STABILITY OF FIELD RESISTANT SLASH PINE TO SELECTED ISOLATES OF FUSIFORM RUST FUNGUS

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Abstract.--In a series of 6 experiments, 22 open-pollinated families of field resistant slash pine and 6 full-sib families were tested for their reaction to isolates of fusiform rust. There was a greater range and more differences for percent rust infection among pine families than among rust isolates. The coefficient of variation ranged from 13 to 69 percent for the 22 families. The degree of variation was not related to the relative susceptibility. The family x isolate interaction was significant in all six experiments. In tree improvement, rather than determining specific rust isolates-pine family reactions, rust resistance breeding should concentrate on crossing individuals selected for moderate to high resistance to many individual or composite isolates of the rust fungus.

Additional keywords: *Pinus elliottii*, G x E interaction.

From the standpoint of genetic improvement, there is a need to better understand fusiform rust (caused by *Cronartium quercuum* [Berk.] Miyabe ex Shirai f. sp. *fusiforme*) resistance that exists in slash pine (*Pinus elliottii* Engelm. var. *elliottii*). Although there is no evidence of provenance variation for rust resistance (Goddard and Wells 1977), individual resistant trees throughout the range have been identified. In selecting resistant trees, there have been problems in getting consistent responses in field and greenhouse tests. The pollen mix in the orchard (Powers and Zobel 1978), the geographic location of the test (Goddard and Schmidt 1979), and the fungal isolate used for greenhouse inoculations (Snow et al. 1976) have been identified as being responsible for inconsistent results. Family x location and family x fungal isolate interactions reported for slash and loblolly pine (P. *taeda* L.) suggest that in selection of rust resistant trees, many factors must be considered (Powers et al. 1978).

In this greenhouse study, our objective was to characterize the resistance of 22 open-pollinated slash pine families and several full-sib families for their reaction to naturally occurring rust isolates. On the basis of percent infection and stability of the pine families, breeding implications are discussed.

MATERIALS AND METHODS

Seed was collected from 22 open-pollinated slash pine trees already selected and recognized by industries and the U.S. Forest Service as being rust resistant when they were progeny tested under field conditions. Most

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seed came from operational seed orchards where there had been little, if any, roguing for rust resistance. Since all these trees were not included in the same progeny test, we calculated a field performance rating according to the method of Walkinshaw et al. (1980) to compare families. In this rating, higher values indicate greater resistance.

Nine fungal isolates from slash pine in Louisiana, Mississippi, and Florida were selected for this study. The selected isolates varied from weak to strong in their ability to infect slash pine (Walkinshaw and Bey in press).

Aeciospores were collected from individual stem galls, stored according to the methods of Roncadori and Matthews (1966), and later used to produce telia and basidiospores on susceptible water oak (*Quercus nigra* L.). Density of 11-16 basidiospores/mm^2 was maintained with a forced-air apparatus (Snow and Kais 1972) by varying exposure time from 2.5-8 seconds and holding air flow to 3-5 standard liters per minute.

In the six experiments, eight families were tested each year from 1978 through 1980, with family 18-15 common to all experiments. In a split plot design, isolates were whole plots and families were subplots. The nine rust isolates were divided into two groups. Five were used in each inoculation, with isolate MS-15 common to all. Three replications per experiment (except two in experiment 2) were completed in 8 days. Each day we inoculated all pine families with one isolate before proceeding to the next. One seedling from each family was inoculated, and the process was repeated until all 12 seedlings for each family were completed. The 12 trees per family per isolate were considered the experimental unit.

In an adjunct to the main study, the same inoculation procedure was used for seven open-pollinated and six full-sib families, with a weak (MS-15) and a. strong (LA-7) isolate.

Gall incidence was recorded at 6 or 9 months and converted to arc sine $\sqrt{\text{percent}}$ for use in analysis of variance (ANOVA).

RESULTS

The percent of seedlings with galls for pine family-fungal isolate combinations ranged from 0 to 100 percent with an overall average of 59 percent. The averages for families over 9 isolates ranged from 27 to 84 percent while isolate means for the 22 families ranged from 47 to 68 percent (tables 1 and 2). The high infection rates in greenhouse tests do not detract from the high field performance for these field resistant families. During the inoculation and in the growth chamber and greenhouse, the environmental conditions were kept near optimum for the fungus, not like normal field conditions. When we used the rust evaluation index recently developed by Walkinshaw et al. (1980) all families were classified as being field resistant (table 2).

Table 1.--Percentage galled slash pine seedlings listed by isolate of rust used in the greenhouse inoculations

Expt.	Rust isolate					Expt.	Rust isolate				
No.	MS-15	MS-4	LA-8-7	LA-1	FL-4	No.	MS-15	LA-6	MS-10	FL-3	LA-7
1	50a[1]	51a	62a	64a	70a	2	52a	53a	59a	63ab	78b
3	49ab	44a	68ab	66ab	74b	4	45a	**57a**	49a	**57a**	46a
5	50ab	46a	65b	61ab	60ab	6	57a	**59a**	61a	76a	64a
Isolate mean	50	47	65	64	68		51	56	56	65	63
Rank corr.[2]	.72	.58	.43	.78	.79		.61	.66	.77	.70	.79

[1] / Means within the same experiment separated by the same letter are not significantly different at the .05 level.

