

Host Relationships of Fusiform Rust Disease

I. Infection and Pycnial Production on Slash Pine and Nearby Tropical Relatives

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Summary

Seedlings from 8 sources of slash pine (*Pinus elliottii* var. *elliottii* and var. *densa*) and from 2 sources each of Caribbean pine (*P. caribaea* var. *caribaea*) and West Indian pine (*P. occidentalis*) were inoculated using inocula obtained from 2 sources of *Cronartium quercuum* f. sp. *fusiforme*, the causal agent of fusiform rust disease. The percentage of infection, evident primarily as galls and sporulation of pycnia of *C. q. fusiforme*, differed significantly within sources of slash pine and among species. The percentage of infection of all inoculated shoots was highest on slash pine (*P. e. densa*) seedlings from south Florida (92%) and lowest on *P. occidentalis* (30%). Among slash pine seedlings, the general trend was more pycnia sporulation on seedlings from sources nearest the origins of inocula, with the only exception being on those of *P. e. densa* from the most southern source, which showed abundant sporulation of pycnia similar to seedlings *P. c. caribaea* from a nearby source. No sporulation occurred on seedlings of *P. occidentalis*. Differences among families within sources of slash pine were significant for percentages of infection and sporulation of pycnia on all shoots inoculated using *C. q. fusiforme*. Because sporulation of pycnia is a prerequisite for fecundity in *C. q. fusiforme*, the results suggest strong selection on natural inoculum for infection and fertility among and within sources of slash pine. Breeding strategies currently recognize families of slash pine that minimize damage due to fusiform rust disease, but new strategies might consider limiting pathogen reproduction.

Key words: *Pinus elliottii* var. *elliottii*, *Pinus elliottii* var. *densa*, *Pinus caribaea* var. *caribaea*, *Pinus occidentalis*, *Australes*, *Cronartium quercuum* f. sp. *fusiforme*.

FDC: 443; 181.41; 165.53; 172.8 *Cronartium quercuum*; 174.7 *Pinus elliottii*; 174.7 *Pinus caribaea*; 174.7 *Pinus occidentalis*.

Introduction

Slash pine (*Pinus elliottii* ENGELM.) is one of the most commercially valuable pines in the United States. It is commonly planted inside and outside its natural range in the southeastern United States (BOYER and SOUTH, 1984), as well as in exotic plantings in Africa and Australia (MULLIN et al., 1978), Asia (PAN, 1989), and South America (PICCHI and BARRETT, 1967).

Taxonomically, slash pine has obvious affinities with other Caribbean hard pines, and before 1954 (LITTLE and DORMAN), slash pine and *Pinus caribaea* MORELET were not considered separate species. Today, slash pine is divided into 2 varieties, the "typical" variety (*P. elliottii* var. *elliottii*) and the south Florida variety (*P. elliottii* var. *densa*) and *Pinus caribaea* is a separate species. The transition between the 2 varieties of slash pine was mapped in the central Florida peninsula by

LITTLE and DORMAN (1954; *Figure 1*), although SQUILLACE (1966) found no distinct transition and considered the variation to be continuous along the peninsula. Furthermore, there is some evidence for introgression of *P. caribaea* genes into slash pine in southern Florida, based on analysis of cortical monoterpenes (SQUILLACE et al., 1977) as well as chloroplast DNA (WAGNER et al., 1992; NELSON et al., 1994).

Fusiform rust, caused by *Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f. sp. *fusiforme* is the most damaging disease on slash pine as well as loblolly pine (*P. taeda* L.) in the southeastern United States (POWERS et al., 1981). Previous studies on host relationships showed evidence of pathogen variability for fusiform rust disease on slash and loblolly pine (HEDGCOCK and SIGGERS, 1949 [as *P. caribaea*]; SNOW and KAIS, 1970; POWERS, 1972; KAIS and SNOW, 1972; and SNOW et al., 1975). Geographic variation in the disease has a distinct pattern on loblolly pine (WELLS and WAKELEY, 1966), but no such pattern is apparent on slash pine (SNYDER et al., 1967). However, previous studies of geographic variation of fusiform rust on slash pine have included only sources from within the range of the "typical" variety and have not included the south Florida variety. The purpose of our study was to further evaluate the patterns of variation in fusiform rust disease on seedlings of slash pine from throughout its range and on seedlings of its nearby tropical relatives in the subsection *Australes*.

Materials and Methods

Seedlings

Seeds from 8 sources of slash pine (*P. e. elliottii* and *P. e. densa*) and 2 each of Caribbean pine (*P. caribaea* var. *caribaea*) and West Indian pine (*P. occidentalis* SWARTZ) were obtained for study (*Table 1*). Origins (*Figure 1*) of 6 sources of slash pine (FL1 to FL6) ranged from 24.5°N latitude on Big Pine Key, Monroe County, at the southern tip of Florida (*P. e. densa*), to 30.25°N, at Olustee, Baker County, in north central Florida (*P. e. elliottii*). FL1 to FL3 are from within the range of *P. e. densa*, and FL4 to FL6 are from within the range *P. e. elliottii*. Each of these sources represented a natural stand or parts of a few adjacent natural stands. At each source, 25 to 30 wind-pollinated cones were collected. Trees from which cones were collected at sources were spaced at least 50 m apart. Two sources of slash pine outside Florida, from southern Georgia (GA) and southern Mississippi (MS), were also tested. Both sources were trees growing in the clone bank on Harrison Experimental Forest (HEF, Southern Institute of Forest Genetics), Harrison County, Mississippi. The families represented by source GA were fusiform-rust-free trees selected in heavily infected plantings of south Georgia origin (specifics unknown) in Wayne and Stone Counties, Mississippi. The families varied widely in performance in artificial inoculations (DINUS, 1971). Several of the families received additional

