PITCH CANKER RESISTANT SLASH PINE IDENTIFIED BY GREENHOUSE SCREENING

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Abstract.--A new greenhouse-based method was used to screen for pitch canker resistance in open-pollinated slash pine families representing a range in relative resistance as determined by field tests. Four-month-old seedlings of 12 families plus a susceptible check family were inoculated by severing the stem just below the bud and spraying the cut surface with a water suspension of *Fusarium moniliforme* var. *subglutinans* conidia. Seedling responses were evaluated 3, 4, and 5 months after inoculation. Highly significant family differences were observed on all three reading dates, but the best family separation occurred 5 months after inoculation, when family mean infection exceeding the response standard ranged from 0 to 80 percent (mean 42 percent). The susceptible check was always correctly ranked. Field and greenhouse resistance class groups were nearly identical, and standardized performance scores were highly correlated. Operational greenhouse screening for pitch canker resistance appears feasible.

INTRODUCTION

Slash pine *(Pinus elliottii* Engelm. var. *elliottii)* is one of the most susceptible southern pine species to damage caused by pitch canker disease. Epidemics have periodically devastated forest plantations of this species in Florida and Georgia. The most recent widespread episode occurred during the mid-1970's, when over 1 million acres of Florida slash pine plantations were affected (Phelps and Chellman 1976), and several thousand acres were harvested prematurely to salvage damaged stands (Dwinell and Phelps 1977). Damage has also been severe in some slash and loblolly pine seed orchards (Phelps and Chellman 1976, Kuhlman et al. 1982, Kelly and Williams 1982), and in some plantations of loblolly pine (Kuhlman and Cade 1985).

Pitch canker, caused by *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. & Rienk. (FMS), is a shoot-killing disease in slash pine. Losses accrue mainly from reductions in volume growth, but stem deformities sometimes occur, reducing opportunities for high-value, solid wood products (Arvanitis et al. 1984, Phelps and Chellman 1976, Schmidt and Underhill 1974). Associated mortality is usually minor, but has been known to exceed 25 percent (Blakeslee and Oak 1979).

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Evidence exists for variation in pitch canker resistance among slash pine genotypes (Blakeslee and Rockwood 1978, Dwinell and Barrows-Broaddus 1979, Lowerts et al. 1985, McRae et al. 1985). Disease-free trees are commonly present in devastated plantations, as well as in diseased seed orchards and progeny tests. This variation has been quantified through controlled inoculation experiments and the potential genetic gains estimated for various selection strategies. Results indicate that meaningful improvements can be made in pitch canker resistance (Rockwood et al. 1987). A rapid and reliable screening procedure for identifying resistant genotypes would greatly advance development of pitch canker resistant planting stock.

Artificial screening methods used to date to identify relative resistance in the field and greenhouse have required tedious hand wounding and inoculation procedures (Blakeslee and Rockwood 1978, Dwinell 1978, Dwinell and Barrows-Broaddus 1979, McRae et al. 1985) that are difficult to apply consistently to individual seedlings. Seedling morphology, wound size and depth, the types of tissues exposed, and inoculum exposure can vary considerably within and between tests. A new screening method that can be more quickly and uniformly applied was tested to determine if differences in relative resistance could be detected for a group of slash pine families and if the results were comparable to those obtained from field progeny tests.

MATERIALS AND METHODS

Twelve half-sib slash pine families were selected for the greenhouse test. Based on their responses in one or more of four artificially inoculated tests of field-grown trees ranging in age from 3 to 22 years, these families represented a range in relative pitch canker resistance (White et al. 1987). Selected families fell into three relative resistance groups (resistant, intermediate, and susceptible); relative resistance classifications were identical for families common to different tests. After the greenhouse test was initiated, information from an additional artificial inoculation field test and a naturally infected field test became available. Results from these tests were also used in subsequent comparisons of field and greenhouse resistance. The 12 families were given arbitrary letter codes (A-L), and the greenhouse test was conducted without knowledge of the family identities or relative resistance classification. An additional susceptible check family (B2) was also included. Selection of this check family was based on our (Oak and Blakeslee) knowledge of its propensity for severe damage in infected seed orchards and in past greenhouse inoculation experiments.

Prior to inoculation, the seedlings were grown in Ray Leach Super Cells for 4 months in a greenhouse at the USDA Forest Service Resistance Screening Center (RSC) near Asheville, NC. When they were inoculated, seedlings in all families had similar morphology. Stems were succulent with only primary needles, basal diameter averaged 2 to 3 mm, and heights averaged 75 to 100 mm. The seedlings were inoculated by severing the stem just below the bud and spaying the exposed cross-section with a water suspension of FMS conidia (1 x 10 conidia/ml) until visibly wet. The inoculum was an equal mixture of three separate proven pathogenic isolates originating from diseased slash pines growing in Liberty County, Florida. It was prepared in the manner described by McRae et al. (1985).

The experimental design was identical to that used for fusiform rust screening at the RSC (Anderson et al. 1983). Three trays of 20 seedlings of each family were inoculated on each of 2 consecutive days. At 3, 4, and 5 months after inoculation, the percentage of available stem length (cut surface to cotyledons) killed by FMS was estimated for each seedling. Two response standards were used--50 and 75 percent of available stem length killed. The percentage of trees that exceeded the response standard in each tray was subjected to analysis of variance procedures to determine family, run, and family-by-run (experimental error) interactions. Duncan's Multiple Range test was used to compare family test means.