[2] / Spearman rank correlation. Values show how well the individual rust isolates ranked the pine families compared to the overall pine family rankings. All values significant at .05 level.

There were significant differences among families in five of the six experiments (table 3). Within experiments, the family means for percent galled ranged from 25 percent for LA-11 to 92 percent for A-31. For statistical significance, family means generally had to be separated by at least 20 percent to be different. For most experiments, this separated only families with the lowest and highest infection rates. The level of infection for families was dependent on the fungal isolate, but in general five isolates seemed to adequately rank families and provide a reliable average for percent of seedlings infected. Families that were best with the first set of isolates generally were best with the second set. The generally uniform results in all six experiments of common isolate MS-15 on common family 18-55 adds further reliability to tests with a small number of fungal isolates.

Compared with pine family differences, there were relatively few differences among isolates. There were statistically significant isolate differences in three (2, 3, and 5) of the six experiments. In general, isolate means had to be separated by 20 percent or more to be statistically different. Means for isolates on eight families (within experiments) ranged from 44 to 78 percent, considerably less than the wide range for means for the pine families. The five isolates maintained their relative ranking from experiment to experiment (1 versus 3 versus 5 and 2 versus 4 versus 6). Isolates MS-15, MS-4, and LA-6 tended to be low while LA-1, FL-4, FL-3, and LA-7 tended to be high. Isolate LA-8-7, known to be highly virulent on progeny of pine parent 8-7 (Snow et al. 1976), was intermediate in this test. Some of the other isolates were just as virulent as LA-8-7 on pine family 8-7.

Table 2.--Percent galled, predicted field performance and corresponding coefficient of variation for 22 pine families. Values are based on plot means over two experiments

Pine family	Expt. no.	Percent galled		Rank correlation [1]	Rust evaluation [2] index		Pine family	Expt. no.	Percent galled		Rank correlation [1]	Rust evaluation [2] index	
		Mean	CV		Mean	CV			Mean	CV		Mean	CV
LA-11	5,6	27	66	NS	16.7	19	J-17	3,4	63	31	.71	7.4	48
J-1-5	3,4	42	56	NS	11.9	31	FA-7	3,4	64	34	NS	6.8	48
18-55	3,4	43	31	NS	12.0	23	35-55	3,4	65	30	NS	7.9	31
M-707	1,2	43	31	-.71	15.4	15	316-56	5,6	68	28	.45	9.2	36
24-54	3,4	44	33	.47	12.2	21	36-55	3,4	69	27	NS	7.1	41
179-55	5,6	45	39	NS	13.6	23	J-10	5,6	70	29	NS	7.3	53
18-55	1,2	46	43	-.38	13.9	22	H-7	1,2	70	34	NS	8.7	36
71-58	1,2	47	47	NS	13.4	20	PR-1	5,6	71	36	NS	8.7	60
18-55	5,6	50	31	-.35	12.6	19	A-31	5,6	71	34	NS	7.1	52
H-28	3,4	54	63	.71	12.2	63	7-55	5,6	78	29	NS	6.4	72
8-7	1,2	55	69	.39	12.3	63	FA-2	1,2	80	20	NS	7.1	40
18-27	1,2	59	28	NS	11.7	28	A-20	1,2	84	13	NS	7.1	23

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[1] Spearman rank correlation. Values show how well the individual pine families ranked the isolates compared to the overall rust isolate rankings. All values shown are significant at the .05 level.

[2] From Walkinshaw et al. 1980. High resistance is associated with seedlings showing initial purple spot symptoms but few developing galls that are fat or smooth. With the forced-air system used in this study there were more seedlings with initial symptoms and no swelling than in the controlled basidiospore suspension system used at the Resistance Screening Center where the rust evaluation index was developed.

Table 3.--Mean squares from analysis of variance for infection of slash pine by rust isolate 1/

	Df	Experiment number					
		1	2	3	4	5	6
Replications (days)	2	64	281	12	204	78	611
Isolates	4	1184^{NS}	1061*	2224*	486NS	821*	698NS
Error a (RxI)	8	27	188	159	78	112	176
Families	7	2117*	1202*	1097NS	865*	1795*	3817*
Family x isolate (FxI)	28	667*	295*	581*	333*	283*	614*
Error b	70	134	111	92	89	148	89

1/ *Denotes significance at .05 level. The FxI mean square was used for testing family and isolate main effects. Degrees of freedom for experiment 2 with only two replications were 1, 4, 4, 7, 28, and 35 accordingly.

