Evaluation of Pathogenic Variability of Cronartium fusiforme on Loblolly Pine in the Southern USA

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ABSTRACT

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Isolates from 56 individual rust galls from seven southeastern states were used to inoculate three loblolly pine families rated as resistant, intermediate, and susceptible to infection by *Cronartium fusiforme*. Significant variation appeared among the rust collections from different states and among collections within individual states. Even the most resistant pine family was highly susceptible to infection by inocula from certain individual galls. Because this high variability occurred in a limited sample of the fungus population, it is clear that pathogenic variability of the fungus should be taken into account in all tree-improvement programs involving southern pines. The high levels of virulence of specific inocula, even on resistant hosts, also mean that efforts must be intensified to find new sources of resistance, and to develop a wide genetic base of resistant pine material in breeding programs.

Additional key words: *Pinus taeda*, fusiform rust, disease resistance, southern pines.

Fusiform rust, which is caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm., is causing widespread (5, 6) and increasingly heavy (8) losses on loblolly (*Pinus taeda* L.) and slash (*Pinus elliottii* var. *elliottii* Engelm.) pines, the tree species most commonly planted in the southeastern USA. Because other field control measures are ineffective, selection and breeding of these two species for resistance to fusiform rust have been emphasized. Effective resistance to fusiform rust has been demonstrated in both slash (2) and loblolly (13) pines. Resistance in bulk collections of loblolly pine seed from certain geographic areas also has been demonstrated (12). If the breeding programs are to succeed, however, the pathogenic variability in the fungus population must be evaluated.

The results of preliminary work indicated that pathogenic variability was expressed within *C. fusiforme* when isolates were tested on several pine hosts (10). Subsequent studies have shown significant variation in infection of specific families of slash pine by several isolates of *C. fusiforme* (9). Since loblolly pine is the most widely planted pine species throughout the South, information regarding pathogenic variability of *C. fusiforme* on this species is needed. This study was designed to investigate the pathogenic variability of a wide range of collections of *C. fusiforme* from across the South by artificially inoculating susceptible, intermediate, and resistant loblolly pine seedlings.

MATERIALS AND METHODS

Loblolly seedlings were grown from seed from two half-sib families and one bulk collection of seed from a specific geographic area. Half-sib family 11-20 was rated as resistant and 11-23 as susceptible to fusiform rust. Both families were derived from superior trees selected in Georgetown County, S. C., by the West Virginia Pulp and Paper Company as part of the N. C. State-Industry Tree Improvement program. The bulk collection was from Livingston Parish, Louisiana, and seedlings from this source were intermediate in rust resistance. Relative resistance ratings of these pine families were based on many previous artificial inoculation tests and also on performance in field plantings. Although the host material used in this study included two half-sib families and one bulk collection, hereafter they will be referred to as families.

Aeciospores were collected separately from 10 individual galls in each of seven southern states from North Carolina to Louisiana. In most states, the spore collections were made within a single county, but collections in North Carolina were from two counties. No two spore collections were from galls located less than 30 m apart. Spore collections were handled separately and processed and stored according to the procedures outlined by Roncadori and Matthews (7).

All aeciospore collections from each of the seven states were individually checked for germination prior to inoculation. From each state, the eight collections with the highest germination percentage were used in this

study; thus, a total of 56 collections were included. To produce inoculum, seedlings of northern red oak (*Quercus rubra* L.) were inoculated with aeciospores from each collection. Basidiospores were harvested later from the oak leaves and used in a concentrated spray for pine inoculation (3, 4). In brief, pine seeds were germinated, and the seedlings were transplanted immediately after emergence into flats containing 20 seedlings each. At 4 wk of age, the seedlings were inoculated with 75×10^3 spores/ml dispensed in 8-ml aliquants per flat as seedlings moved on a conveyor under a spray nozzle. In vitro basidiospore germination immediately prior to inoculation was at least 85%. Each inoculum source was tested on four flats of 20 seedlings for each of the three pine families, with each flat constituting one replication. A total of 13,440 seedlings were inoculated. Immediately after inoculation the seedlings were placed in a mist chamber and held at 21 C for 24 hr. Then they were grown in the greenhouse for 9 mo.

