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ABSTRACT

Three field progeny tests in Greene County, Georgia, of 28 loblolly pine seed sources with possible resistance to fusiform rust indicated that all sources had moderate to high resistance after 10 to 14 years in the field. Susceptible check sources had 86 and 78 percent rust infection after 13 and 14 years, while the various seed sources had infection percentages ranging from 1 to 57. In one test, field infection of rust-free seedlings surviving the concentrated basidiospore spray (CBS) inoculation system had a correlation coefficient of r=0.85 with the original CBS infection results. Generally, the CBS system correctly classified resistant and susceptible sources; however, seven sources with field resistance were classed as susceptible in the CBS system. Therefore, field progeny tests will continue to play an important role in evaluating rust-resistant pine sources.

Keywords: Pinus taeda, Cronartium quercuum, disease resistance, artificial inoculation.

The identification and selection of loblolly (Pinus taeda L.) and slash (P. elliottii Engelm. var. elliottii) pines with genetically controlled resistance to fusiform rust, caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme, has been underway for more than 25 years (Barber 1961; Jewell and Mallett 1967; Wells and Wakeley 1966). Several methods have been used to identify sources of resistance. In the Southwide Seed Source Study (Wells and Wakeley 1966), mass collections of loblolly pine seeds from some geographic areas were more resistant to fusiform rust than collections from other areas. Others observed performance of progeny of rust-free phenotypes in field tests or in artificial exposures to the rust fungus (Dinus and Griggs 1975; Jewell and Mallett 1967; Powers 1980; Zobel and others 1971). A major breakthrough was the development of the concentrated basidiospore spray (CBS) system, a standardized artificial inoculation system that made it possible to evaluate hundreds of pine families (Matthews and Rowan 1972). Miller and Powers (1983) showed that results with the CBS system correlate closely with field test results for loblolly pine. Testing by the CBS system is provided at the Resistance Screening Center, maintained near Asheville, NC, by the Southern Region of the Forest Service, U.S. Department of Agriculture. Forestry organizations across the South routinely send seedlots to the Center for evaluation of fusiform-rust resistance.

In this paper, we describe three tests involving seedlings from 24 half-sib families, 1 full-sib family, and 3 bulk collections of loblolly pine that were outplanted in Greene County, Georgia, to determine their relative field susceptibility to fusiform rust. All seed sources were originally selected because field or greenhouse test results indicated at least some rust resistance. In one of the studies, rust-free survivors of CBS inoculations were outplanted, whereas in the other two studies seedlings were not inoculated prior to outplanting. Field performance of each family was compared with its susceptibility as estimated by the CBS system.

Methods

The three field tests were established in Greene County on land owned by the Georgia Kraft Corporation. Two plantings were contiguous and the third was less than 1.6 km away. Sites were clearcut and roller-chopped prior to planting. Seedlings were all planted at a 1.8- x 3.0-m spacing.

Test 1. Progeny of 11 half-sib families and one geographic seed source were inoculated with basidiospores in 10 early tests of the CBS system. Only seedlings without galls after 1 year in a greenhouse were selected for outplanting. Seedlings from the 13th source, the susceptible check, were not subjected to artificial inoculation prior to planting in the field. However, in the 10 prior CBS inoculations, 75 percent of seedlings of the susceptible source got galls. Seedlings were hand-planted in March 1973. There were eight replications with each replication containing one 10-tree row of each family. Families were randomly assigned to rows within replications in a randomized block design. In November 1981 at age 9, galled trees and numbers of galls per tree were tallied for each family. In March 1987 at age 14, branch and stem galls and d.b.h. were recorded.

Test 2. Six sources of uninoculated seedlings were hand-planted in November 1973. Four sources are half-sib families and two are bulk collections. Each of 12 replications consisted of an 11-tree row of each of the 6 sources. At age 8 (1981), numbers of galls per tree were tallied for each family. At age 13 (1987), the presence of branch and stem galls and d.b.h. were recorded for each tree.

