

## Variation in Pathogenicity of Diverse Sources of *Cronartium fusiforme* on Selected Slash Pine Families

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### ABSTRACT

In three experiments, open-pollinated slash pine families with known degrees of fusiform rust resistance were inoculated with *Cronartium fusiforme* basidiospores from diverse sources. In one experiment, ten families were exposed to inocula collected along a transect across Florida, Georgia, Mississippi, Louisiana, and Texas. Two families were resistant to all inocula; three were uniformly susceptible, and the remaining five were resistant to certain inocula. In a second experiment, inocula were from a north-south transect in Mississippi, and another in western Florida and Georgia. Responses of families from two resistant and two susceptible parent trees demonstrated variation in pathogenicity not only between eastern and western collections, but also among

inocula from within both transects. In a third experiment, virulence of cultures from eight individual galls in the Mississippi transect was highly variable on three resistant pine families. Two cultures were more virulent than others on all families, while one family was immune to two of the cultures. These findings demonstrate great genetic diversity in both the fungus and host populations. It is clear that inoculum source and inoculum sampling methods must be considered in evaluating resistance of slash pine to southern fusiform rust. Further, proper deployment of several forms of stable resistance seems necessary for long-term control of the disease.

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*Additional key words:* disease resistance, rust, *Quercus nigra*, artificial inoculation, *Pinus elliottii* var. *elliottii*.

Because fusiform rust caused by *Cronartium fusiforme* Hedgc. & Hunt ex Cumm. constitutes a major threat to efficient management of slash pine (*Pinus elliottii* var. *elliottii* Engelm.) and loblolly pine (*P. taeda* L.), resistance is an important trait in the selection and breeding of these species. To accelerate development of resistant pines, tree improvement programs increasingly are employing large-scale testing via artificial inoculation of young seedlings (4). Evidence of pathogenic variability, and its extent, therefore has both applied and theoretical significance. Not only must the source of inoculum be considered in evaluating resistance, but the ability of the pathogen to specialize over time must also be taken into account.

In an earlier investigation (7, 9), open-pollinated slash pine seedlings from a south Mississippi parent tree were more resistant to *C. fusiforme* from Mississippi and Texas than to isolates from Alabama and Florida. These variable host responses were interpreted as evidence of pathogenic variability. This finding suggests a potential problem with rust-resistant trees similar to that encountered with disease-resistant agricultural crops when new races of plant pathogens evolve. At present, foresters are dealing with genetic diversity that has evolved in natural populations of both the host and pathogen. Natural populations of southern pines are much more heterozygous than agricultural crops, and the pathogen has not yet been exposed to any great selection pressure. So far, plantings of resistant materials are small and widely separated.

Research has therefore been directed toward characterizing pathogenic variation in *C. fusiforme* rather than simply searching for potentially dangerous races of this organism. The hope is to obtain enough information about the diversity of the pathogen so that tree breeders can avoid production of genetically vulnerable materials.

This report summarizes three experiments undertaken to further examine pathogenic variability in *C. fusiforme*, as well as the nature and range of responses in slash pine. The first experiment compared the pathogenicity of rust cultures collected east to west from Georgia to Texas. The second compared rust cultures obtained from north-south transects in Florida and Georgia and in Mississippi. The third experiment evaluated cultures from individual pine galls in Mississippi.

### EAST-WEST COMPARISON OF *C. FUSIFORME*

**MATERIALS AND METHODS.**—In 1970, aeciospores were collected from a fusoid gall on each of three slash pines in five widely separated areas (Fig. 1): Nacogdoches County, Texas (NT); Livingston Parish, Louisiana (LL); Harrison Experimental Forest, Harrison County, Mississippi (HM); Decatur County, Georgia (DG); and near Perry in Taylor County, Florida (TF). Aeciospore collections were processed separately according to the methods of Roncadori and Matthews (6), and used to establish 15 cultures on water oak (*Quercus nigra* L.) seedlings. To minimize the possibility of selection by the oak host, each rust culture was propagated on at least five oak seedlings derived from a mixed lot of seed from several trees. Inoculum for pines was then composited from leaves of the five oaks.