Infection data were based on the number of seedlings with actively growing galls after 9 mo. The statistical design was a nested-factorial analysis of variance with individual gall collections nested within states. Means separation was done with Duncan's multiple range test (1).

RESULTS

The analysis of the data showed highly significant differences in: (i) overall differences among levels of rust infection on the three host families, (ii) differences among the average levels of infection of the rust collections from different states, (iii) interactions between host families and states, (iv) differences among individual collections within states, and (v) differences between host families and individual collections within states.

The average percent infection of the three host families was as expected (Table 1). Inocula from almost every state produced about half as much infection on family 11-20 as on 11-23, and Livingston Parish was always intermediate. These differences in infection on host families, however, were strongly influenced by both the family × state interaction, and particularly the family × collections-within-states interaction.

The average levels of infection by states, measured across all three host families, ranged from a low of 49% for Georgia to a high of 63% for Mississippi (Table 1). These differences, however, are not considered definitive because of the limited sample of the population within individual states and the confounding of the interactions with families.

The interaction between host families and states was highly significant (Table 1). The inocula from North and South Carolina were significantly more pathogenic on family 11-20 than those from Louisiana and Georgia. The inocula from Louisiana and Georgia also had the lowest level of infection on the susceptible family 11-23. The most striking result was the high level of infection of Livingston Parish seedlings produced by inocula from Mississippi in contrast to all others.

TABLE 1. Influence of inoculum source on pathogenicity of *Cronartium fusiforme* to three loblolly pine families (host family × state interaction)

Pine family	Seedlings (%) with galls 9 mo. after inoculation with spores from: La.	Miss.	Ala.	Ga.	Fla.	S. C.	N. C.	Host mean
11-20 (Resistant)	33 ij[z]	42 gh	40 hi	32 j	42 gh	45 fgh	45 fgh	40
L. P. (Intermediate)	53 de	67 bc	55 de	49 efg	57 d	50 def	49 efg	54
11-23 (Susceptible)	65 c	79 a	74 ab	66 bc	77 a	75 ab	79 a	74
Mean	50	63	56	49	59	57	58	

[z]Means followed by the same letter do not differ significantly at $P = 0.05$ as determined by Duncan's multiple range test.

TABLE 2. Pathogenicity of *Cronartium fusiforme* spores collected in Georgia and North Carolina on loblolly pine seedlings (average of three host families)

Georgia		North Carolina	
Collection no.	Seedlings with galls after 9 mo (%)	Collection no.	Seedlings with galls after 9 mo (%)
G-1	45 c[z]	NC-1	64 ab
G-2	51 bc	NC-2	63 ab
G-3	67 a	NC-4	53 bc
G-4	48 bc	NC-5	58 abc
G-6	37 c	NC-7	50 c
G-7	41 bc	NC-8	48 c
G-9	45 bc	NC-9	69 a
G-10	57 ab	NC-10	57 abc

[z]Within columns, infection percentages followed by the same letter do not differ significantly at $P = 0.01$ as determined by Duncan's multiple range test.

TABLE 3. Influence of inoculum source on pathogenicity of *Cronartium fusiforme* to three loblolly pine families

Rust collection		Pine family 11-20	Pine family Livingston Parish	Pine family 11-23
Louisiana	1	43[z]	56	79
	2	34	44	73
	3	25	45	53
	4	40	73	71
	5	33	50	58
	6	26	65	66
	7	33	31	54
	10	33	56	66
Average		33	53	65
Mississippi	1	43	78	81
	2	43	61	76
	3	44	63	81
	5	38	64	81
	6	48	68	76
	7	41	70	84
	9	44	59	78
	10	38	76	74
Average		42	67	79
Alabama	1	44	61	70
	2	35	56	73
	3	39	61	78
	5	49	41	80
	6	46	57	63
	7	40	48	81
	9	33	63	70
	10	35	51	73
Average		40	55	74
Georgia	1	25	43	66
	2	38	58	58
	3	40	78	83
	4	36	43	65
	6	28	26	58
	7	28	41	54
	9	25	43	68
	10	34	63	74
Average		32	49	66
Florida	1	38	63	79
	2	44	63	73
	3	38	56	76
	4	54	54	75
	5	38	56	81
	6	35	59	74
	9	43	45	70
	10	48	63	89
Average		42	57	77
South Carolina	1	36	61	80
	2	33	40	78
	3	48	55	80
	5	39	41	79
	6	39	53	70
	8	40	49	70
	9	41	41	60
	10	86	56	84
Average		45	50	75
North Carolina	1	54	51	88
	2	55	50	83
	4	36	45	79
	5	43	51	80
	7	33	43	73
	8	33	41	71
	9	68	58	80
	10	39	55	78
Average		45	49	79

