

FUSIFORM RUST RESISTANCE OF PROGENY FROM  
NATURAL LOBLOLLY X SHORTLEAF PINE HYBRIDS

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ABSTRACT

Seedlings of thirty-nine open-pollinated shortleaf, loblolly and putative loblolly x shortleaf pine families were inoculated with fusiform rust and scored for stem gall formation after six and nine months.

Arcs in of square root percent rust infection of progeny was regressed against five morphological traits and hybrid index scores of the parents. Hybrid index appeared to be the best predictor of progeny response to fusiform rust, shortleaf pines with low and loblolly pines with high rust susceptibility. Some parent trees with intermediate hybrid index scores appeared to be hybrids and produced progeny with intermediate responses to rust inoculation. Most putative hybrids resembled shortleaf pine morphologically and in their response to rust.

Results prompted speculation that introgression of loblolly and shortleaf pine occurs in East Texas but frequency of individuals which could be definitely classed as hybrids is low.

INTRODUCTION

Loblolly (*Pinus taeda* L.) and shortleaf (P. *echinata* Mill.) pines are sympatric throughout much of their ranges. Shortleaf pine is resistant to southern fusiform rust (*Cronartium fusiforme* Hedge. and Hunt ex Cumm.); loblolly pine is highly susceptible but appears to be more resistant west of the Mississippi River (Wells and Wakeley, 1966).

It has been proposed that the western provenances of loblolly pine derive their increased resistance to fusiform rust from introgression with shortleaf pine. A study by Cotton, Hicks and Flake (in press) showed that natural hybridization occurs in East Texas, but the frequency of identifiable hybrids is low.

The present study was designed to sample and classify trees from native loblolly-shortleaf pine populations in East Texas and evaluate fusiform rust resistance of their open-pollinated progeny. Fusiform rust

<sup>1</sup> Financial support for this project was made available to Stephen F. Austin State University by McIntire-Stennis and the State of Texas. Controlled inoculation tests for fusiform rust were conducted by the U.S.D.A., Forest Service, Fusiform Rust Testing Facility, State & Private Forestry, Bent Creek, NC.

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trials were conducted by the United States Department of Agriculture, Forest Service, Fusiform Rust Testing Facility, State & Private Forestry, Bent Creek, NC, Subsequent analyses were performed to determine what relationships may exist between parental morphological classifications and rust response of their progenies.

#### LITERATURE REVIEW

During recent years, loblolly pine has become the most important commercial pine species of the southern United States and has received increasing attention from tree breeders representing all sectors of southern forestry. The genetic variability found in natural stands of loblolly pine, its rapid growth, and its capacity to survive in a wide range of habitats has contributed to loblolly pine's popularity (Dorman and Zobel, 1973).

Fusiform rust is the most detrimental pathogenic pest of loblolly pine and may infect 90 percent or more of the trees in plantations (Czabator, 1971; Zobel, Blair, and Zoerb, 1971). The commercial impact of the disease has recently been reviewed for loblolly and slash (*P. elliottii* Engelm.) pines (Powers, et al. 1974), Many practices designed to increase volume production, including clearcutting, planting species off-site, fertilization, and cultivation, may also encourage fusiform rust (Dinus and Schmidtling, 1971; Siggers, 1955; Westberg, 1951).

During the past twenty years considerable attention has been focused on selection and conservation of rust resistant germ plasm in the southern pines. Wells and Switzer (1971) have noted that resistance to fusiform rust has been found to exist at higher frequencies (1) west of the Mississippi River (2) in the Florida parish of Louisiana and (3) on the eastern shore of Maryland. Genetic sources of rust resistance in loblolly pine proposed by Henry and Jewell (1963) are selections of rust-free individuals from areas of high rust incidence, and hybridization with shortleaf pine.

Selection for apparent resistance from natural stands has met with varying degrees of success (Kinloch and Stonecypher, 1969; LaFarge and Kraus, 1967; Goddard and Strickland, 1970).