The family x isolate interaction was significant in all six experiments. In every experiment, certain family-isolate combinations stand out against the general trends. For example, in experiment 1 isolate LA-1 was generally virulent, but not on family M-707, while isolates MS-15 and MS-4 were disproportionately weak on family 8-7. In experiment 6, MS-15 was unusually high (100 percent) on family 7-55, while isolate LA-6 was unusually low (14 percent). Such interaction might be responsible for some of the family x location interaction reported in field tests (Goddard and Schmidt 1979). As in field tests, certain families in these experiments possessed high resistance levels to most isolates.

The coefficient of variation (CV) is a measure of family stability to the nine rust isolates. It measures differences due to scale effects but not isolate rank changes. For the 22 families, the CV's ranged from 13 to 69 percent. The most variable families had low to moderate average infection rates. The slash pines in these experiments exhibited a wide diversity in average level of infection and in variation to the nine isolates (table 2). There were degrees from low infection and low variability to high infection and high variability. The CV's were computed across the experiments using plot means, and therefore include isolate differences, between experiment differences, and plot-to-plot within experiment differences. Accordingly, the values were unusually high.

In addition to the pine family differences for percent infection and coefficients of variation, there were differences among pine families in the way they ranked the isolates (table 2). For a comparison of individual families, we used as a standard the isolate rankings over all families. Using Spearman rank correlations, 16 out of 22 families did not rank the isolates according to their overall rank. Only five families had a significant positive rank correlation, and one had a significant negative correlation. There was no relationship between the families with significant correlations and percent galled or the CV.

In contrast to the general lack of individual families correctly ranking the isolates, the individual isolates all ranked the pine families about equally (table 1). The rank correlations for isolates varied from 0.43 to 0.79 and were all significant.

DISCUSSION

In terms of breeding strategy, this is a "good news" paper. The range of average infection of families was much greater than for the range of average infection of isolates. Some of the highly resistant families had high CV's, but when expressed for predicted field performance values, the CV's for the highly resistant families were substantially reduced and the CV's for the more susceptible families were increased (table 2). Statistically, this suggests that in the development of the predictive equation, there was less variation in ratings for the resistant families than for the more susceptible ones. Biologically, it appears that high resistance and low variability may occur more frequently in the field than in these greenhouse tests. Perhaps trees with galls in the more resistant families are more likely to recover than trees with galls in the more susceptible families.

Preliminary results from the adjunct inoculations to the main study and results from another study (Griggs and Walkinshaw 1981) suggest that it is unnecessary to know specific family x isolate responses to make additional gains through crossing. Griggs and Walkinshaw have shown that general combining ability for percent infection is high. In this study, infection rates for the control-cross progeny were always lower than the average infection rate of the two wind-pollinated parents (table 4). The same relationship was noted for other families by Griggs and Walkinshaw. It appears that there are many host genes that control resistance for each rust isolate, and that they may be different in different pine families.

Table 4.--Percent infection for open- and control-pollinated progeny for fungal isolates MS-15 and LA-7

Open-pollinated pine family	Fungal isolate MS-15	Fungal isolate LA-7	Control-pollinated pine family	Fungal isolate MS-15	Fungal isolate LA-7
8-7	8	71	8-7 x 7-55	21	71
18-27	44	87	8-7 x 35-55	15	41
18-62	96	88	18-27 x 18-62	63	79
9-2	46		18-27 x 9-2	33	69
7-55	86	92	18-27 x 7-55	58	79
9-55	75		18-27 x 9-55	46	63
35-55	75	36			

For pine breeding, it is important to develop resistance to a wide variety of rust isolates. But since the exact isolates that will be encountered in the field are unknown, screening to match the host to the pathogen will be impossible. More important than determining the specific rust isolate-individual family reactions is the need to select for moderate to high resistance over many individual or composite isolates. From the standpoint of improvement for resistance, the breeding scheme among the resistant clones or families is probably not critical. However, crossing of many such resistant parents and planting mixtures of progeny from these crosses should be done to provide an ample buffer against the array of fungal isolates in the field.

Although we did not test for buildup of virulent isolates nor for erosion of resistance, these will not likely be serious problems in tree improvement programs. Snow et al. (1976) list five factors that will moderate the rate of increase of virulent strains. Although this study shows that many naturally occurring isolates can infect all "resistant" pine families, the situation in the field is far more complex. Where mixtures of pine families are used, infection of "resistant" families in plantations will likely take place from many isolates in the fungal population on slash and loblolly pine in the area. This is supported from a slash pine test (Snow and Griggs 1980) which showed that inocula from infected individual resistant families were neither consistently virulent on these families nor on seedlings grown from commercial seed.

In summary, the balance seems to be tipped in favor of the tree breeder and the trees. The potential for selection of resistant pine families is large, a procedure for identifying resistant families is available, and a breeding system for making gains appears to be reasonably straightforward.

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