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testing in field experiments (GRIGGS, 1984). Families of the source MS were selected from natural stands in Harrison County, Mississippi. The families represented a range of fusiform rust disease based on quantitative analyses. The origins of *P. c. caribaea* and *P. occidentalis* were Cuba and Hispaniola, respectively.

Seeds were germinated during August 1992 in boxes containing damp vermiculite. Germinated seedlings were transplanted during September 1992 into peat:vermiculite (1:2) potting mixture and grown in Ray Leach fir cells. The transplants were grown at 10°C to 29°C in a glasshouse. Metal

halide supplemental lighting provided an 18 h photoperiod. Four weeks after transplanting, seedlings were fertilized using 9-45-15 (200 ppm elemental nitrogen) liquid fertilizer. Seedlings were hedged during November 1992 when the epicotyls had elongated 5 cm to 8 cm to produce multiple shoots for inoculation.

Inocula

Two inocula of *C. q. fusiforme* were studied. Both originated as aeciospores collected from galls on trees approximately 10 years old planted in Madison County, north central Florida;

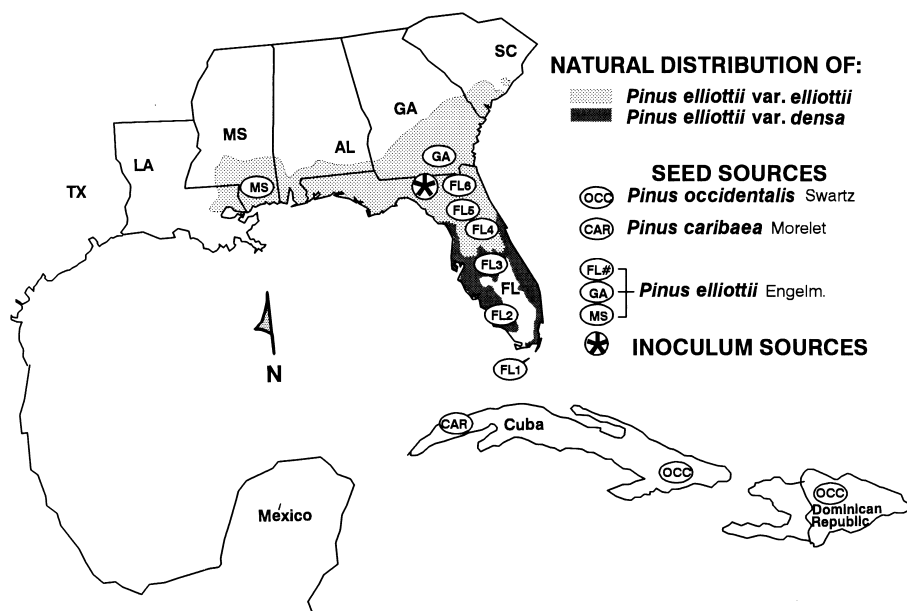


Figure 1. – Map of the southeastern United States, México, Cuba, and Hispaniola showing location of seed sources and inocula described in text. Distribution of *Pinus elliotii* adapted from CRITCHFIELD and LITTLE (1966).

Table 1. – Origins of *Pinus occidentalis* (OCC), *P. caribaea* var. *caribaea* (CAR), and *P. elliotii* var. *densa* (P.e.d.) and var. *elliotii* (P.e.e.) seedlings tested for development of fusiform rust disease using 2 inocula *Cronartium quercuum* f. sp. *fusiforme*.

Prov ^a	Species	°N lat	Provenance	Parent ID	Cooperator	Families	Avg/Family
OCC	<i>P. o.</i>	19.1	Jumunco, Dom. Rep.	Lot 38/77	Oxford Univ	(20) ^b	
		20.2	Bayamo, Cuba	Lot 7/78	Oxford Univ		
CAR	<i>P. c. c.</i>	22.5	Marbajitas, Cuba	Lot 51/83	Oxford Univ	(63) ^b	
		22.5	Marbajitas, Cuba	Lot 61/83	Oxford Univ		
FL1	<i>P. e. d.</i>	24.5	Monroe Co, FL	N/A	USFWS	9	10.6
FL2	<i>P. e. d.</i>	26.25	Collier Co, FL	N/A	Collier Ent.	12	14.1
FL3	<i>P. e. d.</i>	27.5	Polk/Highlands Co, FL	N/A	USAF	11	14.0
FL4	<i>P. e. e.</i>	28.25	Orange Co, FL	N/A	Disney World	14	16.3
FL5	<i>P. e. e.</i>	29.25	Marion Co, FL	N/A	USFS	15	18.0
FL6	<i>P. e. e.</i>	30.25	Baker Co, FL	N/A	USFS	16	18.6
GA	<i>P. e. e.</i>	30.5-31	GA	H-28XWind	USFS		18
				W-1-5XWind			20
				W-1-7XWind			19
				W-1-18XWind			20
				W-1-19XWind			17
				W-1-20XWind			18
MS	<i>P. e. e.</i>	30.6	Harrison Co, MS	8-7XWind	USFS		20
				9-2XWind			19
				18-26XWind			20
				18-27XWind			19
				18-62XWind			18

^a) The location of each seed source is pictorially presented in figure 1.