Ten open-pollinated families from slash pines growing at several locations in Mississippi, Alabama, and Florida (Table 1) were tested. Parents chosen were such that materials varying in resistance were available from much of the area sampled for inoculum. Resistance of the parents had been estimated previously via artificial or natural exposure of open-pollinated progenies to inoculum from sites near where the parents were growing.

Pines were inoculated singly with a forced-air

apparatus (8) that deposited basidiospores over an area about 5 mm in diameter; the point of impact was the junction of the stem with the base of the terminal tuft of juvenile needles. Inoculum densities were maintained within the range of 10 to 20 basidiospores/mm<sup>2</sup>. Twenty-four 5- to 7-week-old seedlings from each family were inoculated with basidiospores from one culture on a given day; thus, there were 15 separate inoculations. The culture, and the order in which families were inoculated on any given day, was chosen randomly. Pine seeds had been planted in sequence to provide seedlings of approximately the same age for all inoculations. Oaks had been inoculated in a similar sequence, and 3-week-old telia were used.

Seedlings were grown in the greenhouse in individual pots and examined for disease symptoms 6 months after inoculation. We have observed that resistance can be expressed by formation of small, inactive galls as well as by the absence of galls (3); hence only galls more than 1 cm in length were classed as active. In this paper, our references to resistance or susceptibility of a family refer to the relative percentage of individual seedlings with or without active galls.

Observations were summarized as proportions of seedlings with active galls per culture-family combination, and transformed to arcsine  $\sqrt{\text{proportion}}$  by the Freeman-Tukey method (5). The experimental design was a randomized complete block with split plots. Variance analyses were made on the entire experiment; separate analyses evaluating response of individual families to the several inocula were performed in addition to those involving all families. The three individual cultures from each area were treated as replications. All tests of significance were calculated at  $P = 0.05$ .

**RESULTS.**—A significant inoculum-source  $\times$  family interaction (Fig. 2, Table 2) confirmed previous evidence of variation in pathogenicity among collections from widely separated areas. While most families considered resistant on the basis of previous information remained more resistant to all cultures than susceptible families, virulence of inocula did not conform to any geographic pattern. That is, the nature of the interaction was complex in that western sources were not necessarily more or less



Fig. 1. Collection points (by county) for *Cronartium fusiforme* aeciospores used in 1970, 1971, and 1972 with the location of parent trees for the 14 pine families that were inoculated. Dotted line indicates the northern border of the natural range of slash pine.

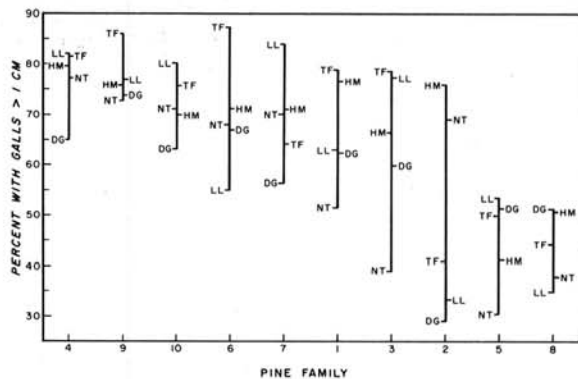


Fig. 2. Ten slash pine families ranked in decreasing order of susceptibility to *Cronartium fusiforme* isolates from sources in five States. Values are percentages of seedlings with galls exceeding 1 cm in length 6 months after inoculation. Inoculum sources are: NT, Nacogdoches County, Texas; LL, Livingston Parish, Louisiana; HM, Harrison County, Mississippi; DG, Decatur County, Georgia; and TF, Taylor County, Florida.

