Fusiform Rust Infection of Loblolly Pines that Survived Resistance Screening and of Their Progeny

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ABSTRACT

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Rustfree loblolly pine seedlings previously inoculated with *Cronartium quercuum* f. sp. *fusiforme* in a tent-style inoculation chamber were used to establish two plantings. After 10 yr, incidence of infection in the first planting was 22% compared with 29% in previously uninoculated checks. In the other planting, incidence of infection was positively correlated with rust incidence in the same families after the original tent inoculations. In both plantings, rustfree survivors of families with low rust incidence in the inoculation test generally remained rustfree after planting in the field; in contrast, many gallfree survivors of susceptible families became infected in the field. For a second-generation test, seedling progeny of eight open-pollinated rustfree survivors in the two field plantings were artificially inoculated. The only highly resistant second-generation family was derived from a family that was resistant in the original inoculation. The remainder were susceptible.

Fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, is a serious disease of loblolly (*Pinus taeda* L.) and slash (*P. elliottii* var. *elliottii*) pines in the southeastern United States. Heritable resistance to the disease is well known and has been incorporated into most loblolly and slash pine improvement programs in the South.

Unlike most important heritable traits in southern pines, genetic variation in resistance to fusiform rust can be detected at an early age. Heritable variation in cotyledon-stage seedlings has been shown in artificial inoculation tests (4). Resistance to fusiform rust should be an ideal trait for rapid genetic improvement if juvenile-mature correlations are high and genetic interactions with environment and pathogen are limited.

Borlaug (1) and Dinus and Griggs (2) suggested that survivors of artificial inoculation would be valuable breeding material. Using rustfree survivors (RFS) of inoculation tests has become operational in one tree improvement program (5).

Our study was initiated in 1967 to determine how well rustfree survivors of inoculation tests perform in the field and to examine the feasibility of using RFS seedlings in a seed orchard.

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MATERIALS AND METHODS

Test plantings, 1967 and 1968. After a mass-inoculation procedure for fusiform rust was developed by Jewell (3), a program was initiated at the Southern Institute of Forest Genetics at Gulfport, MS, to screen loblolly pine families for resistance to fusiform rust. Cotyledon-stage seedlings were inoculated in a tent-style inoculation chamber (3), then planted in a nursery bed. At the end of the first growing season, seedlings were lifted and scored for the presence of galls. Up to 10 gallfree seedlings per family were subsequently planted at the Harrison Experimental Forest in southern Mississippi at a spacing of 3 × 3 m. Plots were single trees planted in a completely random design.

Two plantings that differed only in origin of seedlings were established in successive years using rustfree survivors. The 1967 planting consisted of progeny from 19 full-sib crosses of randomly selected trees from southern Mississippi and southeastern Louisiana, 21 families from open-pollinated national forest selections in central Louisiana plus uninoculated checks (local bulk seed collections) (Table 1). The 1968 planting was adjacent to the 1967 planting and consisted of 114 families from open-pollinated national forest selections that covered the natural range of loblolly pine (Table 2). There were no uninoculated checks in the 1968 planting.

Heights were measured in 1977. Fusiform rust galls (stem and branch galls) were counted in 1972, 1977, and 1982. Analysis of variance for the hierarchial design was used to test family and seed source differences.

Second-generation test. In 1977, cones were collected from 10 open-pollinated trees in the two RFS plantings. Two of the 10 trees were from the original bulk checks and the remaining eight were rustfree survivors. None of the 10 had fusiform rust infection when the cones were collected. Seedlings from these trees were grown in peat pots, and three replicates of eight seedlings per family were inoculated with basidiospores when 6 wk old, following the method of Walkinshaw and Bey (8). Seedlings were scored individually for purple spots (indicating infection), planted in 13-cm pots, and scored for presence or absence of galls at 9 mo. The mean percentage of seedlings with galls in each eight-tree plot was analyzed in a randomized complete block design. Tests of significance were at $P = 0.05$.

RESULTS

Test plantings, 1967 and 1968. Field infection in families in the 1967 planting ranged from 0 to 100% incidence of trees with rust galls. The RFS seedlings averaged 22% with rust galls, and the uninoculated checks averaged 29%. The three families from southeastern Louisiana (Livingston Parish) had no rust galls, verifying the known resistance of that source (9). These families also developed the least rust after the original inoculations (Table 1).