The feasibility of successful artificial hybridization between shortleaf and loblolly pines has been recognized since the early 1930's (Critchfield, 1962; Little and Righter, 1965). The existence of natural shortleaf x loblolly pine hybrids and their potential as sources of rust resistance has also been proposed (Bilan, 1965; Hare and Switzer, 1963; Zobel, 1953). Premating isolating mechanisms appear the most probable causes for the infrequency of natural hybridization of shortleaf and loblolly pines (Critchfield, 1962; Mergen, Stairs, and Snyder, 1963, 1965; Schmidtling, 1971), yet some workers have concluded that reproductive isolation is not complete in certain parts of the species' natural ranges (Schmidtling, 1971). A phenology study by Dorman and Barber (1956) showed both loblolly and shortleaf pine to be releasing pollen concurrently in Nacogdoches County, Texas. This was confirmed again in 1971 by Hicks, Jones, and Cotton (1972), who found ten percent

of the shortleaf pines observed to be producing pollen simultaneously with more than half of the loblolly pines in the study.

Hicks (1973) determined that shortleaf and loblolly pines in East Texas could be distinguished from one another on the basis of five morphological traits, Cotton, et al, (in press) incorporated these (needle length, fascicle sheath length, number of needles per fascicle, terminal bud width, and cone length) into a hybrid index to test 164 trees from 16 different natural populations of loblolly and shortleaf pine in East Texas. Their results indicate that natural hybridization probably occurs but hybrids constitute a relatively small proportion of the combined population.

#### PARENT TREE SELECTION AND INOCULATION OF PROGENY

Mature cones, secondary needles, and terminal buds were sampled from 52 reproductively mature trees growing in four natural loblolly-shortleaf pine stands of East Texas during the fall of 1972. Forty-five of these parental selections were used for this study.

Materials were collected from the upper portion of the crown. The apical bud was avoided. Size of the within-tree sample for the five morphological characters measured was determined using Stein's two-stage sample technique (Steel and Torrie, 1960).

Seeds were extracted and stratified on moist filter paper in petri dishes at 3°C for approximately forty days. Following stratification, seeds from each of the 45 study trees were shipped to the U. S. Forest Service, Forest Pest Management Unit, Asheville, North Carolina, for fusiform rust inoculation.

During field selection we tried to obtain individuals varying morphologically from typical shortleaf to typical loblolly types, including intermediates. The hybrid index technique (Anderson, 1949) was used to classify trees as loblolly, shortleaf or intermediate. In calculating the hybrid index scores, values for trees exhibiting the minimum and maximum means for a given morphological character were assigned scores of 0.0 and 5.0, respectively. Means for other individuals were transformed to this linear 0.0 to 5.0 scale by interpolation. The hybrid index value for a tree was obtained by summing its character scores.

Figure 1 illustrates the frequency distribution of sample trees by hybrid index. As expected, there were two major groups reflecting typical shortleaf and loblolly pine and a small number with intermediate index scores. Because there appeared to be a secondary peak at hybrid index 8, trees with scores between 6 and 16 were classed as intermediate. Seven of these 10 trees also fell in intermediate groups of a cluster analysis. A similar hybrid index range for individuals in intermediate cluster analysis groups was found by Cotton et al. (in press). It is obvious from Figure 1 that most of the trees classed as intermediate in this study were morphologically closer to shortleaf than to loblolly pine. Seed samples from two loblolly pine families were provided as controls by the Fusiform Rust Testing Facility. One was a Livingston

