Comparative Seedling Resistance of *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa* to *Cronartium quercuum* f. sp. *fusiforme*

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ABSTRACT

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Basidiospores of *Cronartium quercuum* f. sp. *fusiforme* were used to inoculate 1-mo-old seedlings from 21 families of *Pinus elliottii* var. *elliottii*, resistant and susceptible to fusiform rust, including several families of undetermined (unimproved) resistance, and seven unimproved bulk seed lots of *P. elliottii* var. *densa*. After 6 mo, seedlings from bulk seed lots of unimproved *P. elliottii* var. *densa* generally were more resistant to fusiform rust than the resistant, susceptible, or unimproved selections of *P. elliottii* var. *elliottii*. These results may partially explain the absence of fusiform rust in central and south Florida.

Additional key words: slash pine, south Florida slash pine

Fusiform rust (*Cronartium quercuum* (Berk.) Miyabe: Shirai f. sp. *fusiforme*) is the most economically serious disease of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) in the southeastern United States, yet the disease is only of minor importance within the natural range of south Florida slash pine (*P. elliottii* var. *densa* Little & Dorm.) (8). We refer to these taxa hereafter as "variety *elliottii*" and "variety *densa*." The range of variety *elliottii* extends from southern South Carolina through Georgia and north Florida to eastern Louisiana, whereas the range of variety *densa* extends from the Gulf and Atlantic coastal areas of north central Florida southward to Dade County (7). The natural ranges of the two varieties are allopatric except in north central Florida (6).

Variety *densa* is not managed commercially for fiber or timber production in central and south Florida because it grows slower and has poorer crown and stem form, eg, flattened crowns and forked stems, than variety *elliottii*. Intolerance to frost also limits the northern range of variety *densa*. When grown on sandy loam soils in Argentina, however, variety *densa* selections from south Florida (Hendry County) have shown greater average volume than variety *elliottii* or loblolly pine (*P. taeda* L.).

The incidence of fusiform rust in 8- to 12-yr-old plantations of variety *elliottii* at the southern limit of its natural range in north central Florida is approximately 10% (17). Rust incidence is negligible in the northern range of variety *densa* and nil throughout the remainder of its range. The absence of fusiform rust among variety *densa* in central and south Florida may be owing to the absence of suitable alternate (oak) hosts (18), inherent physiological or anatomical resistance, or a combination of these factors. Climatic differences seem insufficient to account for the lack of rust in variety *densa* (2,13).

Field trials and laboratory inoculations have given conflicting evidence for resistance of variety *densa*. Fusiform rust incidence in a southern Mississippi plantation was evaluated after 2 yr, and disease incidence in variety *densa* was lower (4%) than in two provenances of variety *elliottii* (20 and 24%) or Caribbean pine (*P. caribaea* Morel.) (66%) (1). In one inoculation experiment (15), rust incidence was 76–85% for seedlings of variety *densa* and 42–58% for seedlings of variety *elliottii*. The conflicting evidence for resistance to fusiform rust by variety *densa* and the relative absence of the disease in central and south Florida were the stimuli to screen and compare selected seed lots of both slash pine varieties.

MATERIALS AND METHODS

Approximately 400 seeds from each of 21 open-pollinated families of variety *elliottii* and seven bulk seed lots of open-pollinated variety *densa* were collected. The sources, status of rust resistance, and codes of variety *elliottii* and variety *densa* seed lots are shown in Table 1. The seed lots of variety *elliottii* and variety *densa* were maintained throughout this study at the USDA Fusiform Rust Resistance Screening Center in Asheville, NC.

