Fusiform Rust Resistance in Loblolly Pine: Artificial Inoculation vs. Field Performance

THOMAS MILLER, Chief Research Plant Pathologist, USDA Forest Service, Southeastern Forest Experiment Station, Gainesville, FL 32611, and H. R. POWERS, JR., Chief Research Plant Pathologist, USDA Forest Service, Southeastern Forest Experiment Station, Athens, GA 30602

ABSTRACT

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Percentage of infection by *Cronartium quercuum* f. sp. *fusiforme* on seedlings of seven half-sib families of loblolly pine (*Pinus taeda*) following artificial inoculation were compared with those of the same families after 5 yr in a high-hazard rust area. Family ranking based on percentage of seedlings galled were essentially the same, demonstrating the reliability of artificial inoculation as a means of evaluating rust resistance in loblolly pine.

The identification of slash (*Pinus elliottii* var. *elliottii*) and loblolly (*Pinus taeda*) pines with genetic resistance to fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, is one of the most important components of research programs addressed to reducing losses caused by this destructive disease. The first step in evaluating rust resistance is the selection of phenotypically resistant parent trees in the field, followed by an evaluation of their progeny exposed to natural inoculum in field tests and by artificial inoculation of seedlings ranging in age from 6 wk to 1 yr (1,5,7).

For several years, a large proportion of artificial inoculations has been made using the concentrated basidiospore spray (CBS) system (2). This inoculation system is used by the Resistance Testing Center, USDA Forest Service, Southeastern Area, State and Private Forestry, at Asheville, NC, where tree improvement programs in the South routinely send seed lots for evaluation of fusiform rust resistance. The CBS system has been highly effective in differentiating levels of resistance between pine families, particularly in the case of slash pines. Recently, the correlation between the results of the CBS system and subsequent field performance of slash pine has been strengthened by the development of a prediction method based on additional symptom types that appear after artificial inoculation (6). In loblolly pine, however, a question remains about the correlation between infection in field tests and the results obtained from the CBS tests.

Possible reasons for discrepancies between field and artificial inoculation methods have included differences in types of resistance operating at different ages of the pine hosts and differences resulting from the relatively high CBS inoculum densities, which are considered to be so much higher than natural field inoculum that mechanisms of resistance could be overpowered (1,7).

In this study, percentage of infection on selected half-sib families of loblolly pine inoculated by the CBS system was compared with that on seedlings of the same families outplanted in a high-hazard fusiform rust area in central Georgia.

MATERIALS AND METHODS

The loblolly pine seedlings used in this study were grown from seed of seven open-pollinated families that showed a range of responses to rust infection, from susceptible to resistant, in previous field and CBS screening tests. One family (10-5) ranked consistently resistant, and another (10-8) showed consistent susceptibility. Two families (12-9 and 12-12) were reported as resistant in field tests, but preliminary artificial inoculations indicated a high degree of susceptibility. The remaining three families (10-25, 7-56, and 5-33) were considered intermediate in resistance but varied considerably in previous greenhouse and field evaluations.

Seed were sowed in flats containing a soil-sand-vermiculite (2:1:1, v/v/v) mixture. Shortly after germination, some of the seedlings were transplanted into plastic flats (33 × 13 × 11 cm) with the same soil mixture, 20 seedlings per flat. Other seedlings were transplanted individually into the same medium in plastic pots (20.3 cm diameter). The seedlings in flats were placed in a greenhouse for 6 wk and then inoculated using the CBS system. The potted seedlings were maintained in a greenhouse for 8 mo and moved to an outdoor shadehouse after all danger of natural rust infection had passed. After 7 mo in the shadehouse, they were outplanted in a high-hazard rust area in Houston County, GA.

Aeciospores of *C. quercuum* f. sp. *fusiforme* from two geographic areas were used in this study. In March 1974, eight single-gall collections were made in Georgetown County, SC, and Shelby County, AL, and processed by the techniques of Roncadori and Matthews (4). Aeciospores from these two areas were used because the seven pine seed lots were from parent trees that originated in central Alabama or in coastal South Carolina and Georgia.