^b) Total number of seedlings evaluated.

2 galls on loblolly pine (CCA-LP), origin Livingston Parish, Louisiana, and 3 galls on slash pine (SC) of local origin.

The two inocula were uniform mixtures of basidiospores derived from the several collections of aeciospores. The general methods for producing inocula were described previously (DOUDRICK et al., 1993), except techniques for single spore isolation were not applied. Briefly, seedlings of Shumard oak (*Quercus shumardii* BUCKL.) were inoculated using aeciospores from single galls. Inoculated oak seedlings were misted with water, enclosed in a plastic bag, and placed in a dark chamber for 24 h at 20 °C. Uredinia were induced by incubation at 20 °C in constant light. Successive inoculations produced enough urediniospores for storage and subsequent inoculations. Urediniospores and the original collections of aeciospores were dried and stored under vacuum at 4 °C using procedures of RONCADORI and MATTHEWS (1966). Throughout the study, oak seedlings were kept isolated from sources of contaminating spores of *C. q. fusiforme* to maintain purity of the cultures derived from single galls.

Inoculation

Seedlings were inoculated during January 1993 when new shoots emerged 0.5 cm to 2 cm. Inoculation was by the method of SNOW and KAIS (1972) using the forced-air system for directed application of basidiospores. Inoculum density was 21 spores to 28 spores per mm², verified after each set of 10 seedlings. The hedging produced seedlings having using multiple shoots. One regenerated shoot was inoculated using an equal

mix of CCA-3-LP and -4-LP, and another shoot was inoculated with an equal mix of SC-1, SC-4, and SC-8 (Figure 2). Each seedling had at least one additional shoot that served as an uninoculated control. Although growth of all regenerated shoots was not equal within or between seedlings, the physiological maturity of shoots were matched as nearly as possible for all inoculated and control shoots. The 2 inocula were applied in 2 replications to 1,536 hedged seedlings.

Inoculated seedlings were kept in growth chambers for 24 h before being returned to the glasshouse. Growth chamber conditions were dark, 19 °C to 22 °C, and 100% relative humidity. After incubation, inoculated seedlings were returned to the glasshouse and grown under the same temperature and light regime described above. Two weeks after inoculation, seedlings were fertilized once using 9-45-15 NPK fertilizer, then every second week thereafter using 20-10-20 (200 ppm elemental nitrogen). In March 1993, 12 weeks after inoculation, seedlings were planted in the nursery at HEF where supplemental water was provided. Seedlings were lifted during November 1993 and scored for fusiform rust disease.

Analysis

The infection percentage (presence or absence of galls) after 9 months as well as the sporulation of pycnia, was observed for CCA inoculated shoots, SC inoculated shoots, or "either" (a seedling having a gall produced by CCA, SC, or both was considered to be in the "either" category). Data were analyzed by a general linear models procedure and by pairwise comparisons using TUKEY's studentized range (HSD) test (SAS, 1990). For the purposes of comparative analyses, inoculated shoots were considered to represent ramets of each seedling, because every seedling scored received both inocula, and each seedling was considered a clone.

Results and Discussion

Symptoms of fusiform rust disease (primarily presence or absence of a gall) and signs of *C. q. fusiforme* (sporulation of pycnia) developed on 1,181 (76%) inoculated seedlings. Nearly 60% (915 and 918 shoots for CCA and SC, respectively) showed infection for one of the inocula, thus many seedlings developed symptoms using either inoculum. No uninoculated shoots developed symptoms of disease or signs of infection of *C. q. fusiforme*.

The shapes of galls were constant within each family of pines for each source of inoculum and relatively constant within each species, but somewhat different among species. Galls varied from being fusoid having long tapering ends to being nearly absent with signs of the pathogen, i.e., pycnial sporulation by *C. q. fusiforme*, the only external evidence of infection. All shoots showing pycnium development, whether immature, actively sporulating, or senescent, were scored positive for infection, even in the absence of an obvious gall.

Variation among species and seed sources

There was a significant difference for infection percentage ($p \leq 0.05$) among sources of seedlings inoculated using one or either inoculum. The percentage of seedlings that were inoculated and developed an infection ranged from a high of 72% for seedlings from source FL1 inoculated using inoculum SC to a low of 15% for *P. occidentalis* (Figure 3). Significant differences were readily apparent when seedlings of *P. c. caribaea*, FL1, FL2, FL3, and FL4 that had been inoculated using inoculum CCA were compared with similarly inoculated *P. occidentalis* and MS seedlings (Figure 3). For inoculum SC, the differences were significant between seedlings of *P. c.*



Figure 2. – Hedged seedling of slash pine. An equal mix of inoculum from collections of origin CCA-LP was applied to one regenerated shoot. Another shoot was inoculated using an equal mix of collections of origin SC. Arrows indicate inoculated shoots.

