FUSIFORM RUST INCIDENCE AND VOLUME GROWTH IN A FIRST-GENERATION BACKCROSS POPULATION, (SHORTLEAF X SLASH) X SLASH

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Abstract.--The lack of fusiform rust resistance has restricted slash pine planting to sites with a low rust hazard. An interspecies backcross breeding study was undertaken to investigate the applicability of transferring the rust resistance of shortleaf pine into slash pine. Rust resistance and volume growth of a first-generation backcross population were evaluated after 7 years of field growth. The population consisted of 12 backcross families formed by mating 1 of 6 elite slash pine clones to 2 of 7 shortleaf X slash pine F1 hybrids. Rust incidence was moderate (25%), but differences among slash pine parents were highly significant (range 11% to 54%). Differences among F1 parents were not significant, however, 4 of the 12 backcross families were less rusted than the slash pine controls. Differences in tree volume among slash pine parents and among F1 parents were significant, and half the backcross families were larger in mean tree volume than the slash pine controls. From this test, no conclusions about the applicability of transferring rust resistance from shortleaf to slash pine with backcross breeding can be made, although the volume growth of slash pine was recovered in several backcross families. In future breeding cycles, multiple rust inoculation tests, coupled with early selection and accelerated breeding, will be required to conclusively evaluate this breeding method. In practice, the careful integration of early testing, accelerated breeding, and DNA marker-assisted selection may alleviate many of the traditional problems of backcross breeding in forest tree species.

Key words: backcross breeding, interspecies breeding, fusiform rust resistance, marker-assisted selection.

INTRODUCTION

Interspecies backcross breeding is a method for transferring favorable alleles of a trait from one species (donor) to another (recipient). Usual requirements for employing this method include a zero or suboptimal level of trait expression in the recipient (and vice versa in the donor), qualitative inheritance of the trait, and a moderate degree of fertility between the two species. Within commercially important southern pines, these requirements appear to be present in several instances. For example, Brown (1964) recommended using the method to transfer genes for early height growth from slash pine (Pinus elliottii var. elliottii Englm.) to longleaf pine (P. palustris Mill.) as a means of eliminating the grass stage in longleaf. A second example, initiated by a USDA Southeastern Forest Experiment Station research unit, is aimed at transferring fusiform rust resistance from shortleaf pine (P. echinata Mill.) to loblolly pine (P. taeda L.) (Kraus 1986).

Slash pine is a host of the obligate biotrophic fungus, Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme. The severity of the resulting fusiform rust disease has limited the planting of slash pine to sites with a low rust hazard. Currently, only intraspecies sources of resistance are available to slash pine breeders. This resistance is not well characterized and may be vulnerable to changes in the pathogen and environment (Snow et al. 1976). Alternative sources of resistance would greatly benefit the long-term breeding effort. Because it is apparently resistant to all cultures of fusiform rust (f. sp. fusiforme) (Kraus et al. 1982, Kraus and Powers 1984), shortleaf pine is a potential source. These factors, together with a knowledge of moderate interspecies fertility (Synder and Squillace 1966), suggest that a backcross breeding program may be an effective way to transfer rust resistance from shortleaf to slash pine.

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In a study reported by Wells et al. (1978), interspecies hybrids were made between shortleaf pine parents from 8 different geographic sources and a 21-tree slash pine pollen mix from north Florida. These hybrids and shortleaf and slash pine checks were tested at eight locations across the natural range of shortleaf pine. After 10 years, hybrids with north Georgia and southeast Arkansas parents were consistently taller than the other hybrids and the shortleaf pine checks. Rust incidence for all hybrids was well below (0% to 19%) the midparent level and in most cases nearly as low (0% to 6%) as the shortleaf pine checks. **In** the present study, hybrid trees with north Georgia and southeast Arkansas shortleaf pine parents were selected in a south Mississippi test planting and mated to elite slash pine clones. The resulting backcross progenies were grown in south Mississippi to test the shortleaf pine sources and the slash pine clones for their value as parents in a backcross breeding program and to provide a backcross population for further selection and breeding.

MATERIALS AND METHODS

Fl hybrid trees with north Georgia (Southwide Southern Pine Seed Source [SSPSS] 413) and southeast Arkansas (SSPSS 429) shortleaf pine maternal parents were phenotypically selected at age 16 years. The trees were growing in the south Mississippi test planting of a shortleaf X slash pine interspecies hybrid study (Wells et al. 1978). Slash pine parents had previously been progeny tested and selected for a fast growth rate by the Cooperative Forest Genetics Research Program (CFGRP). These clones also ranged from slightly below average to well above average for rust resistance. The original mating plan was to pollinate 8 to 10 hybrids from the 2 sources with an individual slash pine clone and then pool the seed by hybrid source. However, only one tree from source 429 and six from source 413 could be productively pollinated. As a result, the pollinations produced 12 backcross families (BC1)--6 single-tree test crosses for source 429 and 6 single crosses for source 413. Based on cone-collection data, these families consisted predominantly of full-siblings (>70%) and partially of paternal half-siblings (<30%).