TABLE 1. Origin and relative resistance of open-pollinated slash pine families

Family no.	Parent code	Relative resistance <sup>a</sup>	Origin	Experiment
1	PC-STA-24	S	Bay Co., Fla.	1970, 1971
2	PC-FA-2	R	Covington Co., Ala.	1970, 1971
3	10-13	S	Harrison Co., MS	1970
4	10-122	S	Harrison Co., MS	1970
5	24-54	R	Taylor Co., Fla.	1970
6	15-55	S	Taylor Co., Fla.	1970
7	23-55	S	Taylor Co., Fla.	1970
8	35-55	R	Taylor Co., Fla.	1970
9	43-55	R	Taylor Co., Fla.	1970
10	48-55	S	Taylor Co., Fla.	1970
11	18-62	S	Harrison Co., MS	1971
12	8-7	R	Harrison Co., MS	1971, 1972
13	18-27	R	Harrison Co., MS	1972
14	9-2	R	Harrison Co., MS	1972

<sup>a</sup>Relative resistance (R) or susceptibility (S) as determined by previous artificial or natural inoculation.

TABLE 2. Analyses of variance for response of slash pine families to diverse sources of *Cronartium fusiforme* inocula

	Degrees of freedom			f values <sup>a</sup>			
	1970	1971	1972	1970	1971(a)	1971(b)	1972
Blocks	...	2	2		2.99	0.95	1.55
Inoculum source	4	5	7	0.46	4.62* <sup>b</sup>	3.51	16.11*
Error I	10	10	14				
Families	9	3	2	10.40*	84.41*	72.78*	67.30*
Inoculum × Family	36	15	14	1.64*	2.19*	3.82*	4.15*
Error II	90	36	25				
Total	149	71	64				

<sup>a</sup>Variables for 1970 and 1972 are percentage of plants with galls > 1 cm. Variable for 1971(a) is percentage of plants with galls > 2 cm and variable for 1971(b) is the mean ratio of gall length to seedling height.

<sup>b</sup>Asterisk denotes statistical significance at  $P = 0.05$ .

virulent than eastern sources.

Separate analyses indicated significant variation among inocula for Families 2, 6, and 7. An example of differential response is provided by Family 6 (Fig. 2), which was resistant to LL, but susceptible to TF. Family 7, by contrast, was more susceptible to LL than TF inocula. Hence, even for these two families from approximately the same origin, the virulence of inocula was extremely variable. In contrast, Families 4, 9, and 10 consistently were susceptible, and Families 5 and 8 were resistant, to all inocula.

Families 1 and 2 were also included in the 1971 experiment, and inoculated with DG and HM inocula. Results from the two trials were similar.

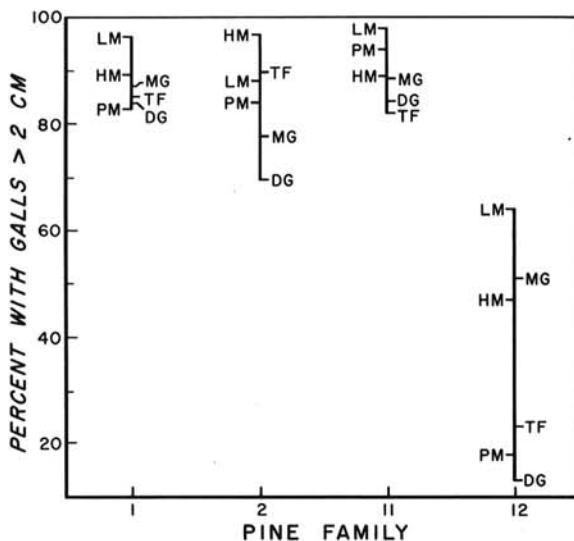


Fig. 3. Results of inoculating four slash pine families with three isolates of *Cronartium fusiforme* from a north-south transect in Mississippi, and three isolates from a similar transect in Florida-Georgia. Values are percentages of seedlings with galls more than 2 cm in length 6 months after inoculation. Inoculum sources are: PM, HM, LM, Hancock, Harrison, and Jones and Wayne Counties in Mississippi; TF, Taylor County, Florida; DG, Decatur County, Georgia; and MG, Bleckley County, Georgia.