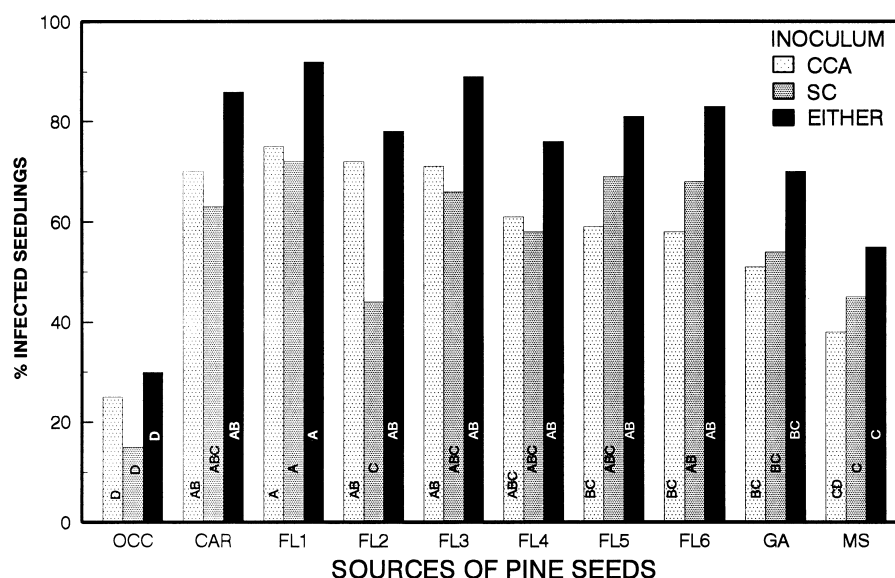


Figure 3. – Infection percentages of seedlings of *Pinus occidentalis* (OCC), *P. caribaea* var. *caribaea* (CAR), *P. elliottii* var. *densa* (FL1 to FL3), and *P. elliottii* var. *elliottii* (FL4 to FL6) when inoculated using 2 inocula of *Cronartium quercuum* f. sp. *fusiforme*. Origins of seeds and inocula are presented in figure 1. Bars marked by the same letter do not differ (within inoculum type) according to TUKEY's Studentized Range Test (0.05 level).

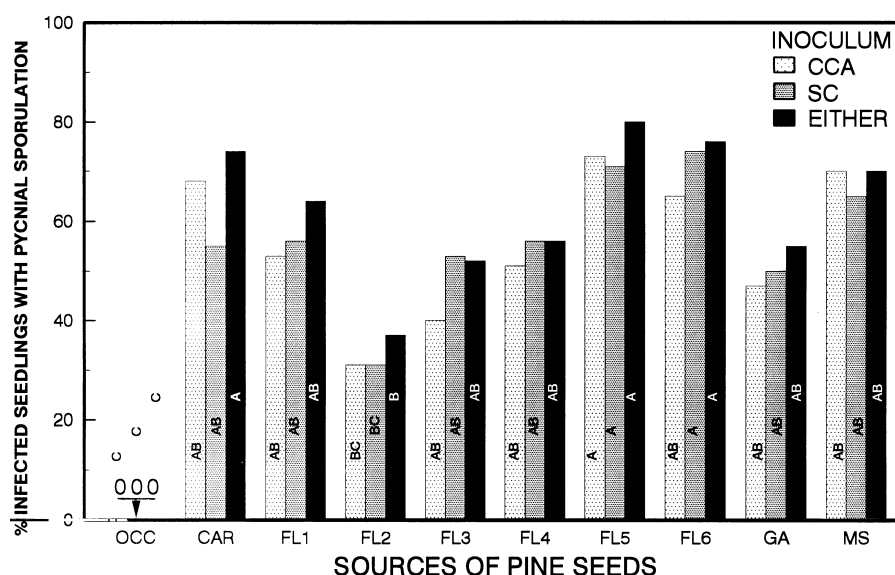


Figure 4. – Pycnia sporulation percentages of seedlings of *Pinus occidentalis* (OCC), *P. caribaea* var. *caribaea* (CAR), *P. elliottii* var. *densa* (FL1 to FL3), and *P. elliottii* var. *elliottii* (FL4 to FL6) when inoculated using 2 inocula of *Cronartium quercuum* f. sp. *fusiforme*. Origins of seeds and inocula are presented in figure 1. Bars marked by the same letter do not differ (within inoculum type) according to TUKEY's Studentized Range Test (0.05 level).

caribaea, FL1, FL3, FL4, FL5, and FL6 and those of *P. occidentalis* (Figure 3). Differences were significant also when the "either" inoculum category was considered in the analysis. Seedlings of *P. c. caribaea*, FL1, and FL3 differed from those of FL2, GA, and MS, and the latter 3 were different from *P. occidentalis*.