[z]Figures represent percentage of seedlings developing galls 9 mo after inoculation.

There also were highly significant differences in the levels of pathogenicity among single-gall collections from within individual states. Inocula from Georgia and North Carolina were particularly variable (Table 2). Among the collections from Georgia, G-3 produced almost twice as much infection as G-6, and was significantly higher in pathogenicity than all other collections from Georgia except G-10. Among the rust collections from North Carolina, NC-9 was significantly more pathogenic than collections NC-4, -7, or -8. In contrast, the inocula collected from galls in Alabama, Mississippi, and Florida were remarkably uniform in the levels of infection produced (Table 3).

The interaction between host families and inocula from collections within states was highly significant. All other components of the analysis were influenced by this interaction. Analysis of the variance components contributing to this interaction showed that the variation involved was primarily due to the rust collections from Louisiana and South Carolina. For example, collection L-6 was highly variable in pathogenicity, depending on host family (Table 4). On the resistant family 11-20 this inoculum was one of the least infective, but on the intermediate Livingston Parish seedlings it was considerably above average in pathogenicity. The South Carolina collections showed even more striking variability. Collection SC-10 was significantly more pathogenic on family 11-20 than on Livingston Parish, whereas the reverse was true for SC-1 (Table 4).

DISCUSSION

The significant differences in percent infection of the three pine families were expected. It was encouraging, however, to see that the resistant family 11-20 performed well when exposed to the widest range of inocula with which it has ever been tested. The intermediate Livingston Parish also held up reasonably well in comparison to the susceptible 11-23. The susceptibility of family 11-23 in past tests has been about equal to nonimproved commercial seed lots. Consequently, this material should not be considered for planting in any area where fusiform rust is a problem.

The differences in levels of infection among inocula from the different states, though statistically significant, are based on a small sample of the fungus population within each state. Therefore, it is not possible to make any general statements about state-to-state differences. The state averages shown in Table 1 should be considered in relation to the family × state interaction shown in the same table. For example, it is obvious that the average percent infection for Mississippi was weighted upward by

the high level of infection on Livingston Parish seedlings.

The host family × state interactions provided insight into problems that specific sources of resistance may encounter in different geographic areas. The level of infection caused by Mississippi inocula on the Livingston Parish material was in the same range as that caused by inocula from four of the seven states on the susceptible family 11-23 (Table 1). The inocula from North and South Carolina were significantly more pathogenic on family 11-20 than the inocula from Louisiana and Georgia. There were also significant differences among levels of infection produced by the various inocula on family 11-23, but in every case at least two-thirds of the seedlings became infected.

The variation observed among the inocula collected within given states, when averaged across all three host families, was substantial. For example, the inocula from North Carolina produced levels of infection ranging from 48% to 69%, with three of the eight collections producing over 60% infection (Table 2). This indicates that even in North Carolina, where fusiform rust is not a severe problem, the rust population contains enough genes for virulence to produce heavy localized damage on specific host genotypes. The inocula from Georgia also were quite variable, producing infection levels ranging from 37% to 67%. These differences provide evidence of extreme variation in pathogenic capabilities of *C. fusiforme* on loblolly pine.