Test 3. Uninoculated seedlings from 18 sources were hand-planted in March 1977 in four replications, each containing one 20-seedling row of each source. Seventeen sources are half-sib progeny, while one source is Georgia Kraft Corporation's improved loblolly pine, which was used as a commercial check. At age 10 (1987), the presence of stem and branch galls and d.b.h. of each tree were recorded.

Data for each test were analyzed separately. Prior to statistical analysis, percentages of trees with rust were transformed to arc sine values. Analysis of variance (ANOVA) and Duncan's multiple range tests were carried out on both percentage and transformed data.

Results

Overall tree survival was 92 percent at age 14 in test 1 and 88 percent at age 13 in test 2. Test 3 was on a poorer site, and survival averaged 74 percent at age 10. The ANOVA indicated that both incidence of rust galls and d.b.h. values varied significantly by family and seed source in each of the three tests. There was an interaction between diameter and replications in tests 2 and 3, but there was no interaction of rust and replications in any test.

Test 1. The incidence of rust galls after 14 years ranged from 78 percent on the susceptible checks to 1 percent on trees in family SML-9 (table 1). All pine seed sources had significantly less rust than the susceptible check source. These results verified those from the CBS inoculations, which indicated that all 12 sources were more resistant than the susceptible check source. The correlation coefficient between field infection on these families at age 14 and the CBS inoculation results was $r=0.85$. Families with high percentages of trees infected also had high numbers of galls per infected tree at age 9 years and high incidences of stem galls at age 14.

There was no relationship between diameter growth and rust susceptibility. Some of the largest average d.b.h.'s occurred in both the most susceptible and the most resistant seed sources. Similarly, seed sources with small average d.b.h. were found at both ends of the susceptible-resistant spectrum.

Test 2. The amount of rust infection was similar to that in test 1. Infection at age 13 ranged from 3 percent in

family SML-9 to 86 percent in the susceptible check source (table 2). The correlation coefficient between field infection on these families and the CBS inoculation results on other seedlings of the same families was r=0.88 at age 13.

Test 3. There were no families with very high levels of rust infection as was the case in tests 1 and 2. One reason was that a commercial control (Georgia Kraft improved loblolly) was included in place of a susceptible check. The commercial check was found to be less susceptible to rust than we had anticipated. The highest incidence of rust (55 percent) occurred in family 601 (table 3). However, four half-sib families that were planted in both tests 1 and 3 had very similar percentages of galls: 31 and 38 percent for 4625-3, 28 and 32 percent for 10-5, 27 and 29 percent for 11-20, and 12 and 13 percent for 10-6 in tests 1 and 3, respectively. The correlation between the field results for this test and the CBS inoculation results on the same families (r= 0.48) was not as good as in the two other tests.

Discussion

Of 28 possibly resistant sources of loblolly pine planted in the three tests in Greene County, all showed moderate to high resistance to fusiform rust after 10 to 14 years in the field. Several of these sources (10-5, 11-20, 7-56, 15-42) have been shown to have field resistance when planted on other sites in the Southeastern United States (Miller and Powers 1983; Powers and Kraus 1983; Zobel and others 1971). Rust-free survivors of CBS inoculations from six families (SML-9, 10-6, 11-9, 11-20, 10-5, and 29R) used in test 1 were also planted in a seedling seed orchard in Baldwin County, Georgia (Powers and Kraus 1983). All six families demonstrated good resistance on both sites. After 5 years in the seedling seed orchard, progeny of SML-9 had only 2 percent infection, whereas those of 29R had 48 percent infection (Powers and Kraus 1983). The 0.85 correlation coefficient between field and greenhouse results in test 1 indicates that survivors of highly resistant families will have higher field resistance than those from moderately resistant families. Since the 28 resistant sources come from a variety of geographic locations and tree improvement programs, they should have sufficient variation in mechanisms of resistance to provide a broad genetic base of resistance for use in diverse plantings.