#### NORTH-SOUTH LOCATIONAL COMPARISON OF *C. FUSIFORME*

**MATERIALS AND METHODS.**—In 1971, aeciospores were collected from five galls on individual slash pines at each of three locations in Mississippi (Fig. 1). One collection was made near the Gulf of Mexico, at the mouth of the Pearl River in Hancock County (inoculum hereafter designated PM); one 32 kilometers inland on the Harrison Experimental Forest in Harrison County (HM); and one about 150 kilometers north of the Gulf, in Jones and Wayne Counties near Laurel (LM). Collections were also made along a similar north-south transect near Perry in Taylor County, Florida (TF); in Decatur County, Georgia (DG); and south of Macon in Bleckley County, Georgia (MG).

Collections were processed as in the 1970 experiment. Five cultures were established for each location and composited by placing equal numbers of telia-bearing water oak leaves in the forced-air inoculation apparatus. Inocula HM, TF, and DG are related to those similarly labeled in the 1970 experiment in that aeciospores were collected at or near the same sites.

Four open-pollinated slash pine families were tested—one from Florida, one from Alabama, and two from Mississippi (Table 1). Performance of Families 11 and 12 has been confirmed in extensive artificial and field trials (2). Families 1 and 2 were common to both the 1970 and 1971 tests.

Inoculations were performed as in 1970 with 3-week-old telia, except that densities were maintained at a higher level (25 to 35 basidiospores/mm<sup>2</sup>). Each day, 24 pine seedlings from each family were inoculated with basidiospores of *C. fusiforme* from two randomly chosen sources. After the source was selected, families were inoculated at random. Treatment combinations were replicated three times.

Pine seedlings were grown as in 1970, and scored for the presence of galls 6 months after inoculation. Gall lengths and seedling heights were also measured. The four variables examined in analyses were: I—proportions of seedlings galled; II—proportions of seedlings with galls > 1 cm long; III—proportions of seedlings with galls > 2 cm long; and IV—mean ratio of gall lengths to seedling heights, exclusive of seedlings without galls. Variables II and III were employed to account for seedlings which

formed only small, inactive galls, since experience in 1970 indicated that the 1-cm limitation did not include all inactive galls. Variable IV was used as an expression of gall length after adjustment for seedling size. Statistical analyses were similar to those of the 1970 experiment.

**RESULTS.**—In analyses of all four variables, significant inocula  $\times$  pine family interactions demonstrated the existence of pathogenic variability among collections from the six locations. Two analyses are presented in Table 2. Separate analyses indicated significant differences among inocula with all four variables, but differences among inocula and families were more evident with Variables III and IV; major emphasis is placed on the outcome of analyses involving only those.

In terms of Variable III (proportions of seedlings with galls longer than 2 cm) Family 12 was more resistant to all inocula than any other family (Fig. 3); Families 1 and 11 were least resistant. As in 1970, Family 2 was more susceptible to TF and HM inocula than to DG. Inocula from the Florida-Georgia transect tended to be less virulent than those from Mississippi on all families, but the difference was significant only for Family 2. Variation among inocula from locations within transects was even more pronounced. For example, PM was far less virulent on Family 12 than was LM. Also, TF was more virulent on Family 2 than other Florida-Georgia inocula.

Although differences in virulence on Family 1 were not significant in the 1970 test, a substantial degree of variability was apparent (Fig. 2). The variable response of Family 1 to diverse inocula was confirmed in 1971, as significant differences were observed in terms of Variables II and III. Only Family 11 was susceptible to all inocula regardless of their origin.