The differences between sources of seedlings for evidence of pycnia sporulation also were significant. Pycniospores were produced on 57% of infected seedlings inoculated using inoculum CCA, 62% of those using SC, and 65% of those using either inoculum. For one or either inoculum, seedlings of *P. c. caribaea*, FL1, FL5, FL6, and MS differed significantly from those of *P. occidentalis* and FL2 (Figure 4); seedlings of the

latter 2 showed much less sporulation. Seedlings of *P. occidentalis* showed the least evidence of sporulation, 0% for both inocula. Among sources of slash pine, the highest proportion of infected seedlings having pycnia sporulation was FL5 (74%) and the least was FL2 (37%).

When inoculated using inocula derived from 2 northern Florida sources of *C. q. fusiforme*, seedlings of *P. elliottii*, *P. c. caribaea*, and *P. occidentalis* developed fusiform rust disease. However, the infection percentage of *P. occidentalis* seedlings (30%) was significantly less than that of the other species (Figure 3 and 4). Seedlings of *P. c. caribaea* had nearly the highest percentages for one or either inoculum (70%, 63%, and 86% for CCA, SC, and either inoculum, respectively). TAINTER

and ANDERSON (1993) showed that 87% of seedlings of *P. c. caribaea* developed galls when inoculated using a mixture of inocula from different sources. This percentage is approximately the same as reported here.

Although the relative lack of infection of *P. occidentalis* seedlings is obvious in figure 3, there is no clear pattern of geographic variation for overall infection percentage. The slightly lower percentages for seedlings of sources GA and MS are probably due to inclusion of a few families known to have relatively low infection percentages, as shown in figure 5. There is, however, an interesting shift in the relative infection percentages of the 2 inocula sources by pine seed source from south to north (from left to right in Figure 3). For seedlings of *P. occidentalis*, *P. c. caribaea*, and FL1 through FL4, infection percentages were slightly higher for inoculum CCA, which was collected from nonlocal loblolly pine. On the other hand, for seedlings from sources close to the inocula sources (FL5, FL6, GA, and MS) percentages were slightly higher for inoculum SC, which was collected from local slash pine. This shift may show a tendency of the pathogen towards specialization for local slash pine. The seed source by inoculum interaction was not statistically significant in this analysis, but was significant in a heritability analysis of sources FL1 through FL6 (see companion paper by NELSON et al.).

An interesting pattern of geographic variation is evident in the data on pycnial sporulation. Sporulation of pycnia was most abundant on *P. elliotii* seedlings from sources nearest the origin of the inocula. Pycnia sporulation was highest for seedlings from sources FL5 and FL6 (73% and 71%; 80% and 65%; and 74% and 76% for seedlings inoculated using CCA, SC, and either inoculum, respectively). Seedlings of source FL2 showed low pycnia sporulation using one or either inoculum (33%, 28%, and 38% for seedlings inoculated with CCA, SC, and either, respectively). The frequency of pycnia sporulation was generally low for GA and MS seedlings. The exception to the pattern was the moderate-to-high percentages of sporulation of pycnia on seedlings from source FL1 (53%, 56%, and 64% for seedlings inoculated using CCA, SC, and either, respectively), especially when compared with those of nearby

sources FL2 and FL3. Infection and sporulation of pycnia percentages also were high on *P. c. caribaea* seedlings (68%, 55%, and 74%).

Species of *Quercus* suitable as alternate hosts of *C. q. fusiforme* do not presently occur within the natural ranges of *P. c. caribaea* and *P. occidentalis* nor do they occur as far south as source FL1 (BURNS and HONKALA, 1990). The high infection percentages on *P. c. caribaea* seedlings appears to correspond to basic host species compatibility as defined by HEATH (1981). The high percentage of infected seedlings for source FL1 may be due to similar genetic constraints. Indeed, there is evidence for introgression of *P. c. caribaea* into south Florida slash pine, based on analysis of cortical monoterpenes (SQUILLACE et al., 1977) as well as chloroplast DNA (WAGNER et al., 1992; NELSON et al., 1994). The sources FL2 and FL3 (also *P. e. densa*) are from more northerly latitudes and farther from contaminating pollen of *P. c. caribaea*, and well within the natural ranges of *Quercus* spp. suitable as alternate hosts of *C. q. fusiforme*.

The relatively high infection and pycnial sporulation percentages observed on seedlings of *P. c. caribaea* is somewhat more difficult to explain. *P. c. caribaea* is obviously a suitable host for the inocula tested. In the phylogeny of the genus *Pinus*, it is commonly believed that *P. c. caribaea* is closely related to and of common ancestry with *P. elliotii* but that *P. occidentalis*, although related, is of a different lineage. Like its relative *P. elliotii*, *P. c. caribaea* is readily attacked by *C. q. fusiforme*, but no evidence of specificity was observed in this study. It is assumed that a common ancestor of *P. elliotii* and *P. c. caribaea* with common genetic background was host for an ancestral pathogen biotype related to *C. q. fusiforme*.