Rust incidence in the 1968 planting averaged 18%. Infection of individual families ranged from 0 to 100% incidence (Fig. 1, Table 2). There was considerable variation among geographic sources. Texas and southwestern Mississippi sources were relatively resistant; Georgia and Alabama sources were susceptible, which corresponds to expected geographic patterns derived from provenance research (9). Coastal South Carolina and northern Mississippi sources were resistant in this test but generally are not resistant in provenance tests. All trees in the 1968 test were from a tree improvement program that specified that all candidate trees be free of fusiform rust when originally selected. The trees in this test may not correspond closely in disease susceptibility to the random selections used in provenance studies, especially in areas where substantial natural infection exists.

A positive relationship appeared between incidence of infection in the nursery after artificial inoculation and in the field after outplanting (Fig. 1). The RFS seedlings from families that were not heavily infected in the inoculation test tended to be infected less often in the field.

Substantial differences occurred in heights in the 1968 test, but these were not related to rust infection. For example, the two best sources for rust resistance, the South Carolina coastal and the northern Mississippi source, had the tallest and the shortest trees, respectively (Table 2).

Second-generation test. The second-generation inoculation test results were similar to those of the field tests (Table 3). Seedlings from open-pollinated progeny of families that were heavily infected in the original test were also heavily infected in the second generation. The progeny from an RFS of a resistant family, FM66, had only 17% incidence of seedlings with galls (Table 3).

Two kinds of check lots were inoculated. One was from a bulk seed collection of loblolly pine from southern Mississippi. The other checks, 1 and 2, were from seed of two open-pollinated uninoculated checks in the 1967 test and were included as a measure of resistance in the families that provided the pollen in the area. (No pollen was produced within the plantings.)

In the second-generation inoculation, seedlings from the bulk check lots averaged 58% incidence of galls. The two background pollen checks averaged 65% incidence, not significantly different from the 70% incidence among seedlings of rustfree survivors from the susceptible lines.

Early symptoms, i.e., purple spot lesions, were recorded in the second-generation test (Table 3). The proportion of seedlings with early symptoms was uniformly high, indicating that the inoculations were successful. Even in the family with the lowest incidence of galls after 9 mo (17%) ((FM66 × W) × W), 75% of the seedlings previously showed purple spots.

Table 1. Field performance and rust inoculation data for loblolly pine families in the 1967 test planting, summarized by geographic source

Source	Families (no.)	Inoculation test: Incidence of galls[a] (%)	Field performance: Incidence of galls[b] (%)	Field performance: Height[c] (m)
Mississippi (southern)	16	84	28	10.4
Louisiana (southeastern)	3	50	0	10.9
Louisiana (central)	21	66	20	10.4
Average[d]	40	72	22	10.5
Uninoculated checks	Bulk	···	29	10.9

[a]At 9 mo.
[b]Cumulative to 15 yr.
[c]At 10 yr.
[d]Weighted by families.

Table 2. Field performance and rust inoculation data for loblolly pine families in the 1968 test planting, summarized by geographic source

Source	Families (no.)	Inoculation test: Incidence of galls[a] (%)	Field performance: Incidence of galls[b] (%)	Field performance: Height[c] (m)
North Carolina (Piedmont)	19	73	15	8.9
South Carolina (Piedmont)	10	81	18	8.3
South Carolina (coastal)	7	55	6	9.4
Georgia (northern)	21	85	32	8.2
Mississippi (northern)	6	72	7	7.4
Alabama (northern)	6	81	31	8.3
Mississippi (southwestern)	22	68	10	8.5
Alabama (central)	6	86	36	7.8
Texas (eastern)	17	50	8	8.7
Average[d]	114	71	18	8.5

[a]At 9 mo.
[b]Cumulative to 14 yr.
[c]At 9 yr.
[d]Weighted by families.

DISCUSSION

When the experiments were originally established, we anticipated that many of the RFS seedlings from the heavily infected families would not be escapes and might be a source of resistance to fusiform rust. The relatively poor performance of many of the rustfree survivors in the field and in the second-generation inoculation was therefore a disappointment. One might assume that they should all be relatively resistant since they survived the intense tent inoculation procedure. However, if the rustfree survivors were all equally resistant, there should not have been such large and significant observed differences among families. Powers and Kraus (5) also made the observation that RFS seedlings from

