Susceptibility of Longleaf Pine Seedlings to *Cronartium quercuum* f. sp. *fusiforme* in Greenhouse Tests

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ABSTRACT

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Seedlings from families of 13 wind-pollinated longleaf (*Pinus palustris*), 2 loblolly (*P. taeda*), and 2 slash (*P. elliottii* var. *elliottii*) pines were inoculated with basidiospores of *Cronartium quercuum* f. sp. *fusiforme*. Inoculum was derived from galls on 30 naturally infected longleaf pines. Incidence of infection in families of longleaf pine seedlings ranged from 16 to 38% compared with 33 to 86% for families of slash and loblolly pines. Screening longleaf pine families by inoculation of seedlings with basidiospores appears feasible.

An inoculation system employing concentrated basidiospore suspensions (CBS) (3) is used at the Resistance Screening Center (RSC), Asheville, NC, to evaluate slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pine seedlings for resistance to the fusiform rust fungus, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*. Thousands of loblolly and slash pine seedlings are inoculated each year and evaluated for resistance to the disease. Clients use these results in their tree selection and breeding programs.

Recently, there has been renewed interest in planting longleaf pine (*P. palustris* Mill.), which is more resistant than loblolly or slash pine to fusiform rust (4). However, the incidence of fusiform rust in longleaf pine appears to increase when this species is intensively managed (5). More than 82% incidence was reported recently by Kraus (1) in one 10-yr-old longleaf pine plantation. Walkinshaw (6) found that longleaf pine can be readily infected with *C. quercuum* f. sp. *fusiforme* in the greenhouse.

The purposes of this study were to determine if the controlled basidiospore suspension system currently used at the RSC could provide rapid, uniform screening of longleaf pine families for fusiform rust resistance and to obtain additional information on the susceptibility of longleaf pine to fusiform rust.

MATERIALS AND METHODS

Seeds from 13 wind-pollinated longleaf pines were collected by the Florida Tree Improvement Cooperative. Two loblolly seed lots (Livingston Parish and 10-8) and two of slash pine (FA-2 and Georgia Slash) were included as standards. CBS methods closely paralleled those used previously (2,7).

Seeds were germinated, transplanted, and grown at the RSC as tubelings in a 5:3:1 mixture of vermiculite, peat moss, and perlite. Two replicates of three 20-tree trays containing 8-wk-old seedlings were inoculated 1 day apart with basidiospores of *C. quercuum* f. sp. *fusiforme*. Basidiospores were obtained from telia on northern red oaks (*Quercus rubra* L.), which had been inoculated with a composite of aeciospores by the technique of Matthews and Rowan (3). The composite was collected from 30 stem galls on longleaf pines in Georgia. The concentration of the inoculum was 35,000 basidiospores per milliliter. Twenty thousand is normally used for slash pine and 50,000 for loblolly pine.

After inoculation, seedlings were held in a chamber at 21 C and 100% relative humidity for 24 hr, then were moved to a greenhouse and maintained for 9 mo. Natural lighting was supplemented to maintain a 16-hr photoperiod. Trees were fertilized to maintain active growth during the 9-mo period.

The percentage of symptomatic seedlings per seed lot was determined after 9 mo. Typical fusoid stem galls did not form on young longleaf seedlings. Because the seedlings lacked elongated stems, a new evaluation procedure was needed for measuring rust incidence. We classified a longleaf seedling as infected if it had an unusual bud formation, upper root swelling, or internal necrosis. Each seedling was cut in half to assess necrosis. Beet-shaped galls often formed on root collars of seedlings that showed these symptoms. To verify these symptoms as rust-induced, 15 longleaf pine seedlings from the RSC and 188 from parallel tests at the Forest Science Laboratory, USDA Forest Service, Gulfport, MS, were examined microscopically (6) for rust hyphae. Two standard seed lots of loblolly and two slash pines were also examined for galls.

RESULTS AND DISCUSSION

All longleaf, slash, and loblolly pine families had some infected seedlings (Table 1). Infection of longleaf pine was confirmed by microscopic examination. On the average, seedlings with rust hyphae in their cambial zones showed a mean increase of 34% in root-collar diameters. The lowest incidence of infection was 16% for longleaf pine seed lot 53-80, and the highest was 38% for seed lots 29-80 and 21-73. Slash and loblolly pine standards ranged from 33 to 86% galled, as anticipated on the basis of previous tests. Resistant and susceptible standard families ranked normally.

Analysis of variance revealed significant variability in rust incidence due to seed lot but not due to test or to an interaction of test and seed lot. Duncan's multiple range test showed significant differences

Table 1. Incidence of fusiform rust in 13 longleaf, 2 slash, and 2 loblolly pine seedling families inoculated with concentrated basidiospore suspensions

Seed lot[x]	Incidence of rust[y] (%)
53-80	16 a[z]
14-80	23 ab
Bulk-check	27 abc
3-65	28 abc
57-50	28 abc
L-58	29 abc
LLP-MS	30 abc
52-71	30 abc
RP-LA	32 bc
FA-2 (slash)	33 bc
26-66	37 bc
50-80	37 bc
29-80	38 bc
21-73	38 bc
Liv Par (loblolly)	42 c
10-8 (loblolly)	66 d
GA SL (slash)	86 e

[x] All seed lots not labeled slash or loblolly are longleaf pines.

[y] Data are means of two tests, each with three replicates of 20 seedlings per seed lot.

[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

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between longleaf seed lots (Table 1). The resistant and susceptible loblolly and slash pine checks ranked normally. Moreover, the mean percent infection of longleaf pines at the RSC paralleled values obtained at Gulfport, MS (34–52% incidence).

These data demonstrate that the CBS inoculation procedures developed by the USDA Forest Service, Athens, GA (3), and used operationally at the RSC for loblolly and slash pines can be used to screen longleaf pine families for fusiform rust resistance, with the exception of infection assessment. Unusual bud formation, upper root swelling, and internal necrosis were used. The relationship between greenhouse inoculations and field performance of longleaf pines is unknown.

The observation that composite inoculum from field-infected longleaf pines readily infects loblolly and slash pine controls demonstrates a lack of specialization. Thus, field-infected longleaf pines could serve as potential reservoirs for strains of *C. quercuum* f. sp. *fusiforme* that can infect loblolly and slash pines.

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