A relatively low percentage of *P. occidentalis* seedlings (30%) were infected using the inocula tested in the current study. The infection percentage is equal to or less than percentages reported for incompatible, non-host interactions using inoculum of *C. q. fusiforme* on *P. banksiana* LAMB., *P. echinata* MILL., *P. glabra* WALT. and *P. virginiana* MILL. (HEDGCOCK and SIGGERS, 1949; KAIS and SNOW, 1970; TAINTER and ANDERSON, 1993). These data and the absence of pycnia on infected seedlings suggest that the interaction of *C. q. fusiforme* and *P. occi-*

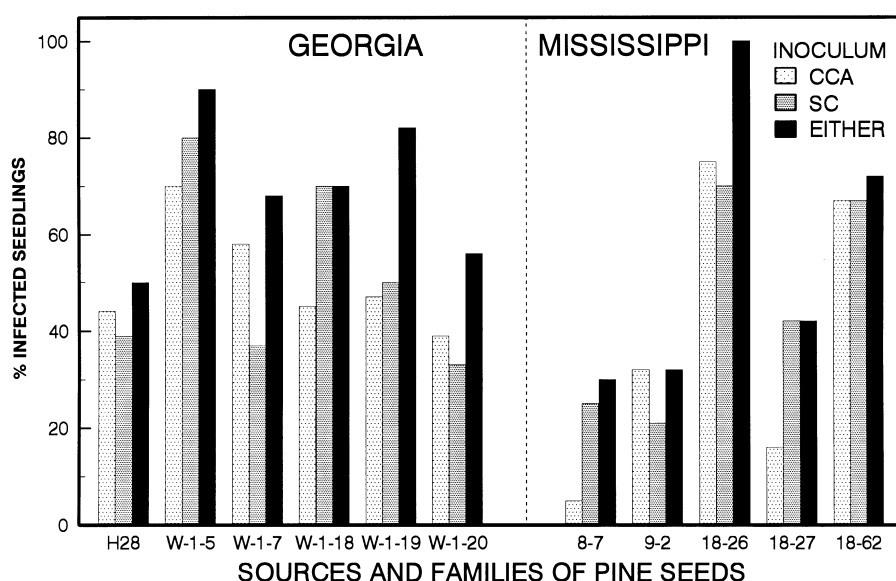


Figure 5. – Infection percentages of *P. elliotii* var. *elliotii* seedlings from open-pollinated families inoculated using 2 inocula of *Cronartium quercuum* f. sp. *fusiforme*. Origins of seeds and inocula are presented in figure 1.

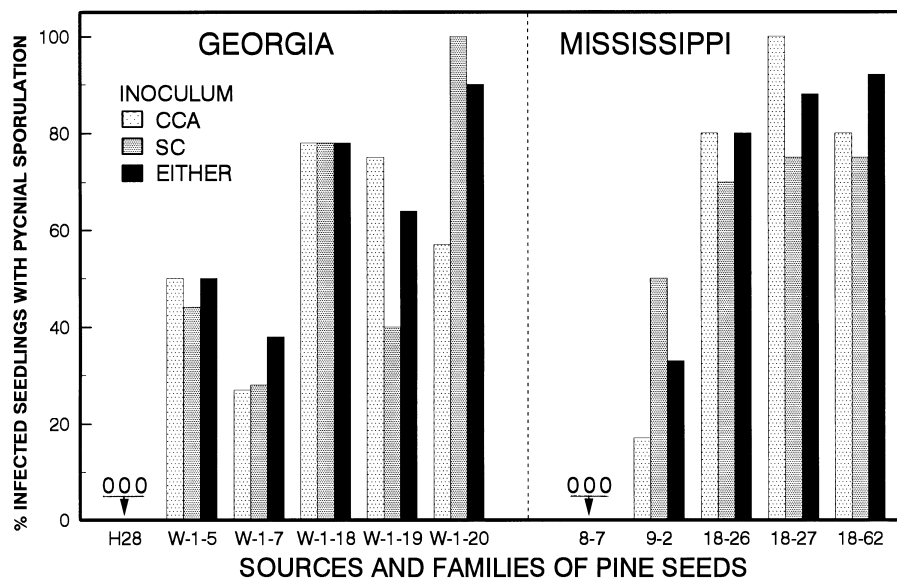


Figure 6. – Pycnia sporulation percentages of *P. elliotii* var. *elliotii* seedlings from open-pollinated families inoculated using 2 inocula of *Cronartium quercuum* f. sp. *fusiforme*. Origins of seeds and inocula are presented in figure 1.

dentalis also is an incompatible, nonhost interaction. *Pinus occidentalis* and *P. c. caribaea* both occur in Cuba, but their current natural ranges are separated by more than 500 km (CRITCHFIELD and LITTLE, 1966).

There have been suggestions (DINUS and SCHMIDTLING, 1971) that infection of seedlings of *P. elliotii* by *C. q. fusiforme* may be correlated with growth rate. The data presented for the current study does not support such a hypothesis on a provenance basis. Seedlings of source FL1 had the slowest growth rate but the highest infection percentage and a high pycnia sporulation percentage when compared to seedlings from other sources. Growth rate was next slowest for seedlings of FL2 and greatest for those of more northern sources, thus no consistent correlation is evident for growth rate of *P. elliotii* seedlings and infection percentage.

