nuclei required a lengthy search of the stained mycelium, even when the stained mycelium was allowed to incubate for 3–5 min, as suggested by Burpee et al (2), or when scanned at 1,000× magnification. However, the binucleate condition was observed for all isolates of *Rhizoctonia* sp. from loblolly and longleaf pine seedlings, including those collected in 1986 and 1988; no multinucleate hyphal cells were observed for any isolate. The use of alternative stains (9) or fluorescence microscopy (7) might have provided easier and more accurate methods of determining nuclear condition of these isolates of *Rhizoctonia* sp.

Koch's postulates were fulfilled for the isolates of *Rhizoctonia* sp. obtained from the loblolly seedlings from Virginia in 1988. Both inoculation methods reproduced symptoms of needle infection (discoloration, abscission, and mycelial webbing) characteristic of infection observed in nursery beds, but needle symptoms appeared faster (approximately 3 wk after inoculation) in seedlings sprayed with the inoculum suspension than in seedlings inoculated with mycelial plugs (approximately 5 wk after inoculation). Spread of the disease within and among seedlings was restricted in those inoculated with mycelial plugs. Neither inoculation method produced disease foci, as seen in the New Kent nursery, prior to termination of the experiment in November 1988. A binucleate *Rhizoctonia* sp. was the only fungus reisolated from symptomatic needles and from mycelial webbing.

Isolates of *Rhizoctonia* spp. differing in nuclear condition and/or anastomosis grouping can be obtained from a single host plant, can appear similar in culture, and can incite similar disease symptoms (A. R. Chase, *personal communication*). Therefore, since Koch's postulates were not attempted for the isolates of *Rhizoctonia* sp. obtained in 1989 (used for characterization of anastomosis), it is possible that these isolates differed from those used in the pathogenicity tests in 1988. However, given that isolates used in the pathogenicity and anastomosis tests were all binucleate, were obtained with little contamination from pine needles showing identical disease symptoms, and appeared the same in culture, we believe this possibility to be remote.

Isolate BN-6 (CAG-3) was the only tester isolate that anastomosed with isolates from either loblolly or longleaf pine, and this tester isolate was able to anastomose with all pine isolates tested. All loblolly pine isolates anastomosed with each other, as did all longleaf pine isolates, regardless of nursery of origin. All loblolly pine isolates were also able to anastomose with all longleaf pine isolates.

The fungus causing foliar blight of loblolly pine meets the criteria of a binucleate *Rhizoctonia* sp. in anastomosis group CAG-3. Also, the fungus causing foliar blight of loblolly pine was morphologically indistinguishable from that causing longleaf pine seedling blight. Reciprocal pathogenicity tests using the loblolly and longleaf pine isolates would help confirm that the same fungus is responsible for both pine blights.

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