

Reaction of Field-Resistant Slash Pines to Selected Isolates of *Cronartium quercuum* f. sp. *fusiforme*

Charles H. Walkinshaw and Calvin F. Bey

Principal plant pathologist and principal plant geneticist, respectively, U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Box 2008, GMF, Gulfport, MS 39503.
Accepted for publication 9 February 1981.

ABSTRACT

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.

Nine isolates of *Cronartium quercuum* f. sp. *fusiforme* were pathogenic to 15 field-resistant slash pine (*Pinus eliottii* var. *elliottii*) families in greenhouse tests. Some isolates from random galls were as virulent as isolates derived from galls on a resistant family. Family \times isolate

interactions were significant in four experiments with 15 families but were greatly reduced when certain families were omitted from the analysis. Differences in gall incidence were generally significant among families but not among isolates.

Additional key words: fusiform rust, pathogenic variation, resistance.

Slash pine (*Pinus eliottii* Engelm. var. *elliottii*) in plantations in the South is commonly attacked by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (4). A buildup of fusiform rust over the past 40 yr has coincided with changes in techniques for managing southern forests. Widespread planting of slash pines has mixed types of pine, oak, and rust that formerly never came together.

Before human intervention, oak, pine, and *C. quercuum* f. sp. *fusiforme* probably coexisted without severe rust epidemics. As with other rusts and hosts (1), the fungus most likely evolved with pines and oaks. A natural balance was achieved within small local populations, which was perhaps especially important for slash pine, because it occupied wet flatland sites often separated by many miles (5).

As large numbers of healthy slash pines from diverse areas were planted in the Gulf states, they encountered rust isolates existing on loblolly (*P. taeda* L.) and other pine species. In a sense, in some areas, slash pine was an introduced species and behaved accordingly. Slash pines that previously appeared to be field-resistant were exposed to new isolates of the fungus and became heavily infected. Today, fusiform rust incidence in slash pine is high (4,12).

We tested field-resistant slash pines for their reaction to an array of naturally occurring rust isolates. Previous studies suggested the existence of both stable and unstable resistant slash pine types (9).

MATERIALS AND METHODS

A preliminary artificial inoculation test was established to help select rust isolates to use in this study. Nine of 24 collections tested on field-resistant pine families FA-2 (International Paper Co.) and 35-55 (Buckeye Cellulose Corp.) were selected. The selected isolates originated from slash pine in Louisiana, Mississippi, and Florida. One of the nine isolates, LA-8-7 is known to be virulent on the progeny of the parent pine, 8-7 (2,10).

Seedlings used in the study came from open-pollinated seed (mostly from seed orchards) collected from 15 slash pine clones selected on the basis of progeny tests for field resistance to fusiform rust. Most are considered mainline commercial types and have been planted extensively by the forest industry (Table 1). Five have been used in research by the Southern Forest Experiment Station.

Aeciospores were collected from individual stem galls, stored according to methods of Roncadori and Matthews (8), and used to inoculate susceptible water oak (*Quercus nigra* L.), which in turn produced telia and basidiospores for pine seedling inoculations (9). A density of 11-16 basidiospores per square millimeter was maintained during inoculation with a forced-air apparatus (11) by varying exposure time from 2.5-8 sec and holding airflow to 3-5 L/min.

Because we could not inoculate eight families with nine isolates in a day's time, we divided the rust isolates into two groups and did

TABLE 1. Origin of host lines

Pine parent code	State of origin	Source
J-17	FL	St. Regis Paper Co.
A-20	FL	St. Regis Paper Co.
FA-2	FL	International Paper Co.
FA-7	FL	International Paper Co.
M-707	FL	Florida Division of Forestry
71-58	FL	Buckeye Cellulose Corp.
36-55	FL	Buckeye Cellulose Corp.
18-55	FL	Buckeye Cellulose Corp.
24-54	FL	Buckeye Cellulose Corp.
35-55	FL	Buckeye Cellulose Corp.
18-27	MS	Southern Forest Experiment Station
8-7	MS	Southern Forest Experiment Station
J-1-5	MS	Southern Forest Experiment Station
H-7	MS	Southern Forest Experiment Station
H-28	MS	Southern Forest Experiment Station

TABLE 2. Mean squares from analysis of variance for infection of slash pine by rust isolate^a

Variable	Experiment			
	1	2	3	4
Replications (days)	64	281	12	204
Isolates	1184 ^{NS}	1061*	2224*	486 ^{NS}
Error <i>a</i> (R \times I)	27	188	159	78
Families	2117*	1202*	1097 ^{NS}	865*
Family \times isolates (F \times I)	667*	295*	581*	333*
Error <i>b</i>	134	111	92	89

^a Denotes significance at 0.05 level. Where F \times I mean square was significant, the F \times I square was used for testing family and isolate main effects. NS = not significant.