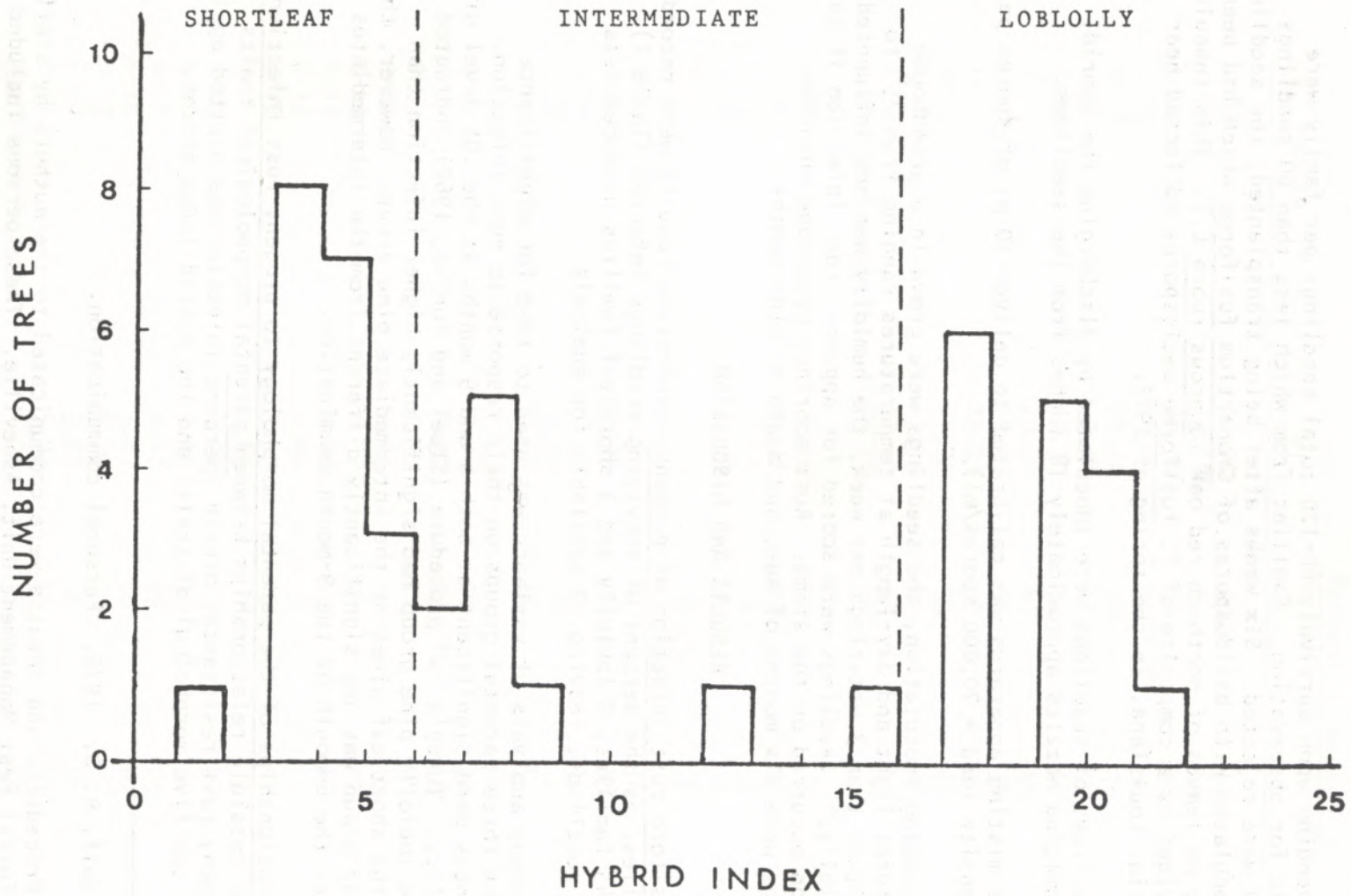


Figure 1.--Frequency distribution by hybrid index of the 45 parent pines.

Parish, LA source, considered relatively resistant; and the other was Westvaco 1123, a seed orchard clone of eastern provenance, considered susceptible. The susceptible check was considered representative of eastern sources of loblolly pine in susceptibility.<sup>3</sup> The rust trials commenced in September, 1973.

Depending upon survival, 60-120 total seedlings per family were available for observation, Families from which less than 60 seedlings survived were rejected. Six weeks after being transplanted, the seedlings were inoculated with basidiospores of Cronartium fusiforme which had been cultured on leaves of northern red oak (*Quercus rubra* L). This inoculum was provided by a composite of C. fusiforme aeciospores collected near Alexandria, Louisiana, in the spring of 1973.

The trays of seedlings were inoculated by discharging the spore from atomizing nozzles approximately 18 inches from the seedlings.

The misting apparatus was calibrated to deliver 10 ml of spores per tray (density load = 70,000 spores/ml).

Following inoculation, the seedlings were grown in a greenhouse with natural light and day-length at temperatures ranging from 75 to 85°F. Other than 3 waterings per week, the humidity was not influenced artificially.<sup>4</sup> Seedlings were scored for apparent rust infection if gall formation occurred on the stems, Rust scorings were made when the seedlings were six months of age, and again at nine months.

#### RESULTS AND DISCUSSION

Fusiform rust infection of progeny.--Infection results were recorded by families, as the percent of surviving seedlings infected (Table I) Of the 45 families, 3 loblolly and 3 shortleaf families produced less than 60 seedlings, leaving 39 available for analysis.