The CBS system is routinely used to evaluate the relative rust resistance of progeny from phenotypically resistant parent trees, and our evaluations are based on galls formed on seedlings 9 months after inoculation. The disease ratio (DR) is computed as the percentage of seedlings with galls in a test family divided by the percentage of seedlings with galls in the susceptible control seedlot. In our studies, a test family with a DR $\leq$ 0.70 is considered resistant; those with a DR $>$ 0.70 are considered susceptible. On the basis of DR $\leq$ 0.70, 21 of the 28 resistant families were properly identified as resistant by the CBS system. However, three families (SML-20 DR 0.79, 1495-18 DR 0.71, and 7-56 DR 0.82) were not identified as resistant in the CBS inoculation tests. In these instances, field performance was definitely superior to the artificial inoculation test results. Since the CBS system is not only a rigorous test but also a conservative one, such families can be misclassified. Families with intermediate levels of resistance in the field, such as SML-20 with 50 percent rust are often difficult to classify by the CBS system. Family 1495-18, which was quite resistant in the field, had a DR of 0.71 and narrowly missed the resistant classification. Family 7-56 also performed better in a field planting in Houston County, Georgia, and in test 3 than in the CBS inoculation (Miller and Powers 1983). In addition, Zobel and others (1971) reported that progeny of this family had good field resistance in seven progeny tests. More research is needed to determine why families like SML-20, 1495-18, and 7-56 are more resistant in the field than in CBS tests. Progress

is being made in enhancing the CBS system by identifying symptoms that are better correlated with field resistance (Walkinshaw and others 1980).

The correlation (r=0.85) of CBS greenhouse test results to the field results in test 1 was very good. There were still strong differences in resistance between families even though a portion of the susceptible population of each family had been eliminated by the CBS inoculation test. These results show that CBS survivors of the most resistant families are superior in resistance to those from families with moderate resistance. Exposing seedlings to the rust by the CBS or other inoculation procedures and outplanting only rust-free survivors eliminates many of the most susceptible trees in a family (Dinus and Griggs 1975; Powers 1980; Powers and Kraus 1983; Schmidtling and Walkinshaw 1985). Powers and Kraus (1983) reported a correlation of r=0.76 between infection in the CBS system and subsequent infection of rust-free survivors. Schmidtling and Walkinshaw (1985) reported r^2=18 percent (r=0.42) for the regression equation of field infection to inoculation test results. Correlations between field infection and inoculation test results tend to be high when there is a wide range in susceptibility of the test families and low when the families are similar in response to rust.

In the present work, some families with relatively low percentages of trees infected, such as 11-16 (19 percent) had a high number of rust galls on the trees that did become infected. In contrast, some families such as 1590-6, had a much higher percentage of trees infected (57 percent) but only about half as many galls per infected tree. The reason for this result is unknown, but the increase in galls per infected tree and stem galls between studies 1 and 2 for sources L.P. and 29R suggests that some very susceptible individuals were eliminated by using survivors of CBS inoculation.

Diameter growth was fair on these relatively poor sites. Granite outcroppings and gulleys in the study area contribute to the poor-site quality. The significant replication effect in tests 2 and 3 was caused by the poor sites. Some of the most resistant sources (Hitchiti Hybrid, 7R, and 10R) have slow growth rates that make them less desirable silviculturally. However, most resistant sources had good growth rates. Their growth is encourging and indicates that using these families in rust-resistance programs will not result in reductions in yield.

Acknowledgment

We thank the Georgia Kraft Corporation for its assistance in providing land for planting sites.

Table 1.--Relative susceptibilities to fusiform rust of progeny from 13 loblolly pine sources (test 1) in a 1973 field planting of rust-free survivors in Greene County, Georgia, and in CBS system seedling inoculations