Variation among families within seed sources

Besides the low infection percentage for seedlings of *P. occidentalis*, seedlings from a few families within *P. e. elliotii* and *P. e. densa* (FL, GA, and MS) showed significantly low infection percentages (Figure 5 and 6). WEBB et al. (1984) also reported low infection percentages for seedlings of *P. e. densa* using a variety of sources of *C. q. fusiforme* inocula. POWERS (1972), however, using inocula from various hosts and geographic origins, reported that infection percentage averaged 80% and higher on seedlings of several sources of slash pine, especially those tested from south Florida. Neither WEBB et al. nor POWERS indicated precisely the south Florida sources of the tested seedlings, but obviously from the current study and those 2 studies taken together, the origin of seedlings appears to be critical to the interpretation of results. If POWERS' source of seedlings was comparable to FL1, the results are similar. In contrast, seedlings from sources FL2 and FL3 showed lower infection rates than POWERS', but similar results to those studied by WEBB et al.. WEBB et al. also reported commonly observing symptoms of fusiform rust disease without swelling on inoculated *P. e. densa* seedlings. In the current study, similar symptomology was observed.

SNOW et al. (1975) reported a significant inoculum-source X family interaction for a series of 3 experiments. The families of

slash pine used in the study by SNOW et al. had origins similar to FL6, GA, and MS in the current study. The responses by seedlings of most of the slash pine families studied by SNOW et al. to the inocula were strongly suggestive of pathogenic variation in *C. q. fusiforme* and specificity in disease development. The results reported by SNOW et al. for inoculum from northern Florida were similar to our results. For the family MS 18-62 in our study, the infection percentages of seedlings inoculated using CCA and SC inocula were 66% for each and 72% for seedlings inoculated using either (Figure 5) as compared to about 80% infection reported by SNOW et al.. Seedlings of family MS 8-7 showed 5% and 25% infection for inocula CCA and SC, respectively, and 30% for either inoculum as compared to about 21% in the study by SNOW et al. The range of percentages reported by SNOW et al. for seedlings of 7 northern Florida families inoculated using inoculum from northern Florida was 45% to 87%. In the current study, using comparable inocula, the range for seedlings of 16 families from source FL6 was 47% to 84% and 25% to 100% for CCA and SC, respectively, and 35% to 100% for either inoculum.

GRIGGS (1984) also studied some families represented in the current study; however her study evaluated natural field infection. The relative infection percentages were similar to the ones reported here. Seedlings of source GA W-1-5 had a relatively high infection percentage, while those of sources GA H-28 and GA W-1-18 had moderate percentages and those of GA W-1-20 showed an intermediate-to-low infection percentage (Figure 5).

Conclusions and Recommendations

There is a distinctive geographic trend in data for pycnia sporulation on *P. elliotii* seedlings consistent with strong selection for pathogen reproductive fitness. Certainly some families within sources of *P. elliotii* represent extremes, but the general trend within and between sources suggests an increase in compatibility for families nearest the sources of the inocula, with the highest infection and sporulation of pycnia percentages on seedlings of sources from the nearby locale. SNOW et al. (1976) reported increased infection percentages associat-

ed with serial inoculation of slash pine families. By holding the host genotype relatively constant, the increased percentages would be a product of selective forces on the pathogen, resulting in a higher frequency of complementary factors in the haploid pathogen population infecting pines. Higher infection and pycnia sporulation percentages for seedlings from sources nearest the origin of inocula as seen in the current study could be explained similarly.

This rationale would be consistent with a hypothesis of coevolution and reciprocal adaptation of complementary genes in the host and pathogen in response to mutual selection pressures. The results of the current study are insufficient to estimate gene frequencies, but, because the biological success of an organism depends on its ability to reproduce, we feel that the data on sporulation of pycnia of *C. q. fusiforme* is especially relevant. The formation of a pycnium is an indication of a successful infection (a compatible interaction of host and pathogen) and is certainly a prerequisite for pathogen fecundity. Increased cultivation of exclusive pine families selects from the pathogen population those genotypes with complementarity; the selected pathotypes increase in frequency because they are the only forms that can infect the improved varieties of pines.

We would, therefore, expand on the warnings of SNOW et al. (1976), who states that a shift in the tentative ecological balance in fusiform rust disease will occur if pines with one or few factors conditioning resistance are planted extensively. We can visualize a functional loss of resistance in the improved stock if the pathogen's capacity to respond to selection pressure is not recognized. SNOW et al. (1975) recommended that efforts should be made to identify interacting factors that produce fusiform rust disease. Therefore, the genetic diversity of southern pines must be maintained so that excessive losses resulting from changes in gene frequencies in populations of *C. q. fusiforme* can be avoided (SNOW et al., 1976). Only limited progress has been made in this effort, but we believe that it is crucial to continue toward that goal.