With few exceptions, results from Variable IV (adjusted gall length) (Fig. 4) corresponded well with those from the other three variables. Variable IV or related measurements therefore provide an added means of comparing host response to *C. fusiforme*. Such comparisons may aid in identifying tolerant materials, or those having more than one form of resistance. The inoculation device employed in these experiments usually resulted in formation of a single stem gall. Gall-length observations would not be applicable where inoculation methods deposit spores on the whole plant or otherwise result in multiple galls.

Galls on seedlings of Family 12 were by far the smallest, regardless of inocula. Maximum variability among inocula in terms of Variable IV occurred on Families 2 and 12, both of which were resistant in one or more previous tests. Mississippi inocula tended to produce larger galls on all families than those from the other north-south transect, but this difference was significant only on Family 2. As with Variable III, responses to inocula varied widely among locations within transects. HM caused the largest galls on Family 12, but LM also produced larger galls than did PM. On Family 2, TF produced larger galls than either DG or MG, and HM produced larger galls than DG.

#### COMPARISONS OF *C. FUSIFORME* FROM INDIVIDUAL PINE GALLS

**MATERIALS AND METHODS.**—In 1972,

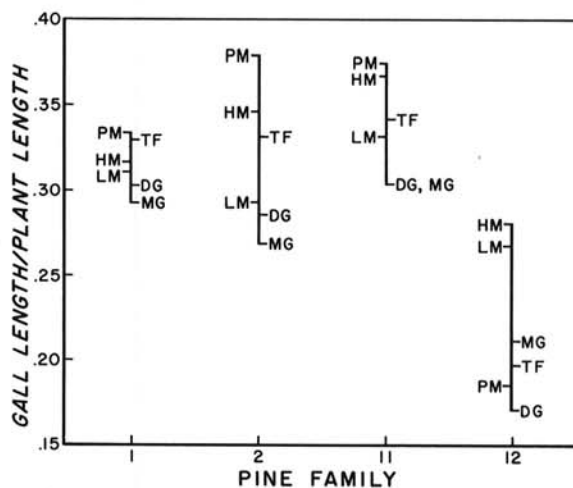


Fig. 4. Mean ratio of gall length to slash pine seedling height 6 months after inoculation with *Cronartium fusiforme*. Four slash pine families were inoculated with three isolates from a north-south transect in Mississippi and three isolates from a similar transect in Florida-Georgia. Inoculum sources are: PM, HM, LM, Hancock, Harrison, and Jones and Wayne Counties in Mississippi; TF, Taylor County, Florida; DG, Decatur County, Georgia; and MG, Bleckley County, Georgia.

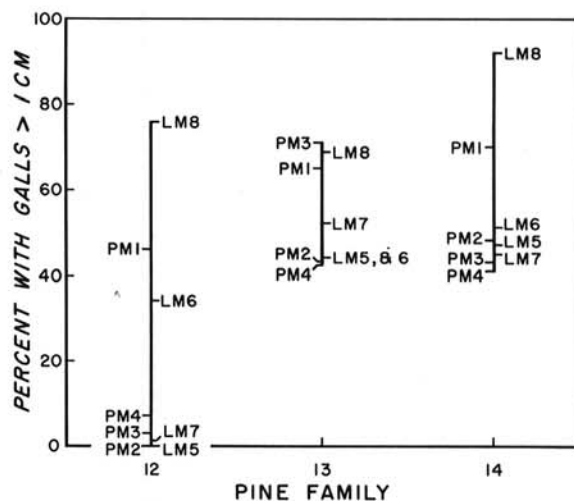


Fig. 5. Results of inoculating seedlings of three slash pine families with isolates of *Cronartium fusiforme* from eight individual galls. Values are percentages of seedlings with galls more than 1 cm in length 6 months after inoculation. Inoculum sources are: PM 1-4, Hancock County, Mississippi; and LM 5-8, Jones and Wayne Counties, Mississippi.

aeciospores were collected from four galls on slash pines near the mouth of the Pearl River in Hancock County, Mississippi, and from four galls on slash pines near Laurel in Jones and Wayne Counties, Mississippi (Fig. 1). These were two of the locations from which inocula were obtained in 1971, but collections were from different trees. Aeciospores from each gall were processed separately and used to establish eight rust cultures on



water oak seedlings. Inocula from the two locations are hereafter referred to as PM 1 through 4 and LM 5 through 8.