The relationship between greenhouse and field test performance was statistically evaluated using rank and product-moment correlations. Family means in field and greenhouse tests were converted to standardized scores by subtracting the test mean from the family mean and dividing by the square root of the error mean square. Correlations were based on 9 of the 12 families for which field tests permitted standardized scoring.

RESULTS AND DISCUSSION

As FMS colonized the succulent shoots, the tissues turned purplish and resin was exuded. Symptoms of infection were evident within 1 month after inoculation, but they were relatively uniform within and among families, being restricted to less than about 1.5 cm of the stem. By 3 months, considerable variation in individual seedling response was evident. Symptoms ranged from just a few millimeters of killed stem to seedling mortality. Adventitious shoots that formed after wounding were frequently killed as the fungus advanced down the stem.

A broad range of family means (percent of seedlings exceeding the response standard) occurred at all reading dates and for both response standards. For the 75 percent response standard, the overall test mean increased nearly 20 percent (from 17 to 35 percent) between the 3- and 5-month readings, while the overall test mean using the 50 percent standard increased 10 percent over the same period (32 to 42 percent). The 5-month reading of the 50 percent response standard provided the best differentiation of family performance, with individual family means ranging from 0 to 80 percent.

Analysis of variance indicated highly significant family differences for both response standards at all reading dates. Table 1 illustrates the results for the 50 percent response standard at 5 months. Significant family-by-run interactions were absent.

Statistical separation of family means allowed grouping of families into relative resistance classes according to the greenhouse test results (Table 2). The susceptible check was the most susceptible family and was correctly ranked in all analyses.

Table 1.--Analysis of variance for mean pitch canker infection response of 12 half-sib slash pine families and a susceptible check for the 50 percent response standard, 5 months after inoculation in the RSC greenhouse test.

Source of Variation	DF	S.S.	M.S.	F.
Families	12	38560.25	3213.35	36.54**
Runs	1	566.69	566.69	6.44*
Families x Runs	12	1055.37	87.95	.97
Among Replicate Trays within Runs	52	4708.19	90.54	

** and * denote significance at the 1% and 5% levels, respectively.

The relative pitch canker resistance classes determined in the greenhouse testing closely agreed with resistance class groupings based on the field tests (Table 2). Only one family (F) did not fall into the same resistance class group in the greenhouse as it had in an earlier field test. The method of pathogen introduction apparently has little influence on detecting relative resistance. The resistance class groupings for four families (B, D, H, I) common to the naturally infected field test, two artificially inoculated field tests (H only one), and the greenhouse test were identical.

One of the 12 test families (B) represented a different collection (different orchard) of the same family used as the susceptible check (B2). Both of these families were grouped in the same relative resistance class in the greenhouse test and in the field tests, but they received statistically different specific rankings (10th and 13th) according to family mean infection in the greenhouse test.

The strong correspondence between the greenhouse and field results suggested by the similar family resistance class groupings was further supported by significant correlations between standardized scores. For the nine families having both field and greenhouse scores, both the rank correlation (.85) and the product-moment correlation (.845) were significant at the 1 percent level.

While the similarities in resistance class groupings and significant correlations between greenhouse and field test scores are encouraging, it must be recognized that this test provides only a single comparison between a relatively small number of families. Additional experiments are underway that will: 1) evaluate the repeatability of this test with the same families plus an additional group of families that represent a range in relative resistance,

Table 2.--Mean percent pitch canker infection, relative pitch canker resistance classes, and standardized scores for 12 half-sib slash pine families, based on field and greenhouse testing.

Family	Field Tests: Resistance class[1]	Field Tests: Current standardized score[2]	Greenhouse Test: Family mean[3]	Greenhouse Test: Resistance Class[4]	Greenhouse Test: Standardized Score[5]
D	R (3)	1.18	0 a	R	1.83
G	R (1)		13 b	R	1.21
I	R (3)	2.07	17 b	R	1.02
J	R (1)	1.30	23 b	R	.74
F	S (1)		38 c	I	.02
K	I (1)		43 c	I	- .21
E	I (1)	- .49	43 c	I	- .21
C	I (2)	- .04	48 cd	I	- .45
L	I (3)	- .13	49 cd	I	- .50
B	S (3)	- .34	58 de	S	- .93
H	S (2)	-1.18	63 e	S	-1.16
A	S (1)	-3.16	67 e	S	-1.35
Suscept. Check - B2			80 f		

[1]Relative pitch canker resistance class (R=resistant, I=intermediate, S=susceptible) based on performance in artificially and/or naturally infected field tests conducted independent of greenhouse test. ( ) indicates number of field tests from which classifications were made.

[2]Current standardized performance score (standard deviations from the overall test mean) based on performance in an artificially or naturally inoculated field test with 40 or more families and more than 30% infection.

[3]Duncan's Multiple Range Test for mean pitch canker infection (50% percent response standard at 5 months). Test means followed by different letters are significantly different at the 5% level.

[4]Relative pitch canker resistance class (R=resistant, I=intermediate, S=susceptible) based on response in RSC greenhouse test.

[5]Standardized performance score (standard deviations from the overall test mean) based on response in RSC greenhouse test.

and 2) evaluate the feasibility of pitch canker resistance screening in loblolly pine. Favorable results may lead to operational pitch canker screening at the RSC.

CONCLUSIONS

The stem severance-inoculation procedure reported in this study accurately detected differences among slash pine families that represent a broad range of relative resistance to pitch canker. This method is quicker, easier, and may be more consistently applied than current screening procedures. The relative resistance class groupings and infection rankings were consonant with existing knowledge of field performance. These results suggest that pitch canker resistance in slash pine may be routinely and reliably evaluated by this greenhouse procedure.

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