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Abstract

Two hundred twenty-four disease-free slash pines with good growth and form were tested for rust resistance. Trees in Alabama, Florida, Louisiana, and Mississippi were selected. After artificial inoculations, a low percentage of open-pollinated progeny of 32 selected trees had galls. In progeny from six of those, the number of gall-resistant trees increased from 50 to 75 percent after one round of crossing. Analysis of seedling height, gall length, formation of adventitious shoots, and pith necrosis suggests that the six families may differ in their response to infection, even though they form similar percentages of galls. This resistance appears to be stable in a variety of fungus field isolates.

Keywords: Breeding for resistance, *Cronartium quercuum f.sp. fusiforme*, greenhouse inoculations, *Pinus elliottii* Engelm. var. *elliottii*.

Introduction

Slash pine (*Pinus elliottii* Engelm. var. *elliottii*), which is noted for straight stems with high specific gravity and rapid early growth, grows well on the imperfectly drained soils of the middle and lower Coastal Plain (Koch 1972). Increased demand for wood fiber in the South between 1950 and 1970 resulted in the expansion of pine plantations in the region. Slash pine was chosen for many locations because of its favorable early height growth, but the uniform tender shoots in extensive plantations provided abundant rust-susceptible host tissue. A full-scale fusiform rust epidemic resulted and eventually spread to other pine species. Infection rates in young stands rose to 90 percent, and mortality was often >50 percent.

Whereas rust-resistant slash pine trees occur throughout the species' range, many families with putative rust resistance grow more slowly than is desirable in commercial or national forest plantations (Snyder and others 1967, Walkinshaw and Bey 1981). In this study, we determined the number of trees with rust resistance in a population selected for its phenotypic growth (height and diameter at breast height [d.b.h.]) and form. Through control pollination, we sought to estimate levels of resistance.

Materials And Methods

Selection of phenotypes—There are 177 663 hectares (439,000 acres) of slash pine and 12 141 hectares (30,000 acres) of mixed slash pine and hardwood in the national forests of the South. When we made selections for this study, roughly half of that area supported 25- to 50-year-old slash pines. Trees were chosen at a rate of one per every 388 hectares of slash pine forest. Fifty trees each were selected in Alabama, Louisiana, and Mississippi, and 78 were selected in Florida. These 228 trees had greater heights and larger diameters than neighboring trees and were free of fusiform rust galls. Their average volume was 26.8 percent greater than neighboring trees, and selected trees were notable for their straightness and better-than-average crown characteristics.

Inoculation of the progeny of selected trees—Three preliminary and 9 standard tests for fusiform rust resistance (including 1 and 2 replications, respectively) were made with open-pollinated seeds from 224 selections (4 did not produce adequate seed). Thirty-eight of the trees were tested more than once. Each of the nine tests consisted of two inoculations 1 day apart. In each run, 3 trays of 20 seedlings were sprayed with 20,000 basidiospores per mm. The inoculum originated from a 150-gall mix collected from Alexandria, LA, Gulfport, MS, Mobile, AL, and Perry and Jacksonville, FL. The percentage of trees with galls was determined 6 months after inoculation. A tree was considered resistant if the mean percentage of seedlings with galls for the 2 inoculations was ≤50 percent. This level is considered low in tests at the Resistance Screening Center near Asheville, NC (Laird and Phelps 1975). All other aspects of the tests were modeled on procedures followed in earlier tests, e.g., Laird and Phelps 1975, Tainer and Anderson 1993, Walkinshaw and Roland 1990.

Breeding selected trees—Six of the rust-resistant trees with the lowest infection rates in the greenhouse were chosen as breeding parents, and, of the 228 superior phenotypes, tree number 214 was included as highly susceptible to fusiform infection. Control-pollinated trees are listed in table 1 (see page 2).

Inoculations of control-pollinated progeny—The cross-pollinated trees listed in table 1 were inoculated in 1992 or 1994. Three inoculations (replications) of 3 trays with 20 seedlings per tray were made according to procedures described in earlier publications, e.g., Laird and Phelps 1975, Tainer and Anderson 1993, Walkinshaw and Roland

Table 1—Test years for crosses between fusiform rust-resistant slash pine clones[a]

	Females						
Males	310	214	304	211	331	224	311
310	1994	R 1994	1992	1994	1992	0[b]	R 1992
214	—	0[b]	1992	1994	R 1994	0[b]	R 1994
304	—	—	1992	1992	1994	1992	0[b]
211	—	—	—	1992	1992	1994	1992
331	—	—	—	—	1994	1992	1992
224	—	—	—	—	—	0[b]	1994
311	—	—	—	—	—	—	1992

R = Reciprocal cross tested.