Twenty seeds of each seed lot were planted in each of six soil-filled trays on 10 August 1981. Northern red oak (*Quercus rubra* L.) leaves inoculated with a north Florida (Duval County) aeciospore source were observed for telial formation. Detached leaves were placed on wire racks over acidified water (pH 2.0) for collection of basidiospores. Viable 1-mo-old seedlings of both host varieties were

Table 1. Seed lots of *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa* screened for resistance to *Cronartium quercuum* f. sp. *fusiforme*

Variety	Source	Status[a]	Code
elliottii	Florida Division of Forestry	Unimproved	FLA-M-1-75S, FLA-M-3-75S, FLA-C-76S, INT-B-76S, FLA-M-1-77S, FLA-M-77S, FLA-S79S
	University of Florida Genetics Cooperative	Resistant	6-56, 52-56, 70-56, 89-57, 165-57
		Resistant check	FA2
		Intermediate resistant	University check
		Susceptible	115-56, 172-58, 244-56, 252-55, 267-55, 299-56
	USDA Forest Service	Susceptible check	GA SL
densa	Florida Division of Forestry	Unimproved	S-FLA-M-77S(1), S-FLA-M-77S(2), S-FLA-1-78S, FLA-L-79-SFS, FLA-M-79SFS(1), FLA-M-79SFS(3), FLA-S-79SFS

[a] Unimproved = no selection for resistance to fusiform rust; check = University of Florida Genetics Cooperative check (intermediate resistance).

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inoculated with a concentrated basidiospore spray (20,000 spores per milliliter) (10,11). The trays of seedlings were processed in two groups of three trays each. Seed lots of the first group were inoculated on 7 October 1981 and of the second group, on 8 October 1981. Seedlings were fertilized 4, 8, and 16 wk after inoculation. Six months after inoculation, seedlings were evaluated for fusiform rust incidence on the basis of several stem symptom types: localized stem pigmentation without swelling, "rough" galls (≥50% of surface dark brown and slightly sunken), typical galls, "short" galls (≤25 mm long), and "fat" galls (diameter twice that of healthy stems) (20).

The incidence of fusiform galls was compiled and analyzed by analysis of variance techniques. An index of relative resistance to fusiform rust also was calculated for each of the 28 seed lots using the information on proportions of rough galls, short galls, symptoms without swelling, and healthy (asymptomatic) seedlings within the seed lots. Frequent occurrence of one or more of these four characteristics denotes resistance (21). The index value calculated for each seed lot was based on the incidence of each symptom type:

$$\text{seed lot index value} = 100(\text{SYMNO} - \text{M1})/(4 \times \text{STD1}) + 100(\text{ROUGH} - \text{M2})/(4 \times \text{STD2}) + 100(\text{SHORT} - \text{M3})/(4 \times \text{STD3}) + 100(\text{HEALTHY} - \text{M4})/(4 \times \text{STD4}),$$

where: SYMNO = mean percentage within seed lot of seedlings with symptoms (eg, localized stem pigmentation) but no swelling, M1 = minimum value among seed lots for mean percentage of seedlings with symptoms but no swelling, STD1 = standard deviation of seed lot means for percentage of seedlings with symptoms but no swelling, ROUGH = mean percentage within seed lot of seedlings with galls having rough bark, M2 = minimum value among seed lots for mean percentage of galls with rough bark, STD2 = standard deviation of seed lot means for percentage of galls with rough bark, SHORT = mean percentage within seed lot of seedlings with short galls, M3 = minimum value among seed lots for mean percentage of short galls, STD3 = standard deviation of seed lot means for percentage of short galls, HEALTHY = mean percentage within seed lot of seedlings with no symptoms, M4 = minimum value among seed lots for mean percentage of healthy seedlings, and STD4 = standard deviation of seed lot means for percentage of healthy seedlings.

A high seed lot index value indicates frequent occurrence of resistant reactions among the seedlings. The index value may range from zero for a seed lot with no symptoms to 400 for a seed lot with maximum incidence of all symptom types.

Table 2. Incidence of four types of fusiform rust symptoms in *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa*