One-way analysis of variance was used to test for significance among the three parental groups on their response to rust infection. Differences were significant at both 6 and 9 months at the .05 level of probability. Tukey's "w" procedure (Steel and Torrie, 1960) indicated that the loblolly pine group had significantly higher infection than either the shortleaf pines or the intermediate pine group. However, the shortleaf group was not significantly different from the intermediates at either the 6-month or the 9-month examination.

#### Relationships of the parental morphology to progeny rust infection.

-To study possible relationships between parental morphological traits and progeny rust resistance, arcsin /percent infection was plotted against each of the five morphological traits and the hybrid index scores.

<sup>3</sup> Wolf, R. D. 1975. Personal communication.

<sup>4</sup> Procedures and results were communicated to the authors by staff at the Forest Pest Management Unit, Asheville. These persons included John Knighten, Peter Laird, and R. D. Wolfe.

Table 1.--Six- and nine-month results of fusiform rust inoculations, and hybrid index scores (HI) of 39 open-pollinated pine families (S=shortleaf, I=intermediate, L=loblolly).

Family	HI	Percent Infection	
		6-month	9-month
L-02	19.71	50%	52%
L-05	19.22	64	67
L-07	20.94	54	58
L-10	19.16	56	58
L-13	20.14	26	30
L-14	17.90	48	52
L-15	17.50	54	61
L-16	20.84	43	48
L-18	19.47	55	58
L-19	19.23	58	62
L-20	17.72	59	63
L-21	17.65	44	44
L-22	17.18	64	67
Loblolly Average	18.97	52	55
I-08	6.36	1	1
I-10	15.78	27	26
I-13	7.96	6	2
I-16	7.78	9	14
I-17	7.29	11	8
I-19	7.95	22	21
I-20	6.56	13	14
I-21	12.39	32	38
I-22	8.21	13	14
I-23	7.24	10	8
Intermediate Average	8.75	14	15
S-03	3.18	12	20
S-04	4.32	18	15
S-07	5.38	11	11
S-08	3.35	10	10
S-10	4.37	24	22
S-11	4.16	15	16
S-12	1.54	9	6
S-13	3.97	13	15
S-14	3.43	7	7
S-15	3.22	16	16
S-16	4.10	17	18
S-17	4.47	13	14
S-18	5.17	10	10
S-19	4.79	9	8
S-20	3.62	8	10
S-21	5.01	10	10
Shortleaf Average	4.01	13	13