[a] Tested in either 1992 or 1994. The first digit is the State code: 1 = FL, 2 = LA, 3 =MS, 4 = AL.

[b] Pollinated over several years but produced inadequate seeds for the test.

1990. A field isolate from a single gall on a commercial pine in Washington Parish, LA, was used to produce the basidiospores for the inoculations. This inoculum was chosen because it caused a mean infection level of 63 percent and a range from 19 to 92 percent in 50 rust-resistant families on industrial and USDA Forest Service land. Bey and Walkinshaw (1981) and Walkinshaw and Bey (1981) provide partial lists of infections. Seedling heights (cm) and lengths of galls (mm) were measured 6 months after inoculation. Proportions of seedlings with galls (those containing more than two adventitious shoots at acute angles, and reddish-brown necrotic areas in the pith) were calculated 6 months after inoculation. Walkinshaw and others (1980) published a detailed description of these variables.

Statistical analysis—Analysis of variance tests on data from the control-pollinated progeny, the results of which were treated as fixed effects, were performed using greenhouse data. Seedlings in trays were arranged in a randomized design within each of the three replications per family. Gall length was plotted to test for normality.

Results

Progeny from 32 of the 224 selections (14 percent) exhibited resistance, i.e., ≥50 percent of seedlings had galls (table 2, see page 3). The number of families that were selected as resistant decreased when the percentage of galls for the test mean increased. Families selected for the control-pollinated group (table 3, see page 4) had growth similar to other open-pollinated families. Family 214 was selected as the susceptible family in the control-pollinated study.

In the 1992 test, 23.5 percent of seedlings from the 12 putative resistant crosses had galls 6 months after inoculation (table 3). Crosses 211 x 211 and 311 x 331 had galls on only 28 and 45 seedlings per replication, respectively. By contrast, 58 to 60 seedlings in susceptible families had galls. In the control-pollinated susceptible seedlots, 87 to 100 percent of seedlings had galls, and in the Resistance Screening Center's slash pine susceptible-composite seedlot, 88 percent had galls. In the 1994 test, 25.2 percent of seedlings in the 6 control-pollinated crosses had galls. Five crosses of slash pine 211 were included from another study as resistant controls and had means of 17, 18, 28, 29, and 35 percent of seedlings with galls.

Four traits were measured to determine any differences in control-pollinated seedlings that had formed galls after 6 months (table 4, see page 4). Families were significantly different for the 4 traits (f = 21, 31, 11, and 8 at the 1-percent level for height, length, shoots, and necrosis). The length of galls for family 211 was 26.9 mm, whereas for family 304, it was 53.9 mm. The majority of galls on family 310 were >50 mm. The distribution of gall lengths for the 2 families is shown in figure 1a and 1b. Skewing toward small galls was common, even in the control-pollinated crosses. Compared to parent 331, twice as many galled seedlings of parent 224 formed adventitious shoots. Progeny of parent 211 were unusual for their pith necrosis (43 percent of seedlings with galls).

Table 2—Percentage of seedlings with galls for open-pollinated progeny of good growth parents[a]

Family code	Percentage with galls: Family means	Percentage with galls: Test means[b]	Number of families in these tests[c]
201	46	60	53
211	40	—	—
219	9	—	—
224	38	—	—
235	43	—	—
314	48	—	—
342	44	—	—
343	46	—	—
422	37	—	—
426	39	—	—
432	36	—	—
426	39	68	25
435	45	—	—
427	50	—	—
433	43	—	—
441	43	—	—
331	42	69	24
310	37	—	—
346	41	—	—
304	50	—	—
311	50	—	—
419	39	81	48
304	48	—	—
211	3	55	12
204	39	—	—
212	49	—	—
222	47	—	—
439	48	—	—
306	41	72	25
111	37	—	—
238	44	—	—
430	50	—	—
428	40	—	—
449	42	—	—
328	48	86	33

[a] Mean of two replications of 60 seedlings each.

[b] A total of 261 seedlots were inoculated including repeats of families among tests. These 32 (3 repeats) resistant families are 14 percent of the original selections.