Seed lot	Variety	No. of trays (20 seedlings per tray)	Index of relative resistance[a]	Symptom type (mean %)			
				Typical galls	Rough galls	Short galls	Symptoms without swelling
FLA-C-76S	*elliottii*	5	198.92	53	1	3	5
S-FLA-M-77S(2)	*densa*	3	181.59	33	0	10	30
FLA-S-79S	*elliottii*	6	162.75	46	0	22	2
FLA-S-79SFS	*densa*	3	156.83	18	0	0	32
S-FLA-M-77S(1)	*densa*	5	148.67	34	0	4	29
FLA-M-79SFS(3)	*densa*	2	129.60	20	0	0	21
FA2	*elliottii*	5	114.26	29	0	0	20
FLA-M-77S	*elliottii*	2	113.68	39	0	6	13
University check	*elliottii*	6	113.05	53	0	11	8
S-FLA-1-78S	*densa*	2	108.64	31	0	0	19
FLA-L-79-SFS	*densa*	4	101.36	36	0	0	18
52-56	*elliottii*	2	94.97	20	0	0	6
244-56	*elliottii*	5	93.11	67	0	12	8
FLA-M-79SFS(1)	*densa*	3	91.15	62	0	9	10
165-57	*elliottii*	6	91.15	40	0	6	4
6-56	*elliottii*	6	81.10	48	0	4	7
FLA-M-1-77S	*elliottii*	4	80.79	48	0	5	5
267-55	*elliottii*	6	78.24	58	0	8	4
70-56	*elliottii*	5	68.50	47	0	4	3
FLA-M-1-75S	*elliottii*	6	66.27	57	0	4	7
FLA-M-3-75S	*elliottii*	5	61.05	61	0	6	3
172-58	*elliottii*	6	59.94	68	0	6	7
GA SL	*elliottii*	6	51.90	65	0	6	2
89-57	*elliottii*	6	43.99	58	0	1	4
INT-B-76S	*elliottii*	6	41.66	58	0	0	5
252-55	*elliottii*	2	40.76	79	0	7	3
299-56	*elliottii*	6	37.62	63	0	0	7
115-56	*elliottii*	4	7.70	78	0	1	0

[a] See text for formula.

Table 3. Analysis of variance for percentage of typically galled *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa*[a]

Source of variation	df	Sums of squares	*F* value	Significance
Between dates of inoculation	55	6.925	...	...
Source	4	2.197	11.16	0.001
Seed lot (source)	23	1.536	1.35	NS[b]
Date	1	1.863	37.86	0.001
Experimental error	27	1.328	1.47	NS
Among replicated trays within dates	71	2.381	...	...
Total	126	9.306	...	...

[a] Analysis based on arc sine–square root transformation of percentage of typically galled seedlings.
[b] NS = not significant.

RESULTS

Seed lot rankings based on the index of relative resistance are listed in Table 2. Index values ranked from a high, ie, most resistant, of 198.92 for variety *elliottii* family FLA-C-76S to a low, ie, most susceptible, of 7.7 for variety *elliottii* family 115-56.

A ranking of seed lots by mean percentages of typically galled seedlings indicated that five of the seven most resistant seed lots were variety *densa*. According to the index of relative resistance, four of the seven most resistant seed lots were variety *densa*. Based on the Mann-Whitney test (16), seed lots of variety *densa* ranked significantly higher than seed lots of variety *elliottii* in overall fusiform rust resistance. When seed lots were ranked according to mean percentage of seedlings with typical galls, the ranks were negatively correlated with seed lots ranked according to the index of relative resistance (Spearman rank correlation $r = -0.756$, $P < 0.0001$).

Analysis of variance (Table 3) revealed significant differences in percentage of typically galled seedlings both among sources and between dates of inoculation but not among seed lots within sources. With the *F* values from Table 3, the Waller-Duncan k-ratio rule (5) (Table 4) was applied using the experimental error term with 27 df; results indicated no significant difference in percentage of seedlings with typical galls between seed lots of variety *densa* and resistant families of variety *elliottii*.

DISCUSSION

The identification of resistant bulk

seed lots of variety *densa* suggests that resistant pine genotypes are partly responsible for the absence of fusiform rust in central and south Florida. Disease escape resulting from the scarcity of host oak species in that region may contribute to the absence of rust. Only bulk seed lots of variety *densa* were available, and each seed lot may have represented a wider array of genotypes than variety *elliottii* seed lots. Because of the pathogenic variability of *C. quercuum* f. sp. *fusiforme* (12), different geographic sources of aeciospores may change the relative resistance rankings.