The most significant results were the interactions between collections within states × families (Table 4). When tested with inocula from Louisiana, family 11-20 maintained its resistance. However, with five of the Louisiana inocula, Livingston Parish seedlings were not significantly better than the susceptible family 11-23. Inocula from South Carolina were even more variable than those from Louisiana (Table 4). A comparison of SC-1 and SC-10 shows a complete reversal of infection levels on the two hosts 11-20 and Livingston Parish. Collection SC-1 had significantly more infection on Livingston Parish, whereas SC-10 produced significantly more infection on 11-20. This is the classic type of reversal of infection levels on two "differential" host lines that results in the classification of physiologic races of the wheat stem rust fungus (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) (11). It would be premature however, to use the term "physiologic races" in this case, since the conditions for designating these collections are much different from those used for other pathogens.

The relatively high levels of infection caused by inocula from North and South Carolina on resistant family 11-20 are also important. For example, collection SC-10 produced more infection on this family than on the susceptible family 11-23 (Table 3). The ortet of 11-20 was from coastal South Carolina, and the local rust population may be adapted to the sources of resistance in that area. If one of eight random samples of rust in coastal South Carolina had the potential of infecting 86% of the seedlings of family 11-20, it would certainly be dangerous to make extensive plantings of this material over wide areas in this region.

It was fortunate that the Livingston Parish material was included in this study, as seedlings from this area are now being planted over large areas of the South. Livingston Parish seedlings were more resistant than the susceptible family 11-23, except against collections from Mississippi. The inocula from two of the eight collections from Mississippi were extremely pathogenic, and were as virulent on Livingston Parish seedlings as they were on

TABLE 4. Pathogenicity of *Cronartium fusiforme* spores collected in Louisiana and South Carolina on three loblolly pine hosts (collections within states × families interactions)

Collection no.	Host family seedlings with galls			Mean
	11-20 (Resistant) (%)	L. P. (Intermediate) (%)	11-23 (Susceptible) (%)	
L-1	43 efg[z]	56 cd	79 a	59
L-2	34 ghi	44 ef	73 ab	50
L-3	25 i	45 ef	53 de	41
L-4	40 fgh	73 ab	71 ab	61
L-5	33 ghi	50 de	58 cd	47
L-6	26 i	65 bc	66 bc	52
L-7	33 ghi	31 hi	54 de	39
L-10	33 ghi	56 cd	66 bc	52
Mean	33	53	65	50
SC-1	36 gh	61 cd	80 ab	59
SC-2	33 h	40 efgh	78 ab	50
SC-3	48 defgh	55 def	80 ab	61
SC-5	39 fgh	41 efgh	79 ab	53
SC-6	39 fgh	53 defg	70 bc	54
SC-8	40 efgh	49 defg	70 bc	53
SC-9	41 efgh	41 efgh	60 cd	47
SC-10	86 a	56 cde	84 ab	75
Mean	45	50	75	57

[z]Infection percentages followed by the same letter do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

susceptible family 11-23 (Table 3). The inocula from North and South Carolina had the lowest level of pathogenicity on the intermediate Livingston Parish, Louisiana, material. There was, however, at least one rust collection from each state with a fairly high level of pathogenicity on Livingston Parish seedlings. This result indicates the necessity for monitoring rust isolates occurring on the extensive plantings of this material already made in the Atlantic and Gulf Coastal Plains.

Although there were certain similarities between these results on loblolly and those of Snow et al. (9) with slash pine, there were also some notable differences. Their results indicated that two families were resistant to all rust inocula used in their study. In our study, fewer host families were exposed to more rust collections, and it was obvious that even the more resistant pine families were highly susceptible to at least one rust collection. Also, in our evaluations of over 13,000 loblolly seedlings, we did not see an immune reaction such as Snow et al. reported for slash pine. Loblolly and slash pines may differ in this respect.

Our results with *C. fusiforme* on loblolly pine confirm the results of Snow et al. (9) on slash pine regarding the high degree of variation among collections from individual galls. The difference among infections caused by individual collections within a state was often much greater than the difference among collections from different states. These results, therefore, emphasize the necessity of testing resistant materials with a wide range of rust isolates from different geographic areas, and particularly from the areas where the material is to be outplanted.

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