Three open-pollinated slash pine families from Harrison County, Mississippi, were chosen; all are considered resistant (Table 1). Family 14 had been employed in related experiments in 1968 (7). Family 12 was used in 1971, and also tested along with Family 13 in extensive artificial and field trials (2).

Inoculations were performed as in 1970 and 1971, with density maintained at 12 to 18 spores/mm<sup>2</sup>. Twenty-four pine seedlings from each family were exposed to each of the eight cultures. After the culture was selected, families were chosen randomly. There were three replications.

Pine seedlings were cultured and inoculated as in the previous experiments, and observed after 6 months for presence of galls and gall length. Statistical analyses were similar to those of the other experiments.

**RESULTS.**—The significant inoculum × family interaction (Table 2) demonstrated substantial pathogenic variability among collections from individual galls. Separate analyses indicated differences among collections for all families. The largest differences occurred in Family 12, where percentage of seedlings having galls > 1 cm ranged from 0 for PM-2 and LM-5, to 76 for LM-8 (Fig. 5). The PM-1 and LM-8 collections were quite virulent in all three families. With exceptions of LM-6 on Family 12, and PM-3 on Family 13, the others were less virulent regardless of family.

**DISCUSSION.**—The variable responses by most of the slash pine families to the diverse inocula in all three experiments confirm previous evidence (7) of pathogenic variation in *C. fusiforme*. The magnitude of genetic diversity in the host and pathogen populations as demonstrated herein has far-reaching implications for breeding resistant slash pines.

All three experiments demonstrate the risks of testing slash pine for resistance without careful regard to inoculum source. For example, resistance of Family 2 might be underestimated were it to be used for reforestation in Georgia, but tested only with inoculum from Mississippi or Texas or even from certain locations in Mississippi (Figs. 3, 4). Likewise performance of Family 12 might be overestimated were it tested only with inoculum from the lower Pearl River in Mississippi. Thus, accurate assessments of resistance depend on testing specific seedlots against inoculum from at least the area or areas in which the trees are to be grown.

In contrast to families having variable responses, Families 5 and 8 (Fig. 2) were resistant to all inocula. This result suggests a phenomenon similar, though perhaps not equal, to horizontal resistance in agronomic crops (10). These families might, therefore, be usable with confidence not only near their Florida origins, but also over a large portion of the commercial range of slash pine—provided they are suitable in terms of other important traits. Parents of families such as these should form the backbone of breeding programs.

Cooperators who furnished Families 6 and 7 considered them susceptible in areas of high rust hazard (Table 1). The 1970 experiment generally supported this view. Their variable responses (Fig. 2) to several inocula collected both near and distant from the origin of the

parent trees nevertheless are further evidence that *C. fusiforme* has undergone substantial differentiation from area to area.

Performance of Family 2 provides further evidence of pathogenic variation and inferences as to its implications. Variable responses to several inocula were observed. In particular, those from Florida, Texas, and at least two Mississippi locations were quite virulent (Figs. 2, 3, 4). The lower overall resistance of Family 2 in 1971, compared to that in 1970 is attributed to differences in inoculum density. In separate experimentation (Dinus and Snow, *unpublished*) resistance rankings varied inversely with density. Previous evaluations indicated that this family possessed moderate resistance (Table 1).

Should large land areas be planted repeatedly with slash pines having only one or a few forms of resistance similar to that observed especially for Families 2, 12, and 14, there is a good chance for an increase in the frequency and range of virulent strains. *C. fusiforme* is heteroecious and is assumed to complete the sexual process on oak before returning to pine. Segregation and recombination of genetic factors as well as stabilizing selection on the oak host may therefore moderate the specialization tendencies of the pathogen. In addition, the practical consequences of pathogenic variability are difficult to assess, since extrapolation from laboratory to field performance is not always accurate (1). In the light of present knowledge, however, proper deployment of several forms of resistance seems necessary to achieve long-term control.