Pine source[b]	Field planting[a]				CBS disease ratio[c]
	Galls/ infected tree at age 9	Trees with rust galls at age 14	Trees with stem galls at age 14	Diameter at breast height	
	Number	Percent	Percent	cm	
SML-9	0.0	1 A[d]	0 A[d]	15.2 BCD[d]	0.40
Hitchiti hybrid	2.0	5 AB	1 AB	13.4 A	.36
10-6	1.2	12 ABC	4 ABC	16.2 CDE	.38
11-9	1.8	14 ABCDE	4 ABC	16.8 E	.47
11-16	7.1	19 BCD	10 ABCDE	16.5 DE	.36
L.P.	1.7	26 CDEF	10 ABCD	16.9 E	.48
11-20	3.6	27 CDEF	13 CDE	14.7 B	.37
10-5	3.6	28 CDEF	16 DEF	16.3 CDE	.46
4625-3	4.5	31 DEF	20 DEF	16.8 E	.51
29R	3.2	32 EF	12 BCDE	14.6 B	.54
1582-11	2.1	38 F	20 EF	16.3 CDE	.52
1590-6	3.7	57 G	29 F	15.1 BC	.51
Susceptible check	5.6	78 H	49 G	16.9 E	1.00

[a]Percentages and d.b.h. based on 8 replications with 10 trees/source/replication.

[b]Susceptible check and Livingston Parish (L.P.) were bulk seed collections. All others were half-sib progeny of the parent clone indicated. Susceptible check seedlings were not first exposed to CBS inoculations and were not rust-free survivors.

[c]Disease ratio = Percentage of trees galled in test family/percentage of trees galled in susceptible check.

[d]Within columns, numbers followed by the same letter are not significantly different according to Duncan's multiple range test, $P \leq 0.05$.

Table 2.--Relative susceptibilities to fusiform rust of progeny from six loblolly pine sources (test 2) in a planting established in 1973 in Greene County, Georgia, and in CBS system inoculations

Pine source[b]	Field planting[a]: Galls/infected tree at age 8	Trees with rust galls at age 13	Trees with stem galls at age 13	Diameter at breast height	CBS disease ratio[c]
	Number	Percent		cm	
SML-9	0	3 A[d]	0 A[d]	13.7 A[d]	0.40
L.P.	2.9	21 B	6 A	14.9 C	.48
1495-18	1.6	26 B	7 A	14.7 C	.71
SML-20	3.8	50 C	27 B	15.0 C	.79
29R	4.6	54 C	27 B	13.8 AB	.59
Susceptible check	5.9	86 D	49 C	14.5 BC	1.00

[a]Numbers based on 12 replications with 11 trees/source/replication.

[b]Susceptible check and Livingston Parish (L.P.) were bulk seed collections. All other sources were half-sib progeny of the parent clone indicated.

[c]Disease ratio = Percentage of trees galled in test family/percentage of trees galled in susceptible check.

[d]Within columns, numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$).

Table 3.--Relative susceptibilities to fusiform rust of progeny from 18 loblolly pine sources (test 3) in a 10-year-old planting established in Greene County, Georgia

Pine source	Trees with rust[a]	Diameter at breast height[a]	CBS disease ratio[b]
	Percent	cm	
B-5-4	2 A[cd]	9.3 ABCD[d]	--
10R	8 AB	8.7 AB	0.51
10-6	13 ABC	10.5 CDE	.52
BA 1-2	16 BCD	9.6 ABCDE	.85
7R	21 BCDE	8.2 A	.83
15-42	22 BCDE	10.9 E	.61
40R	26 BCDE	9.9 BCDE	.65
48R	28 CDE	9.3 ABCD	.74
11-20	29 CDEF	9.0 ABC	.39
29R x 10-5	29 CDEF	9.7 BCDE	.44
10-5	32 DEF	10.4 CDE	.49
3906-21	34 DEF	9.4 ABCDE	.67
7-56	38 DEF	10.6 DE	.82
4625-3	38 DEF	9.9 BCDE	.68
Georgia Kraft Improved	39 DEF	9.6 ABCDE	--
BA 2	41 DEF	10.0 BCDE	.66
3505-16	41 EF	9.4 ABCDE	.82
601	55 F	8.5 AB	.82

[a]Numbers based on 4 replications with 20 trees/source/replication.

[b]Disease ratio = Percentage of trees galled in test family/percentage of trees galled in susceptible check.

[c]Analysis of variance and Duncan's multiple range test were done on transformed data.

[d]Numbers within columns followed by the same letter are not significantly different according to Duncan's multiple range test, $P \leq 0.05$.

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