Seedlings were grown in the Harrison Experimental Forest (Saucier, Mississippi) nursery in 1983 and planted at a field site near Lizana, Mississippi in January 1984. The field site was judged in the moderate-to-high category for fusiform rust hazard. A randomized complete block experimental design was used with 5 replications of I5-tree row plots. In addition to the 12 backcross families, 2 checks were included--slash pine and the F1 parents' open-pollinated progeny (F 1 -op). Two plots of each check were planted in each replication. The source of the slash pine check was not documented, but it is thought to be a bulk of open-pollinated seed collections from 6 to 10 resistant slash pine trees[2/]. The F 1 -op seeds were bulked by source before growing in the nursery and then planted in the field plots by source. A border row of slash pine, presumably from same source as the check, was placed completely around the planting. Also in January 1984, an industrial slash pine plantation was established adjacent to all sides of the test planting. A 30-ft-wide firebreak was maintained between the test planting and the industrial plantation.

Data were collected on each test tree for 1-year survival, 2-year height; and 7-year total height, stem diameter at 1 ft, and rust incidence. Heights and diameters were measured to the nearest 0.1 ft and 0.1 in, respectively. Assuming a cylindrical base (length of 1.0 ft) and a parabaloid top, stem volume (cu ft) was computed for each tree. Rust incidence was scored on a 0 to 3 scale as follows: 0 = no gall, 1 = branch gall(s) only, 2 = branch-into-stem gall(s) and branch gall(s) or no gall, and 3 = stem gall(s) and branch-into-stem gall(s) or branch gall(s) or no gall. Rust score was used to create two binomial variables--gall and stem gall: If rust score > 0, then gall = 1, otherwise gall = 0; and if rust score > 1, then stem gall = 1, otherwise stem gall = 0. Mortality was also analyzed as a binomial variable: if the tree was alive at planting and dead at age 7 then mortality = 1, otherwise mortality = 0. In the surrounding industrial slash pine plantation, rust incidence was scored on 15 randomly selected 15-tree linear plots.

[V] This information was kindly provided by John Pait of the Container Corporation of America, Callahan, Florida.

The data were analyzed using the GLM procedure of SAS (SAS Institute 1985) and the following model: Y = M + R + E + RE + W, where M is the mean, R is replication, E is entry (i.e., families and checks), RE is replication X entry interaction, and W is the within-plot error. All effects were assumed fixed. Replication 5 was heterogeneous in terms of height growth and ground vegetation so it was omitted before the analysis. The entry sums of squares were partitioned into 5 degrees of freedom (df) for males, 1 df for female sources, and 5 df for male X female source interaction. In addition, three linear contrasts were constructed to test for differences between each check and the backcrosses and between the checks. Means of males, females, backcross families, and checks were compared with t-tests at a type I error rate of 0.05.

RESULTS

Means for survival, growth, and rust incidence in the test planting are presented in Table 1. Field observations suggested a possible class of stunted trees. They were not easily scored during data collection but were apparent in a frequency histogram of the 7-year height data. The histogram was strongly skewed to the left with stunted trees ranging from 2.5 to 6.9 ft. Large stem galls appeared to stunt some trees, but in general no reason for stunting was apparent. Thus, all trees less than 7.0 ft were omitted from the growth analysis, but not from the rust or mortality analyses. The percentage of trees under 7.0 ft was not associated with any class of progeny or individual family. Rust incidence in the 225-tree sample of the industrial slash pine plantation was 39.6% for all galls and 30.7% for stem galls.

Table 1. Summary of survival, growth, and fusiform rust incidence in a (shortleaf X slash) X slash test planting near Lizana, Mississippi.

Variable	Age	Mean	Std. Dev.	Min.	Max.
Survival (%)	1	85.6			
	2	80.5			
	7	74.9			
Growth					
Height (ft)	2	2.58	0.95	0.5	6.5
	7	16.95	3.91	7.0	27.2
Diameter (in)a	7	3.81	1.02	0.9	7.1
Volume (cu ft)	7	0.83	0.52	0.02	3.87
Rust					
Stem gall (%)	7	20.5	40.4	0	1
Gall (%)	7	25.3	43.5	0	1

[a] stem diameter at 1 ft.

GLM (type III) results for the 7-year mortality, growth, and rust data are summarized in Table 2. Before GLM analyses, stem volume was transformed to the square root scale to remove positive skewness from the data. For growth traits, replication and entry effects were significant, while only entry effects were significant for mortality and rust incidence traits. Males were highly significant for all traits, and female sources were significant for height, volume, and mortality and nonsignificant for diameter and all rust traits. Male X female source interaction was highly significant for rust traits, but nonsignificant for growth traits and mortality. Linear contrasts comparing the checks and checks versus backcrosses were nonsignificant for all growth traits and mortality. The slash versus backcrosses and slash versus F 1 -op contrasts were significant for the rust traits, with slash significantly less infected than backcrosses or F 1 -op.

Table 2. F-test results from GLM analyses of 7-year mortality, stem growth, and fusiform rust incidence data.

