

## Diallel Analysis of Genetic Resistance to *Cronartium quercuum* f. sp. *fusiforme* in Slash Pine

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### ABSTRACT

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Selfed, intercrossed, and wind-pollinated progenies of five slash pines of varying field resistance were inoculated with three collections of *Cronartium quercuum* f. sp. *fusiforme*. Two collections were from individual galls while the third was a composite from 10 galls. Differences among families and inocula occurred in percent galled seedlings 9 mo after

inoculation. In diallel analyses, general combining ability effects were significant while specific combining ability effects were not. Maternal effects were not apparent. Gall lengths were not significantly different among full-sib families or inoculum sources. The two most resistant parents appear to possess different kinds of resistance.

*Additional key words:* fusiform rust, *Pinus elliotii* var. *elliottii*, stability.

Selection and breeding in loblolly (*Pinus taeda* L.) and slash (*P. elliotii* Engelm. var. *elliottii*) pines have led to the development of resistant planting stock for reducing losses to fusiform rust (1,9). Since forest rotations are long and variability has been demonstrated in the rust fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (11,12), improved planting stock must possess resistance that is effective against diverse strains of the fungus so that the resistance will remain stable over time. The production of such trees requires a knowledge of the inheritance of resistance. In this study, five slash pines with varying resistance were selected and used in diallel crosses to determine the inheritance of fusiform rust resistance to various rust inocula.

### MATERIALS AND METHODS

Five slash pine trees were chosen from the Harrison Experimental Forest in south Mississippi. Previous research had classified the trees as follows: tree 8-7, resistant; trees 9-2 and 18-27; moderately resistant; and trees 18-26 and 18-62, susceptible (2,7,11). These parent trees were control-pollinated in a diallel crossing design. Then, full-sib seed from these crosses and the wind-pollinated seed from the parents were used to produce seedlings in 5-cm-diameter peat pots.

Three aeciospore collections were used for inoculation of water oak (*Quercus nigra* L.). The composite collection consisted of an equal-weight sample of fresh spores from single galls on 10 slash pines in commercial plantings in Mississippi. A second inoculum (LM-5), known to be avirulent on progenies of parent 8-7 (11), consisted of spores from a gall on slash pine near Laurel, MS. A third inoculum (8-7-8), which is virulent on wind-pollinated progeny of parent 8-7 (3), was collected from one such progeny near Bogalusa, LA. Both LM-5 and 8-7-8 are virulent on susceptible seedlots (11,13). Spores from each gall were processed according to the method of Roncadori and Matthews (10). For each replication, a separate group of oak seedlings was inoculated for production of basidiospores.

Six-week-old pine seedlings in 5-cm-diameter peat pots were inoculated by using the forced-air apparatus of Snow and Kais (14). On each of 3 days, eight seedlings of each wind-pollinated (WP)

and full-sib (FS) family were inoculated with each of the three spore collections. For each day (replication), inocula and families were selected randomly. Inoculum density was adjusted at 12-18 spores per square millimeter and verified after each group of eight seedlings (11). Inoculated seedlings were kept at 100% relative humidity for 24 hr, transplanted to soil in 10-cm-diameter plastic pots, and placed in the greenhouse in a randomized complete block design of eight-tree plots with three replications for each inoculum source.

Except for crosses of 18-62 as female, 18-26 × 9-2, and 18-26 × 18-27, enough seedlings were available to test the composite inoculum. For testing inoculum 8-7-8, seedlings were adequate for a half-diallel design using four families. The remaining seedlings were used to test the immunity of parent 8-7 to inoculum LM-5.

Numbers of seedlings with purple spots were recorded at 2 mo to determine successful infection. Occurrence of galls, and length of galls were measured at 6 and 9 mo after inoculation. Only 9-mo gall incidence and length are analyzed in this paper. Verification of 9-mo readings for seedlings without galls was made in field plantings at 3 yr. Analyses of variance ( $P = 0.05$ ) were used to test differences among families for percent galled and gall length for each inoculum.

Two diallel analyses for percent galled at 9 mo were run with the composite inoculum: one with FS families of the five parents, and one with FS and WP families of four parents (excluding 18-62). A diallel analysis was also run for FS families of four parents inoculated with 8-7-8. The Analysis III procedure of Gardner and Eberhardt (4) was used to partition variances and test for significance of differences for WP families versus FS families (average heterosis) and for differences among FS families. General and specific combining abilities were determined with inocula and families assumed to be fixed effects (5). The experimental material was regarded as the population about which inferences were made. Owing to a limited availability of seedlings, diallel analyses were performed only with the composite and 8-7-8 inocula.

Seedling and family numbers in this study were small in order to conform within constraints of the laboratory. However, survival of seedlings at 9 mo was so near 100% that mortality was not considered a problem in inflating the sampling error.