TABLE 3. Percentage of galled seedlings in slash pine families inoculated in the greenhouse with rust isolates²

Family	Rust isolate						Family	Rust isolate					
	MS-15	MS-4	LA-8-7	LA-1	FL-4	Mean		MS-15	LA-6	MS-10	FL-3	LA-7	Mean
Experiment 1							Experiment 2						
71-58	28	42	33	28	61	38 a	M-707	33	41	33	62	42	43 a
M-707	47	33	50	31	55	43 ab	18-55	42	29	50	25	71	43 a
18-55	42	53	31	44	69	48 ab	8-7	0	58	50	83	71	53 a
18-27	44	61	61	53	44	53 abc	71-58	33	41	67	62	70	55 a
8-7	8	11	95	94	78	57 abcd	H-7	79	67	38	46	88	63 ab
H-7	81	42	78	80	80	72 bcd	18-27	75	41	67	58	87	66 ab
FA-2	58	89	72	100	81	80 cd	FA-2	62	71	79	83	100	79 b
A-20	92	77	78	83	92	84 d	A-20	88	71	92	80	91	84 b
Mean	50 a	51 a	62 a	64 a	70 a	...	Mean	52 a	53 a	59 a	63 ab	78 b	...
Experiment 3							Experiment 4						
18-55	44	42	44	50	42	44 a	J-1-5	39	22	17	36	34	30 a
H-28	3	6	83	72	86	50 a	24-54	33	39	44	31	47	39 ab
24-54	53	50	56	36	61	51 a	18-55	44	39	50	47	25	41 ab
J-1-5	22	39	86	64	58	54 a	FA-7	39	86	56	53	47	56 b
J-17	72	31	50	72	92	63 a	35-55	61	72	39	75	36	57 b
FA-7	78	58	53	70	97	71 a	H-28	14	69	72	86	53	59 b
35-55	75	66	80	72	75	74 a	J-17	70	67	58	53	67	63 b
36-55	44	64	92	92	83	75 a	36-55	61	61	55	78	62	63 b
Mean	49 ab	44 a	68 ab	66 ab	74 b	...	Mean	45 a	57 a	49 a	57 a	46 a	...

²Mean 9-mo values for 12 seedlings and three replications in experiments 1, 3, and 4 and two replications in experiment 2. Families or isolates with the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

two experiments each in 1978 and 1979. Five isolates were used in experiment 1 and five in experiment 2; isolate MS-15 was used in both sets of inoculations. All inoculations in an experiment (three replications in experiments 1, 3, and 4, and two replications in experiment 2) were completed within 8 days.

Each day, we inoculated all pine families with one randomly assigned isolate before proceeding to the next isolate. One seedling from each family was inoculated before a second seedling from any family was inoculated. The procedure was repeated 12 times for each family within an isolate. The 12 groups of eight were incubated in plastic chambers (9).

Gall incidence was recorded after 9 mo. The incidence per 12-tree plot was converted to arc sine $(\%)^{1/2}$ for use in analysis of variance.

RESULTS

In all four experiments, family \times isolate ($F \times I$) interactions were significant (Table 2). This indicates that some of the 40 family-isolate combinations in each experiment are at odds with general trends (Table 3). Because pine families 8-7 and H-28 showed near immunity to some isolates and high infection with others, we suspected that these families were the main cause of interaction. When we deleted family 8-7, the $F \times I$ variances were reduced by 60 and 45% in experiments 1 and 2, respectively. In experiment 2, the $F \times I$ was no longer statistically significant at the 5% level. In experiments 3 and 4, family H-28 appeared to be highly variable; eliminating it reduced the variances by 40 and 32%, respectively. Another manifestation of the strong interaction of families 8-7 and H-28 was the unusually high standard deviations associated with these two families (generally twice that for other families) (Table 4).

Significant differences were found among pine families over all isolates in experiments 1, 2, and 4. When families 8-7 and H-28 were eliminated from the analyses, all experiments showed significant differences among the remaining families. Differences occurred despite previous selection of families for high field resistance to fusiform rust. The high infection rate in these tests, compared with the field, suggests that some field growth phenomena are responsible for limiting infection.