Certain elements are critical in the progression of an epidemic. Of those known to affect fusiform rust disease, SNOW et al. (1976) suggest that the most significant contributor is the genetic constitution of the meiotic spore(s) infecting pines. GRIGGS (1984) emphasized the importance of minimizing sporulation of both pycnia and aecia. Pine families considered to be desirable in a breeding program because of low infection percentages may not be so desirable when the wider view of epidemiology is considered – a view including the potential of the pathogen to reproduce. Although KUHLMAN and MATTHEWS (1984) observed frequent occurrence of pycnia on slash pine seedlings having higher percentages of galls, our data show family-specific responses. It is apparent in comparing figure 5 and 6 that the correlation between infection percentage and pycnial sporulation percentage, though positive, is not very close. The qualitative response of *C. q. fusiforme* on seedlings of family MS 8-7 when inoculated using many different methods and densities of inocula has been known for a long time. Our data on the absence of any pycnia on infected seedlings adds significantly to the reputation of family MS 8-7. However, from the standpoint of long-term epidemiology and management of pathogen reproduction, seedlings of family MS 8-7 performed little better than seedlings of family GA H-28 that also failed to produce pycnia but had an intermediate level of infection percentage. In contrast, seedlings of family GA W-1-20 showed an intermediate-to-low infection percentage but abundant sporulation of pycnia (Figure 5 and 6). The point is that widespread deployment of this last phenotype could be a prescription for disaster.

Selecting pine families that limit infection but permit abundant sporulation of pycnia and aecia only serves to increase the frequency of *C. q. fusiforme* pathotypes suitable for subsequent infections. SNOW et al. (1976) predicted that large-scale planting of improved pine stock, especially clonal forestry, will exert further selection pressure, making an increase in the frequency of pathotypes capable of attacking the improved trees highly probable. As a consequence, data on sporulation of *C. q. fusiforme* pycnia and aecia must be considered in screening families of slash pine if disease development is to be minimized, and the genetic plasticity of *C. q. fusiforme* must be evaluated carefully in planning tree-breeding programs.

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Host Relationships of Fusiform Rust Disease

II. Genetic Variation and Heritability in Typical and South Florida Varieties of Slash Pine

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Summary

Seedlings of wind-pollinated families from 6 sources of slash pine (*Pinus elliottii* var. *elliottii* and var. *densa*) were tested for development of fusiform rust disease using 2 sources of *Cronartium quercuum* f. sp. *fusiforme* inocula. The seed source origins ranged from 24.5°N latitude at the southern tip of Florida (*P. e. densa*), to 30.25°N latitude, in north central Florida (*P. e. elliottii*). All seedlings received basidiospores of both inocula; inoculum of each source was applied to a single, separate shoot. Differences among varieties of slash pine were significant, with less pycnial sporulation present on seedlings from southern seed sources. Differences between inocula were significant within *P. e. elliottii* only, suggesting increased specificity for *P. e. elliottii* hosts and these inocula, although inocula x family-within-seed-source interactions were not significant. Heritability estimates for infection or sporulation on an individual seedling basis ranged from 0 to 0.45 within *P. e. densa* and from 0.20 to 0.59 within *P. e. elliottii*. On a family mean basis, heritability estimates were higher, ranging up to 0.58 within *P. e. densa* and 0.71 within *P. e. elliottii*. Diverse sources of reaction to *C. q. fusiforme* appear to be present in *P. e. densa*, suggesting a backcross breeding approach in which genes for reaction are introgressed into fast-growing populations of *P. e. elliottii*. The relatively large individual tree-based heritability estimates in *P. e. elliottii* should help to expedite the introgression process.

Keywords: *Pinus elliottii* var. *elliottii*, *Pinus elliottii* var. *densa*, *Cronartium quercuum* f. sp. *fusiforme*.

FDC: 181.41; 165.53; 443; 165.3; 172. *Cronartium quercuum*; 174.7 *Pinus elliottii*.

Introduction

Fusiform rust, caused by *Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f. sp. *fusiforme*, is the most serious disease affecting the commercially important southern pines. Research efforts have identified sources of genetic resistance to the disease (JEWELL and MALLETT, 1964; WELLS and WAKELEY, 1966; KINLOCH and STONECYPHER, 1969; DINUS, 1971; SCHMIDT et al., 1981; POWERS and KRAUS, 1986; SLUDER, 1986; POWERS and KUHLMAN, 1987; KUHLMAN and POWERS, 1988; HODGE et al., 1989). Breeding programs have attempted to incorporate this resistance into elite families (ZOBEL et al., 1971; POWERS et al., 1979; WEIR and GODDARD, 1986). Recent research efforts have focused on defining the nature of the interaction of host and pathogen with hopes of developing better strategies for controlling the disease in high-production environments (NANCE et al., 1992; NELSON et al., 1993).

In artificial inoculation experiments, disease development is usually scored as the percentage of galled seedlings. Some efforts have been made to quantify the size and shape of galls as a further indication of resistance. SNOW et al. (1990) suggested that round galls on loblolly pine (*Pinus taeda* L.) seedlings indicated restricted pathogen development and, therefore, more host resistance than long, fusoid-shaped galls. WALKINSHAW et al. (1980) identified several symptoms that, when combined in regression equations, served to better predict field resistance of loblolly and slash pine (*P. elliottii* ENGELM. var. *elliottii*) families. In a unique effort to quantify *C. q. fusiforme* aecial sporulation on slash pine, GRIGGS (1984) scored wind-pollinated progenies of several elite parents in 2 field environments and found some families with high percentages of infection and low percentages of sporulation and vice versa. These results were verified in a second test of

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