### RESULTS

**Composite inoculum.** Significant differences occurred among families for percent galled at 9 mo ( $F$  of 5.5 with coefficient of variation (CV) = 31%). Means ranged from 17 to 96 and 54 to 88% galled for FS and WP families, respectively (Table 1). The overall

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mean for the three replications was 57%. Of all crosses tested with the composite inoculum source, crosses with parent 18-27 consistently had the fewest galled trees. Crosses with 9-2 were intermediate between those of parent 8-7 and parent 18-26.

Plot means for gall length ranged from 39 to 64 mm. Crosses with 18-27 had shorter galls at 9, 18, and 24 mo after inoculation. The longest galls developed on offspring of parent 8-7. Gall-length differences among families were not significant.

Because diallel analysis of variance of the FS families from the five parents showed significant differences among data on full-sibs (Table 2), this permitted estimation of combining abilities. General combining ability (GCA) was highly significant while specific combining ability (SCA) was not significant,  $P = 0.05$ . Thus, additive effects of the parents were more important than nonadditive ones in determining performance of full-sib families.

Similar diallel results were obtained for the four WP families and the related FS families (Table 2). Significance among families permitted analysis of WP families, WP families versus FS families, and FS families. The latter two components were significant. The WP families component was not. Variance among WP families was smaller than the variance among FS families. With only four WP families, this nonsignificance is probably sampling error. General combining ability estimates for FS family effects also were significant while SCA effects were not.

General combining ability estimates of percent galled for each parent were similar for both five-parent FS, and four-parent FS and WP analyses (Table 3). The more negative the estimate (ie, lower mean infection) the more resistant the particular parent; thus, parent 18-27 was superior, whereas parents 18-26 and 18-62 performed poorly. Parents 8-7 and 9-2 were intermediate.

Maternal effects could not be analyzed statistically due to the incomplete diallel. However, visual inspection of the data (Table 1) suggested that maternal effects for rust resistance were not significant since reciprocal crosses performed similarly.

**Avirulent inoculum source.** For the avirulent inoculum source (LM-5), there were significant differences among families for percent galled at 9 mo ( $F$  value was 36.0 with a  $CV = 38\%$ ) (Table 1). In all cases except one, parent 8-7 passed its near-immunity to this inoculum to its full-sib offspring. Parent 8-7 was equally effective as female or male parent, except in crosses with 18-26 (female) from which 25% of the trees had typical galls. The reciprocal cross had no infection. Progeny crosses of 18-27 exhibited moderate susceptibility to this inoculum as it had for the composite inoculum. This inoculum, which was avirulent to 8-7 families, was virulent to 18-26 crosses. Gall lengths at 9 mo were not

TABLE 1. Plot means for percentage of slash pine seedlings galled 9-mo after inoculation by three inocula of fusiform rust in a five-parent diallel cross

Inoculum	Male Parents	Percent galling of female parents:			
		8-7	9-2	18-27	18-26
Composite	8-7	50	58	33	67
	9-2	58	72	33	...
	18-27	25	17	25	...
	18-26	67	73	67	92
	18-62	67	75	33	96
	wind	62	71	54	88
LM-5	8-7	0	0	0	25
	9-2	0	...	...	...
	18-27	0	...	...	...
	18-26	0	...	45	88
	18-62	0	...	...	91
	wind	0	25	46	88
8-7-8	8-7	95	100	67	92
	9-2	96	...	...	...
	18-27	42	...	50	...
	18-26	92	...	46	88
	18-62	88	...	69	92
	wind	100	92	46	96

<sup>a</sup>Three dots (...) indicate no seedlings available.

significantly different for families or replications.

**Virulent inoculum source.** There were significant differences among families for the virulent inoculum source (8-7-8) for percent galled at 9 mo ( $F$  of 4.6 with  $CV = 22\%$ ). Crosses with parent 8-7 generally were susceptible to this inoculum whether 8-7 was female or male. However, when 8-7 was crossed with parent 18-27, as male or female, only moderate infection developed (Table 1).

Of all FS families, those of 18-27 had the fewest galled with 8-7-8 inoculum. This reinforced results obtained from other inocula showing the value of 18-27 (Table 1). The virulent inoculum was highly pathogenic on the progeny of crosses with susceptible parent 18-26. As in the composite inoculum tests, FS families of 18-27 had the shortest galls except when 18-27 was crossed to 8-7; families of 8-7 generally had the longest galls.