No differences were found among isolates in experiments 1 and 4, and only a few differences were found in experiments 2 and 3. Infections ranged from 44 to 78% for the nine isolates. Isolate LA-8-7, derived from a gall on pine family 8-7 and previously reported to be highly virulent on pine family 8-7, gave 62%

TABLE 4. Infection of slash pine families inoculated with rust isolates

Pine family	Percentage galled ^a	Standard deviation ^b
J-1-5	42	24
18-55(3,4)	43	13
M-707	43	13
24-54	44	15
18-55(1,2)	46	20
71-58	47	22
H-28	54	34
8-7	55	38
18-27	59	17
J-17	63	20
FA-7	64	22
35-55	65	20
36-55	69	19
H-7	70	23
FA-2	80	16
A-20	84	11

^aAverage over two experiments.

^bBased on plot means over two experiments.

infection for all pine families, about midway in the range of isolates. Percentage infection of pine families by isolate MS-15 was generally uniform in all experiments (45-52%).

DISCUSSION

Fifteen families of slash pine were tested with nine isolates of *C. quercuum* f. sp. *fusiforme*. Although the isolates generally distinguished the families on the basis of percentage galled, the coefficient of variation (standard deviation divided by mean) within a family, due to fungus isolates and error, varied from 13 to 69%.

On the basis of reaction to rust isolates, pine families did not fall into distinct groups. Pine families 8-7, H-28, and perhaps J-1-5 tend to have high variation. Clearly, the variability is highest when certain isolates cause little or no infection; eg, isolates MS-15 and MS-4 on pine families 8-7 and H-28. Before resistant trees, like 8-7 and H-28, with diverse reactions to different rust isolates are incorporated into improvement programs, tests of their crosses with stable types need to be established.

The variation among isolates over all pine families was generally small (12-30%), and only in one case (experiment 2) was a significant difference found among isolates. To establish

differences among isolates, many pine families should be used, particularly if they are random selections.

The uniform results with family 18-55 and isolate MS-15 show that comparisons among experiments and years are valid. Five isolates might provide a reasonable estimate of the susceptibility of a slash pine family and, if the five isolates are carefully selected, they might be sufficient to estimate the stability of the families. Powers and Matthews (6) showed differences among nine half-sib loblolly pine families using five isolates of the rust fungus. Knowing the average susceptibility and the variability should help geneticists develop improved strategies for breeding.

Erosion of resistance, as discussed by Dinus et al (2), may not be a problem with industry-selected trees, such as 18-55 used in this study. Although isolates virulent on both slash and loblolly pine exist in nature (7,9), selective buildup of virulent races might be minimized if seed from trees like 18-55 is mixed with seed from other stable types. This study supports the observations of Goddard and Schmidt (3), who reported that most of their slash pine families were resistant over wide geographic areas in the South. Using resistant material appears to be a promising solution to the fusiform problem in slash pine.

LITERATURE CITED

1. Anikster, Y., and Wahl, I. 1979. Coevolution of the rust fungi on Gramineae and Liliaceae and their hosts. *Annu. Rev. Phytopathol.* 17:367-403.
2. 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. 262 pp.
3. Goddard, R. E., and Schmidt, R. A. 1979. Relative geographic stability of resistance to fusiform rust of selected slash pine families. Pages 99-107 in: Proc. 15th South. For. Tree Improv. Conf., 19-21 June 1979, Miss. State Univ. 186 pp.
4. Griggs, M. M., and Schmidt, R. A. 1977. Increase and spread of fusiform rust. Pages 32-38 in: R. J. Dinus and R. A. Schmidt, eds. Management of fusiform rust in southern pines. Symp. Proc. Univ. Fla., Gainesville. 163 pp.
5. Hebb, E. A., and Clewell, A. F. 1976. A remnant stand of old-growth slash pine in the Florida panhandle. *Bull. Torrey Bot. Club* 103:1-9.
6. Powers, H. R., Jr., and Matthews, F. R. 1979. Interactions between virulent isolates of *Cronartium quercuum* f. sp. *fusiforme* and loblolly pine families of varying resistance. *Phytopathology* 69:720-722.
7. Powers, H. R., Jr., Matthews, F. R., and Dwinell, L. D. 1977. Evaluation of pathogenic variability of *Cronartium fusiforme* on loblolly pine in the southern USA. *Phytopathology* 67:1403-1407.
8. Roncadori, R. W., and Matthews, F. R. 1966. Storage and germination of aeciospores of *Cronartium fusiforme*. *Phytopathology* 56:1328-1329.
9. 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.
10. 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.
11. 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. Publ. 1221. 681 pp.
12. Zobel, B., Blair, R., and Zoerb, M. 1971. Using research data—disease resistance. *J. For.* 68:486-489.