The resistance to fusiform rust shown by variety *densa* seed lots was substantiated by analysis of mean percentages of typical galls as well as by the index of relative resistance. The highly negative correlation between the two ranking systems suggested that symptoms other than typical galls were prevalent in the variety *densa* and in the more resistant variety *elliottii* seed lots. Only the most resistant variety *elliottii* family, FLA-C-76S, had the rough gall symptom, a standard measurement included in the index. If the rough gall symptom were removed from the analysis, the index value would be reduced by approximately half and FLA-C-76S would be ranked in the mid range of the resistance index.

No significant statistical difference in mean percentage incidence of typical galls was observed between seed lots of resistant variety *elliottii* from the University of Florida Genetics Cooperative (45.22%) and seed lots of variety *densa* from the Florida Division of Forestry (33.77%), even though the latter had approximately 12% fewer galls.

Some variation from previous tests of resistance among seed lots of variety *elliottii* was observed. Goddard and Schmidt (3) reported that rust-resistant families of variety *elliottii* showed stable resistance over broad geographic areas in the southeastern United States. Family 89-57, planted in six locations in north Florida, southern Georgia, and Mississippi, had an average fusiform rust incidence of 23% and was considered among the most resistant of the families tested. Walkinshaw et al (20) substantiated the high resistance of 89-57 based on gall formation in field and greenhouse inoculation trials. In our study, 89-57 incurred a mean gall incidence of 58%, similar to the 60% incidence found by Snow and Griggs (14) in Mississippi. Family FA2 ranked highly resistant in our study and is considered one of the most resistant variety *elliottii* selections. Yet, with the high inoculum technique (9), this family had the greatest susceptibility among 13 families tested, with 95% of the seedlings showing galls. Walkinshaw and Bey (19) also emphasized the field resistance of FA2, yet reported 80% gall incidence in a greenhouse inoculation study. Snow and Griggs (14) reported 73% gall incidence for FA2 in another greenhouse inoculation experiment.

Table 4. Waller-Duncan k-ratios for percentage of typically galled *Pinus elliottii* var. *elliottii* and *P. elliottii* var. *densa*

Source	Variety	Status	Mean percentage of gall incidence[z]
Florida Division of Forestry	*densa*	Unimproved	33.77 a
University of Florida Genetics Cooperative	*elliottii*	Resistant	45.22 ab
Florida Division of Forestry	*elliottii*	Unimproved	53.06 bc
USDA Forest Service	*elliottii*	Susceptible check	64.83 cd
University of Florida Genetics Cooperative	*elliottii*	Susceptible	66.56 d

[z]Means followed by the same letter are not significantly different according to the Waller-Duncan k-ratio *t* (LSD) rule, where k-ratio = 100.

The variations in incidence may be due to: 1) the variable resistance component from the male parents of the open-pollinated seed; 2) two types of resistance, eg, one that can withstand high basidiospore inoculum levels and one that cannot (9); 3) variation in virulence of inoculum; 4) unproven reliability of the index of relative resistance; and 5) inconsistencies in gall evaluation. In general, the families of variety *elliottii* previously determined to be resistant were among the more resistant slash pine families in our study. Deviations from reported variety *elliottii* resistance were not explained fully, but this should not detract from the apparent resistance shown by the majority of the bulk seed lots of variety *densa*. Symptoms without swelling were common in seed lots of variety *densa*, which suggests that basing resistance on gall incidence, although appropriate for variety *elliottii*, may be inappropriate for variety *densa*. This warrants further research.

The nature of the apparent resistance of variety *densa* remains unknown. The slow growth rate of variety *densa* within its natural range may contribute to negligible colonization levels, but in our study the greenhouse conditions before inoculation and during incubation should have optimized the likelihood of symptom expression. Perhaps the commercial management of variety *densa* could be expanded into areas of north Florida if selections could be incorporated into longleaf pine (*P. palustris* Mill.) breeding programs to benefit from the latter's frost hardiness and adaptability to dry, infertile sites.

A marked progressive increase in incidence of fusiform rust has occurred among plantations of variety *elliottii* in north central Florida during the past 20 yr (4), but no such increase is recorded for variety *densa*. The results of our study combined with the negligible fusiform rust incidence within the natural range of variety *densa* suggest a degree of host resistance that has been underestimated.