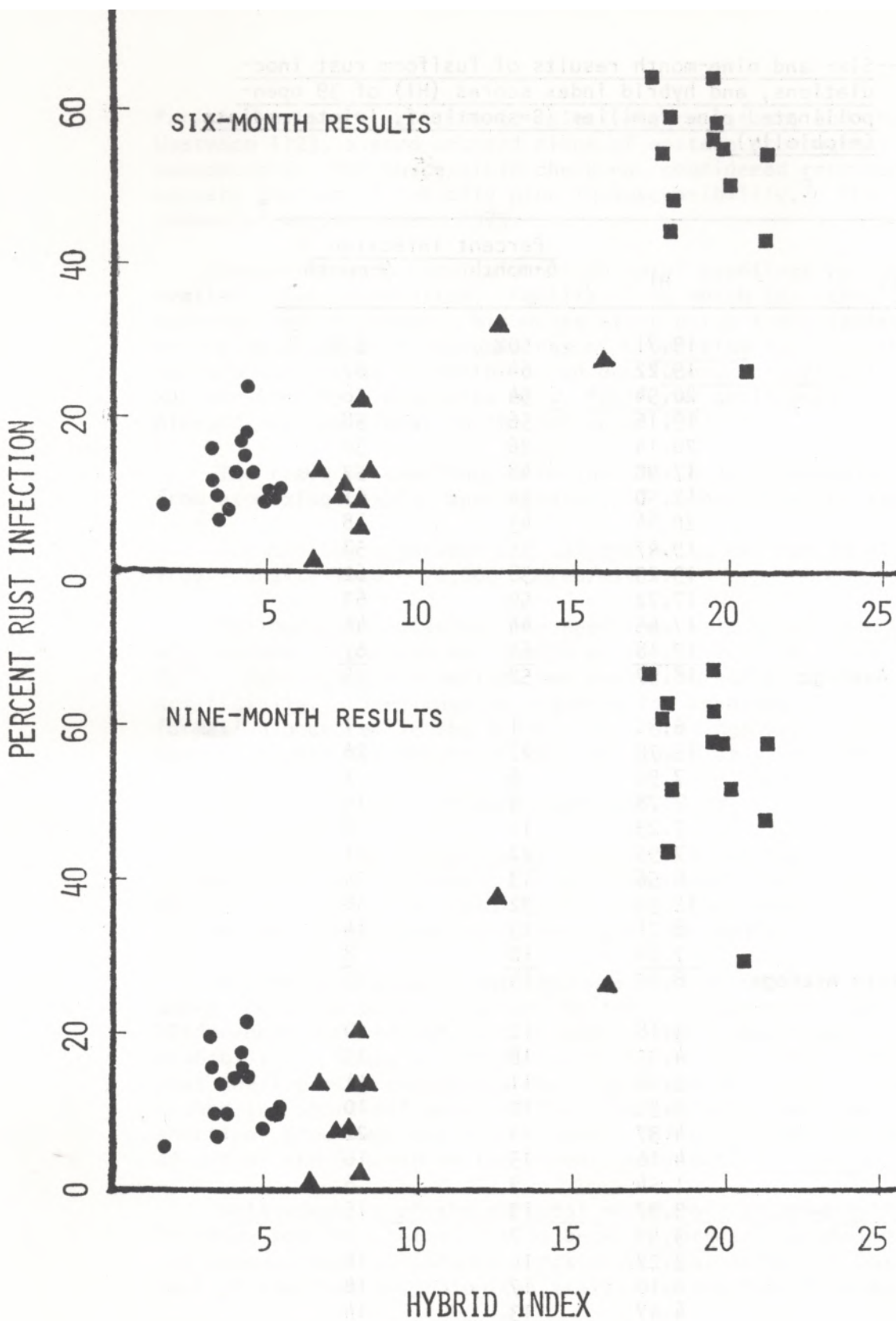


Figure 2.--Plottings of percent fusiform rust infection of progeny versus hybrid index of their respective parent trees at 6- and 9-month evaluation dates. Circles, triangles and squares represent trees (or families) initially classed as shortleaf, intermediate and loblolly pines, respectively.

Plottings suggested linear relationships. Multiple (forward) stepwise regression was then employed to determine which of the variables explained the most variation in fusiform rust infection.

For both the 6- and 9-month rust infection results, hybrid index was included during the first step of regression. It accounted for 77% of the explained variation in the 6-month results, and 76% in the 9-month results. The following regression equations were derived:

$$\text{6-month: Arcsin sq. root percent rust infection} = 11.45 + 1.74 \text{ HI}$$

$$\text{9-month: Arcsine } \sqrt{\text{percent rust infection}} = 10.88 + 1.87 \text{ HI}$$

The difference between these two regression equations is negligible, as there was little change in infection between the two dates (Fig. 2). Although families with similar hybrid indices vary considerably in rust infection, the tendency for loblolly pines to have high indices and infection rates and for shortleaf pines (and most intermediates) to have low values for both characters is apparent on Figure 2. Since 8 "intermediate" families plotting closest to shortleaf pines have infection rates no higher than others of that species, we conclude that they are probably not hybrids. The other 2 families, especially 1-21, which plot midway between the means of the two groups for both characteristics, are of probable hybrid parentage.

#### CONCLUSIONS

Results of fusiform rust inoculations indicate that loblolly x shortleaf pine hybrids exist which produce progeny intermediate to the two species in rust susceptibility. The respective parent trees were also morphologically intermediate. Regression analysis demonstrated that hybrid index of the parent trees was highly related to rust resistance of the progeny. Considerable natural variation exists within both the East Texas shortleaf and loblolly pine populations. Additional variation for potential selection of fast growing fusiform rust resistant genotypes also appears available as a result of relatively infrequent, yet effective, natural hybridization between loblolly and shortleaf pine.

Whether or not the reported rust resistance of loblolly pine from western sources results from hybridization with shortleaf pine was not resolved by this study. On the average, progeny from our loblolly pines were considerably less susceptible (55% infection) than the control from eastern provenance (72%) although somewhat more susceptible than the Livingston Parish, LA source (42%). Since our study has demonstrated that presumed hybrids exist which are intermediate between shortleaf and loblolly pines, there is a definite possibility that the observed resistance of our loblolly pines results from introgression with shortleaf pine.

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