Prior to inoculating oaks in November 1974, equal volumes of aeciospores from each of the eight single-gall collections from each location were combined to provide a South Carolina and an Alabama composite. Spores were rehydrated in a water-saturated atmosphere for 16 hr at 20 C and used to inoculate leaves of potted northern red oaks (*Quercus rubra*). Telia-bearing oak leaves were collected after 3 wk, and basidiospores were harvested.

Three inoculum densities of basidiospores from each aeciospore collection area were used to inoculate the seedlings: 12,500, 31,000, and 75,000/ml as determined with a Coulter electronic particle counter.

Each of the six combinations of two aeciospore sources and three inoculum densities was applied, by the CBS system, to four replicate flats of the seven families. One flat of seedlings of each family was randomly selected and inoculated with an aeciospore source-inoculum density combination until four flats of each of the seven families had been inoculated. This sequence was repeated with the remaining five combinations until all seedlings were inoculated. Between application of each inoculum combination, the spray nozzle and spore reservoir were flushed with a 75% solution of ethanol and thoroughly rinsed with deionized water.

After inoculation, the seedlings were immediately placed in a mist chamber at 20 C for 24 hr, removed from the mist chamber and held in an air-conditioned room for 24 hr, then moved into a

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Table 1. Ranking by percentage of rust-galled seedlings of seven half-sib families of loblolly pine 9 mo after artificial inoculation and 5 yr after outplanting in a high-hazard fusiform rust area

	Artificial inoculation		Outplanted	
Family	Galled after 9 mo (%)[a]	Ranking[b]	Galled after 5 yr (%)	Ranking
10-5	45	1	46	1
10-25	64	2	55	2
7-56	75	3	55	2
10-8	77	4	80	5
5-33	78	5	66	4
12-12	79	6	84	6
12-9	84	7	85	7

[a] Average of infection at three inoculum densities and from two geographic areas.
[b] Ranked from smallest (1) to largest (7) percentage of rust-galled seedlings.

greenhouse. Seedlings were examined for gall development after 9 mo.

The uninoculated, potted seedlings were outplanted in blocks in February 1976. Each block consisted of a single 10-seedling row of each of the seven families. The family planted in each row of each block was selected randomly.

Seedlings were examined for rust galls in January of each year through 1980, and a final examination was made in October 1980. The data were analyzed on the basis of percentage of seedlings per family with rust galls after five growing seasons.

RESULTS AND DISCUSSION

Evaluation of the 9-mo data from the artificial inoculations indicated that, although there were differences in percentage of seedlings infected between the three inoculum densities and two geographic areas, the relative family rankings varied little. Therefore, a single figure for percentage of galled seedlings was derived by averaging across the inoculum density and aeciospore source for each family. The average percentage of galled seedlings within each family 9 mo after artificial inoculation was compared with the percentage of galled seedlings after five rust seasons in the field (Table 1). There were no consistent differences between the percentage of seedlings infected in the greenhouse and those in the field for the seven families. Three families (10-25, 7-56, 5-33) had higher infection levels following artificial inoculation, two families (10-8, 12-12) had higher infection levels in the field, and two families (10-5, 12-9) were essentially the same. Most important, the relative rankings for the two evaluation methods were the same, except for families 5-33 and 10-8, and these differed only by one position (rank correlation, $r = 0.95$).

Field infection in families 7-56, 5-33, and 10-25 was 20, 12, and 9% lower than infection resulting from artificial inoculation. It is possible that these differences indicate some degree of field resistance in these families, but such determinations are beyond the scope of this research. In the case of 7-56, the difference was of such magnitude that it could have both biological and economic significance in our effort to reduce losses caused by fusiform rust. Further research is needed to identify any inherent differences in mechanisms of resistance between the 6-wk-old seedlings inoculated with the CBS system and exposure of older seedlings to natural basidiospore inoculum after outplanting.

Several of the families included in the study were selected from among the most difficult to categorize in terms of greenhouse-field relationships. This test, as well as earlier studies (3), not only identified the most resistant and the most susceptible families, but also accurately ranked the intermediate families that have traditionally been more variable in all types of artificial tests. The CBS system has demonstrated its value in predicting relative differences in rust resistance and can be of great value in programs designed to produce rust-resistant pines.

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