LITERATURE CITED

1. Anonymous. 1950. Thirtieth annual report. U.S. For. Serv. Southeast. For. Exp. Stn. 90 pp.
2. Davis, R. T., and Snow, G. A. 1968. Weather systems related to fusiform rust infection. Plant Dis. Rep. 52:419-422.
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: South. Conf. For. Tree Improv. Proc. 15th.
4. Griggs, M. M., and Schmidt, R. A. 1977. Increase and spread of fusiform rust. Pages 25-31 in: Management of Fusiform Rust in Southern Pines. R. J. Dinus and R. A. Schmidt, eds. Symp. Proc. Univ. Fla., Gainesville. 163 pp.
5. Helwig, J. T., and Council, K. A., eds. 1979. SAS User's Guide. SAS Institute Inc., Raleigh, NC. 494 pp.
6. Langdon, O. G. 1963. Range of South Florida slash pine. J. For. 61:384-385.
7. Little, E. L., Jr. 1971. Conifers and important hardwoods. Atlas of United States Trees. Vol. 1. U.S. For. Serv. Misc. Publ. 1146. 310 pp.
8. Little, E. L., Jr., and Dorman, K. W. 1954. Slash pine (*Pinus elliottii*) including South Florida slash pine—nomenclature and description. U.S. For. Serv. Southeast. For. Exp. Stn. Pap. 36. 82 pp.
9. Lundquist, J. E., Miller, T., and Powers, H. R., Jr. 1982. A rapid technique for determining resistance of slash pine to fusiform rust. Phytopathology 72:613-615.
10. Matthews, F. R., and Rowan, S. J. 1972. An improved method for large-scale inoculations of pine and oak with *Cronartium fusiforme*. Plant Dis. Rep. 56:931-934.
11. Powers, H. R., Jr., Hubbard, S. D., and Anderson, R. L. 1982. Testing for resistance to fusiform rust of pine. Pages 427-434 in: Resistance to Diseases and Pests in Forest Trees. H. M. Heybroek, B. R. Stephan, and K. von Weissenberg, eds. Proc. Int. Workshop Genet. Host-Parasite Interact. For. 3rd, Wageningen, Netherlands. 503 pp.
12. 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.
13. Snow, G. A., Froelich, R. C., and Popham, T. W. 1968. Weather conditions determining infection of slash pines by *Cronartium fusiforme*. Phytopathology 58:1537-1540.
14. Snow, G. A., and Griggs, M. M. 1980. Relative virulence of *Cronartium quercuum* f. sp. *fusiforme* from seven resistant families of slash pine. Phytopathol. Mediterr. 19:13-16.
15. Snow, G. A., Powers, H. R., Jr., and Kais, A. G. 1969. Pathogenic variability in *Cronartium fusiforme*. Pages 136-139 in: South. Conf. For. Tree Improv. Proc. 10th.
16. Sokal, R. R., and Rholf, F. J. 1969. Biometry—The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Co., San Francisco. 776 pp.
17. Squillace, A. E. 1976. Geographic patterns of fusiform rust infection in loblolly and slash pine plantations. U.S. For. Serv. Res. Note SE-232. 4 pp.
18. Squillace, A. E., Dinus, R. J., Hollis, C. A., and Schmidt, R. A. 1978. Relation of oak abundance, seed source, and temperature to geographic patterns of fusiform rust incidence. U.S. For. Serv. Res. Pap. SE-186. 20 pp.

19. 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.
20. Walkinshaw, C. H., Dell, T. R., and Hubbard, S. D. 1980. Predicting field performance of slash pine families from inoculated greenhouse seedlings. U.S. For. Serv. Res. Pap. SO-160. 6 pp.
21. Walkinshaw, C. H., Hubbard, S. D., Triplett, J., and Anderson, R. L. 1981. How to identify six different fusiform rust symptom types on seedlings at the resistance screening center. U.S. For. Serv. Southeast. Area State Private For. Rep. 82-1-5. 6 pp.