This conclusion is reinforced by the magnitude of variability observed among inocula from individual galls (Fig. 5). Complete absence of active galls following exposure of Family 12 to PM-2 and LM-5 constitutes our first observation of an immune reaction. It is in striking contrast to the virulence of these inocula on the other families, and also to the high percentages of galled seedlings in all families after inoculation with PM-1 and LM-8. Family differences in response to LM-6 and PM-3 underscore the large variability among inocula from individual galls.

Comparisons among these, and related, experiments indicate that the extent of pathogenic variability among collections from individual galls approaches, and indeed may exceed, that among inocula from different locations or widely separated areas. For example, the range of responses by seedlings of Family 12 to inocula from individual galls at two locations in Mississippi (Fig. 5) was greater than that to composite collections from the same locations (Fig. 3). Similarly, the percentage of galled seedlings from Family 14 varied from 40 to 98% following inoculation with collections from five states (7). Approximately equal variation was observed among collections from individual galls in the 1972 experiment (Fig. 5).

It follows that accurate evaluations of resistance via large-scale artificial inoculations depend not only upon using inoculum from areas to be reforested with the material under test, but also upon adequate sampling of the fungus population therein. When average performance over all inocula is considered, approximately equal estimates of resistance were obtained for each of those families common to

experiments employing different sampling methods. That is, similar results were found for Families 1 and 2 in 1970 and 1971 (Figs. 1, 2), for Family 12 in 1971 and 1972 (Figs. 2, 4), and Family 14 in a previous experiment (7) and 1972 (Fig. 4). We nevertheless view our sampling methods as inadequate for applied purposes, and have recommended that larger numbers of galls be used in resistance testing.

## LITERATURE CITED

1. DINUS, R. J. 1971. Breeding pest-resistant forest trees: Developments and trends. Pages 10-24 *in* Proc Eleventh South. For. Tree Improv. Conf., 15-16 June 1971, Atlanta, Georgia. 284 p.
2. DINUS, R. J. 1972. Testing for fusiform rust resistance in slash pine. Pages 331-229 *in* Biology of Rust Resistance in Forest Trees. U.S. Dep. Agric. Misc. Publ. 1221. 681 p.
3. JEWELL, F. F., and G. A. SNOW. 1972. Anatomical resistance to gall-rust infection in slash pine. *Plant Dis. Rep.* 56:531-534.
4. LAIRD, P. P., J. L. KNIGHTEN, and R. L. WOLFE. (no date) An evaluation of a method for use in determining relative fusiform rust resistance in loblolly and slash pine families. U.S. Dep. Agric., For. Serv., For. Tree Resist. Test. Facil. (Asheville, N.C.) Rep. 1, 15 p.
5. MOSTELLER, F., and C. YOUTZ. 1961. Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. *Biometrika* 48:433-440.
6. RONCADORI, R. W., and F. R. MATTHEWS. 1966. Storage and germination of aeciospores of *Cronartium fusiforme*. *Phytopathology* 56:1328-1329.
7. SNOW, G. A., and A. G. KAIS. 1970. Pathogenic variability in isolates of *Cronartium fusiforme* from five southern states. *Phytopathology* 60:1730-1731.
8. SNOW, G. A., and A. G. KAIS. 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 p.
9. SNOW, G. A., H. R. POWERS, JR., and A. G. KAIS. 1969. Pathogenic variability in *Cronartium fusiforme*. Pages 136-139 *in* Proc. Tenth South. For. Tree Impr. Conf., 17-19 June 1969, Houston, Texas. 235 p.
10. VAN DER PLANK, J. E. 1968. Disease resistance in plants. Academic Press, New York. 206 p.