[c] The test mean includes all families in an individual test.

Smaller galls in inoculated progeny of parent 211 had less fungal growth in the cortex and cambium than the susceptible control at 21 and 60 days after inoculation. Using routine histological methods with a 12-seedling sample (Walkinshaw 1978, Walkinshaw and Roland 1990), fungal hyphae were seen in only 21 percent of the specimens of 211 but in 100 percent of the controls 21 days after inoculation. When fungus was present, linear growth in the cambium at 21 days was 175 to 750 µm for 211 and 425 to 1400 µm for the susceptible seedlot. The number of rust haustoria per parenchyma (cortical) cell at 60 days was 0.073 for 211 and 0.159 for the susceptible family. There were 15 and 31 haustoria per mm^2 of infected cortical tissue for 211 and the control, respectively.

Slash pine 211 was chosen as a representative to measure stability of the resistant families. Its open-pollinated progenies were inoculated with a number of rust fungus field isolates. The Resistance Screening Center conducted 12 tests with different inocula on its open-pollinated progeny. The mean percentage of seedlings with galls across tests was 14 percent and values ranged from 0 to 40 percent. When 9 other field isolates of the rust fungus were used in Gulfport, MS, an average of 27 percent of the seedlings developed galls. A highly resistant Florida tree, which had a mean of 43 percent and a range of 31 to 62 percent from the same 9 inocula, was the second best tree in these tests. This behavior of 211 paralleled the 25.3-percent mean for the open-pollinated (table 2) and 29.5 percent for the control-pollinated families (table 3).

Discussion

Selection of slash pine phenotypes with good growth and form that are free of fusiform rust is not difficult. Results of this study indicate that 14 percent of such phenotypes are resistant to fusiform rust, and this resistance can be increased to 75 percent by breeding.

The relatively low infection of open-pollinated progeny of parents with good growth is encouraging because most of the parents were probably wind pollinated by susceptible trees. The number of fusiform gall-free clones may be increased to 75 percent when the parents are control-pollinated with resistant trees. A seed orchard of such highly resistant trees has been established in Gulfport, MS. A 0.4-hectare experimental plot of progeny from controlled pollinations with 211, as well as vigorous 9–2 stock, was planted there. The mean d.b.h. after 11 years was 14.5 cm

Table 3—Percentage of slash pine seedlings with galls 6 months after inoculation with fusiform rust fungus[a]

	Females						
Males	310	214	304	211	331	224	311
	Percent						
310	17	54R	28	27	28	—	32R
214	—	—	52	35	57R	—	48R
304	—	—	19	27	28	21	—
211	—	—	—	9	19	30	36
331	—	—	—	—	22	12	33
224	—	—	—	—	—	—	27
311	—	—	—	—	—	—	18

R = Reciprocal cross tested.

Table 4—Mean values for traits of infected seedlings six months after inoculation with the rust fungus[a]

	1992 test			
Family[b]	Height	Length of gall	Proportions with shoots	Proportions with pith necrosis
	cm	*mm*		
211 x 211	30.7	16.2	0.23	0.43
211 x 304	35.6	25.5	.05	.59
224 x 304	34.7	60.2	.66	.09
224 x 331	39.4	52.3	.37	.20
304 x 304	36.8	63.5	.49	.21
304 x 310	37.9	70.4	.66	.04
311 x 211	38.5	28.8	.05	.43
310 x 311	39.5	57.8	.41	.06
311 x 311	38.1	53.5	.28	.16
311 x 331	37.8	57.5	.27	.12
331 x 211	40.7	22.1	0	.52
331 x 310	45.6	59.3	.28	.07
232 x 321(S)[c]	34.2	55.1	.47	.15
211 x 310	35.6	37.0	.15	.27
224 x 211	31.6	28.6	.22	.35
310 x 310	37.8	49.7	.47	.19
311 x 224	38.4	40.2	.40	.23
331 x 304	37.2	49.8	.22	.21
331 x 331	37.9	39.0	0	.42
232 x 321(S)[c]	33.3	44.9	.48	.14

[a] Means and proportions were determined from evaluations of 180 seedlings.

[b] See table 3 for the percentage of seedlings with galls for these families.

[c] (S) is a common treatment susceptible family in both tests.