In the diallel analysis, there were significant differences among FS families from four parents (8-7, 18-27, 18-26, and 18-62) (Table 2). General combining ability was significant, while SCA was not. Additive effects of parents were more important than nonadditive effects in determining performance of the FS families. General combining ability estimates for each parent were similar to composite inoculum estimates (Table 3). Performance of 18-27 was outstanding, but those of 18-26 and 18-62 were poor. The GCA estimates for each parent showed that 18-27 crosses were the most resistant.

TABLE 2. Analysis of variance for percentage of slash pine seedlings fusiform rust galled 9 mo after inoculation performed by using either: a composite inoculum source in a five-parent diallel cross, a four-parent diallel cross incorporating wind-pollinated families, and a virulent inoculum source in a four-parent diallel cross

Inoculum	Source <sup>a</sup>	df	MS	F
Composite	Blocks	2	382.94	1.26
	FS Families	9	1,927.30	6.36 <sup>b</sup>
	GCA	4	3,865.52	12.75*
	SCA	5	376.72	1.24
	Error	18	303.12	...
	Total	29		
Composite	Blocks	2	1,200.33	3.90*
	Families	9	1,459.66	4.38*
	WP families	3	607.64	1.82
	WP versus FS	1	2,200.81	6.61*
	FS families	5	1,822.65	5.47*
	GCA	3	2,581.74	7.75*
	SCA	2	684.01	2.05
	Error	18	333.08	...
	Total	29		
8-7-8	Blocks	2	153.72	0.56
	FS Families	5	1,571.51	5.68*
	GCA	3	2,341.72	8.47*
	SCA	2	416.16	1.51
	Error	10	276.56	...
	Total	17		

<sup>a</sup>FS = full-sib; WP = wind-pollinated; GCA = general combining ability; and SCA = specific combining ability.

<sup>b</sup>\* Significant,  $P = 0.05$ .

TABLE 3. General combining ability estimates (g) for percentage of slash pine seedlings galled 9-mo after inoculation with fusiform rust

Parent	Composite inoculum		Virulent inoculum
	g <sub>i</sub> <sup>a</sup>	g <sub>i</sub> <sup>b</sup>	g <sub>i</sub> <sup>c</sup>
8-7	-5.16	-1.90	3.20
9-2	-1.99	-2.27	...
18-27	-30.40	-23.15	-28.20
18-26	24.04	27.32	7.37
18-62	13.53	...	17.63

<sup>a</sup>Five-parent diallel.

<sup>b</sup>Four-parent diallel incorporating full-sib (FS) and wind-pollinated (WP) families.

<sup>c</sup>Four-parent diallel.

After 3 yr in the field, only two additional trees (0.5%) from all three inocula developed galls at point of inoculation. This precluded the possibility of missed or latent infection and verifies the 9-mo readings.

## DISCUSSION

The patterns of resistance across inocula for parents 8-7 and 18-27 shown in greenhouse tests suggest that different kinds of resistance exist. WP and FS families of 18-27 were consistently resistant to the three inocula; but WP and FS families of 8-7 were highly inconsistent in their response to the three inocula. Percent galled for FS families of 8-7 ranged from 0 to 100% depending on the inoculum source. Past artificial inoculation results also indicated variable performance of WP progenies of parent 8-7 (3,11) and the stable performance of WP progenies of parent 18-27 (11,13). Apparently the resistance of parent 8-7 is more inoculum specific than that of parent 18-27.

When one looks at the field performance of these two parents, one finds that, as expected, the stable resistance of 18-27 has held up very well over time ( $\geq 20$  yr) in field plantings in Louisiana, Mississippi, and Georgia (6). However, the resistance of 8-7 also has held up as well or better than 18-27 over time (6; E. R. Sluder, unpublished). The frequency of spore genotypes similar to the virulent 8-7-8 inoculum, of course, would be much reduced in the field situation compared to our artificial inoculation test situation. The composite inoculum represented spore genotypes a family would most likely face in a field situation. Progenies of 8-7 performed quite well when artificially inoculated with this inoculum source. That the resistance of 8-7 has held up in the field, therefore, was expected. *Extensive or repeated* planting of pines with this type of inoculum-specific resistance, however, may lead to an increased frequency of virulent forms of *C. quercuum* f. sp. *fusiforme* and a gradual erosion of resistance (3). Fortunately, this type of resistance does not appear to be very common. Nevertheless, it is important to know what kind of resistance breeding selections possess.

Both 8-7 and 18-27 had high GCA estimates for resistance with 18-27 having the highest. With additive gene action as high as it is in both parents, crosses among trees like this should yield progeny with increased resistance. This is especially true in approaches to slash pine tree breeding in which additive genetic variation is needed. A similar approach is being used for evaluating rust resistance in loblolly pine (8).