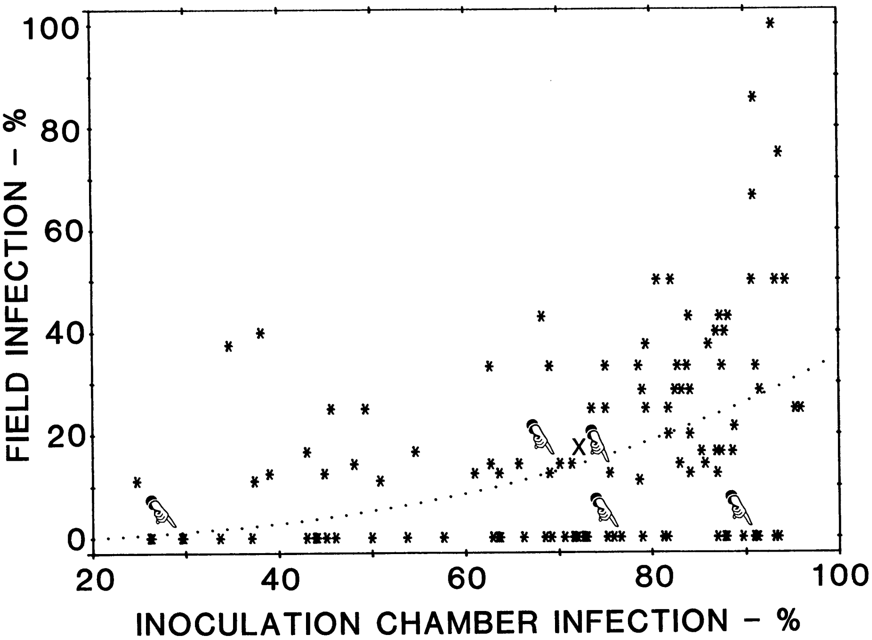

Fig. 1. Scatter diagram of family means for inoculation chamber infection versus field infection of rustfree survivors after 10 yr. Dotted line is a fitted regression equation: field $\% = 3.28 + 0.0000329$ (inoc. $\%)^3$, $r^2 = 18\%$. Pointers indicate family means for individuals used in the second-generation test.

Table 3. Fusiform rust incidence in families of artificially inoculated loblolly seedlings and in the artificially inoculated progeny of survivors of those families

Family[a]	Incidence of rust galls in first generation (%)	Incidence of symptoms in second generation: Purple spots (%)	Galls (%)
Rustfree survivors (RFS)			
FM66 × W	30	75	17
TAL181 × W	91	96	50
1-8 × 1-9	77	100	66
BUDE14 × W	70	92	67
5-7 × 5-10	85	100	79
BUDE25 × W	74	96	79
HOMO4 × W	68	96	79
5-3 × 5-9	75	96	88
Background pollen checks[b]			
Check 1	...	83	61
Check 2	...	92	69
Loblolly bulk			
Mississippi	...	92	58

[a]W refers to wind- or open-pollinated progeny. Second-generation test was conducted using wind-pollinated progeny from individuals of the listed families and checks.
[b]Uninoculated checks in the RFS planting.

the heavily infected families from inoculation tests did not perform well in the field.

There are several possible explanations for the poor performance of many of the RFS seedlings in the field. One is a relatively low juvenile-mature correlation for fusiform rust resistance. Biochemical or morphological traits can change with age. For instance, Squillace (6) found that cortical monoterpenes undergo a change in composition in slash pine seedlings between 1 and 2 yr of age. Squillace and Wells (7) also found some association between cortical monoterpene composition and susceptibility to fusiform rust in loblolly pines. Interactions of genotype × environment or pine genotype × rust genotype could also account for the poor performance of the rustfree survivors.

In spite of the lack of resistance of RFS seedlings in the field to fusiform rust, there was a positive relationship between the family means for infection in the inoculation chamber and in the field (Fig. 1). Even though many of the rustfree survivors were susceptible plants that escaped infection, the inoculation chamber results still predicted the performance of families and geographic seed sources in the field. It seems apparent, however, that family rather than individual tree selection will be most effective in a rust-breeding program. An outstanding family, FM66, was identified in this experiment. It was not only superior in the original tent inoculation and in the field, but its progeny were highly resistant in the second-generation inoculation, even though they were twice diluted genetically by wind pollination.

Rustfree survivors can be useful in establishing seed orchards if the best families are used. Our present rust-screening process uses the more precise inoculation technique used in the second-generation test. In our seed orchards, we are planting only trees that are gallfree and have some evidence of early infection, i.e., purple spot lesions. This should avoid many of the susceptible plants that have escaped infection.

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