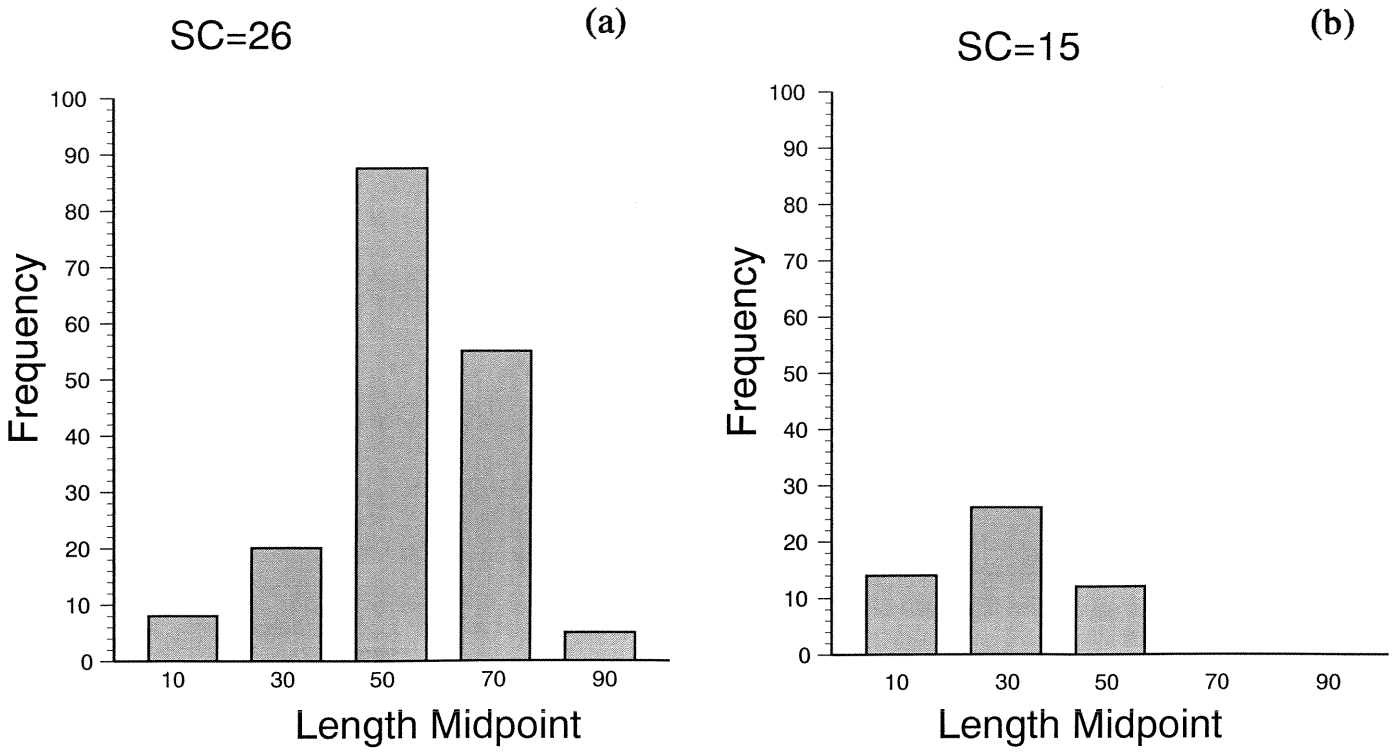

Figure 1—Frequency of gall lengths in (a) susceptible (232 x 321) and (b) resistant families (224 x 211).

(5.7 inches), compared to slash pine 9–2 progeny at 13.0 cm (5.1 inches).

Histological results for the progeny of parent 211 matched gall lengths measured in the greenhouse. Progeny from the susceptible-control family contained twice the amount of fungal hyphae and haustoria as the progeny of parent 211. Slash pines with 1 to 10 mm galls have been shown to have no fungus 9 months after inoculations (Walkinshaw 1978). There is a large proportion of small galls in the frequency plot represented in figure 1b. In other species of pine, small galls—especially those close to the ground—can persist for over 100 years without producing major damage (Walkinshaw and Barnett 1995).

The variation in rust resistance of slash pines as a function of the variability of field isolates remains a problem (Snow and others 1976). However, every effort was taken in this long-term study to expose seedlings to virulent field isolates. The use of many and various field isolates on the progeny of parent 211 had little effect on the percentage of seedlings with galls. Moreover, when a seedling from this highly resistant phenotype became infected, it demonstrated a mechanism for reducing host damage (Bey and Walkinshaw 1981).

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