Although differences in gall lengths were not statistically significant, galls that developed on progeny of parent 18-27 were usually shorter for each inoculum source than the test means. In contrast, galls on 8-7 crosses were generally longer. This suggests that parent 8-7 formed a more compatible relationship with certain fungal genotypes. If reduced gall length is an indicator of less disease, then it serves as an additional indication of resistance of parent 18-27.

Single-gall inocula used in this study were selected for their virulent and avirulent reaction on wind-pollinated progenies of parent 8-7. Inocula such as these can be valuable in distinguishing types of resistance. While the composite inoculum source was valuable in substantiating the greenhouse and field resistance of both 8-7 and 18-27, the two individual gall cultures were useful in discriminating between the types of resistance of these two parents.

Although the results suggest major gene effects, the genetics of inheritance is complicated by observations with our composite inoculum source and nine single-gall inocula in another study (15). Overall, the greenhouse and field data for these two parents favor a more quantitative inheritance pattern.

Inoculum source 8-7-8 has been considered to be family specific (3), but it is clear that it has not evolved to such an extent that it cannot infect other families. Inoculum source 8-7-8 proved as virulent on progenies of 9-2 and 18-26 as on progenies of 8-7. Likewise, the avirulent LM-5 inoculum was quite capable of infecting progenies of 18-26, 9-2, and 18-27 even though it could not cause infection on progenies of 8-7.

## LITERATURE CITED

1. Blair, R. L., and Zobel, B. J. 1971. Predictions of expected gains in resistance to fusiform rust in loblolly pine. Pages 52-67 in: Proc. 11th South. For. Tree Improv. Conf., 15-16 June 1971, Atlanta, GA. (Published by South. For. Tree Improv. Comm.). 284 pp.
2. Dinus, R. J. 1969. Testing slash pine for rust resistance in artificial and natural conditions. Pages 98-106 in: Proc. 10th South. For. Tree Improv. Conf., 17-19 June 1969, Houston, TX (Published by South. For. Tree Improv. Comm.). 235 pp.
3. Dinus, R. J., Snow, G. A., Kais, A. G., and Walkinshaw, C. H. 1975. Variability of *Cronartium fusiforme* affects resistance breeding strategies. Pages 193-196 in: Proc. 13th South. For. Tree Improv. Conf., 10-11 June 1975, Raleigh, NC (Published by South. For. Tree Improv. Comm.). 262 pp.
4. Gardner, C. O., and Eberhart, S. A. 1966. Analysis and interpretation of the variety cross diallel and related populations. *Biometrics* 22:439-452.
5. Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9:463-493.
6. Griggs, M. M., and Dinus, R. J. 1977. Patterns of fusiform rust increase and their implications for selection and breeding. Pages 43-52 in: Proc. 14th South. For. Tree Improv. Conf. 14-16 June 1977, Gainesville, FL. (Published by South. For. Tree Improv. Comm.). 305 pp.
7. Jewell, F. F., and Mallett, S. L. 1967. Testing slash pine for rust resistance. *For. Sci.* 13:413-418.
8. Powers, H. R., Jr., and Duncan, H. J. 1976. Increasing fusiform rust resistance by intraspecific hybridization. *For. Sci.* 22:267-268.
9. Rockwood, D. L., and Goddard, R. E. 1973. Predicted gains for fusiform rust resistance in slash pine. Pages 31-37 in: Proc. 12th South. For. Tree Improv. Conf., 12-13 June 1973, Baton Rouge, LA. (Published by South. For. Tree Improv. Comm.). 352 pp.
10. Roncadori, R. W., and Matthews, F. R. 1966. Storage and germination of aeciospores of *Cronartium fusiforme*. *Phytopathology* 56:1328-1329.
11. Snow, G. A., Dinus, R. J., and Kais, A. G. 1975. Variation in pathogenicity of diverse sources of *Cronartium fusiforme* on selected slash pine families. *Phytopathology* 65:170-175.
12. Snow, G. A., Dinus, R. J., and Walkinshaw, C. H. 1976. Increase in virulence of *Cronartium fusiforme* on resistant slash pine. *Phytopathology* 66:511-513.
13. Snow, G. A., and Griggs, M. M. 1980. Relative virulence of *Cronartium quercuum* f. sp. *fusiforme* from seven families of slash pine. *Phytopathol. Mediterr.* 19:13-16.
14. Snow, G. A., and Kais, A. G. 1972. Technique for inoculating pine seedlings with *Cronartium fusiforme*. Pages 325-326 in: *Biology of Rust Resistance in Forest Trees*. U.S. Dep. Agric. Misc. Pub. 1221. 681 pp.
15. Walkinshaw, C. H., and Bey, C. F. 1981. Reaction of field-resistant slash pines to selected isolates of *Cronartium quercuum* f. sp. *fusiforme*. *Phytopathology* 71:1090-1092.