Source	df	Mort.	Growth			Rust	
			Ht.	Dia.	**Vol.[a]**	Stem gall	Gall
Rep.	3	ns	**	**	**	ns	ns
Entry	14	**	**	**	**	**	**
Male	5	**	**	**	**	**	**
Female[b]	1			ns		ns	ns
Male X Female[b]	5	ns	ns	ns	ns	**	**
Slash vs. F 1		ns	ns	ns	ns	**	
Slash vs. BC	1	ns	ns	ns	ns	**	**
F 1 vs. BC	1	ns	ns	ns	ns	ns	ns
Rep. X Entry	42	ns	**	ns	ns	ns	ns
Within-plot							
Mortality	900						
Growth	633						
Rust	668						

[a] Square root of stem volume.
[b] Source of shortleaf in hybrid female: north GA vs. southeast AR (see text for details).
Notes: ns = not significant at p<.05.
* = significant at p<.05.
** = significant at p<.01.

Table 3 presents the results from the mean separation tests for mortality, volume, and rust incidence. T-tests grouped the males into two groups of three trees for both stem volume and percentage galled (gall*100). The grouping is rather distinct for percentage galled and nondistinct for volume. CFGRP breeding value data for the male clones showed two of the Coop's most resistant clones (males 1 and 4) to be included in the low-incidence group, along with an average clone (male 5). The clones grouped in the high-incidence group ranked intermediate (R40=29) to average (R40=43) in breeding value3/. A comparison of volume rankings showed little or no correlation with the Coop's breeding values. Differences between female sources were significant for volume growth, with the north Georgia source producing larger backcross and F 1 -op progenies.

The significant male X female source interaction for rust incidence is clearly evident in Table 3. For percentage galled, males 3 and 2 interact strongly with the two female sources. Male X female source interaction is essentially absent for mortality and volume. T-tests on entry means for percentage galled show six families equal in rust incidence to the slash checks and nine equal or better than the F 1 -op (Table 4). Entry means for percentage stem galled are also shown in Table 4. Few entry rank-order changes were noted, and each was contained within the upper or lower halves of each ranking (i.e., 2 to 5 and 9 to 12).

R40 is a predicted breeding value for percentage of rust incidence, scaled to an environment in which nonselected slash pine is 40% infected.

Table 3. T-test comparisons of males and female sources for 7-year mortality, stem volume, and percentage galled.

Mortality (%)

	Males							
Females	1	3	5	4	2	6	meana	wind
429 **(AR)**	13	20	17	22	22	30	21a	33
413 (GA)	13	18	23	30	38	40	27b	30
Slash								26
Mean'	13a	19ab	20abc	26abc	30bc	35c		

Volume (sq. rt.)

	Males							
Females	1	4	3	5	6	2	mean[a]	wind
413 (GA)	0.96	0.92	0.91	0.89	0.81	0.76	0.88a	0.91
429 (AR)	0.95	0.89	0.85	0.74	0.75	0.77	0.83b	0.82
Slash								0.89
Mean[a]	0.95a	0.91a	0.88ab	0.81bc	0.78c	0.76c		
Coop rank	6	3	4	1	5	2		
Coop VOL	0.18	1.13	1.08	2.60	0.26	1.34		

Gall (%)

	Males							
Females	5	1	4	6	3	2	mean[a]	wind
413 (GA)	9	8	19	39	31	78	28a	19
429 (AR)	12	15	11	31	58	30	26a	34
Slash								12
Mean[a]	10a	1 la	14a	35b	44bc	52c		
Coop rank	4	1	2	6	5	3		
Coop R40	36	7	15	43	37	29		

[a] Means followed by same letter are not significantly different (p<.05).

Notes: Coop rank is CFGRP rank of breeding values for 15-year volume and 5-year rust incidence. Low ranks indicate high volume and low rust.

Coop VOL is 15-year stem volume breeding value in cu ft deviations.

Coop R40 is 0.80 * R50 (i.e., scaling R50 to a 40% rust environment).

DISCUSSI0N

Various positive and negative aspects of this study must be accounted for in the interpretation of the results. 0n the positive side, several points can be noted, such as a moderate, well-dispersed incidence of rust, reasonably good survival and growth in four of five replications, no replication or replication X entry effects for mortality or rust incidence, highly significant male effects for all traits, and significant male X female effects for rust incidence. Negative points include one test environment, mixed mating design, lack of necessary checks (i.e., susceptible slash and shortleaf), and lack of rust data between planting and age 7 years. In general, the scope of any inferences drawn should be limited to the tested environment, including inoculum source, site conditions and climate, and to the tested parental population.

Table 4. T-test comparisons of backcross families and checks for 7-year percentage galled and backcross family and check means for percentage stem galled.

Female[a]	Male	Gall (%)[b]	**Stem** Gall (%)
413	1	8a	4
413	5	9a	7
429	4		6
429	5	12ab	1**0**
Slash	FL wind	12ab	7
429	1	15abc	13
413	4	19abc	**16**
F1	MS wind	27bcd	23
429	2	30cd	28
413	3	31cd	24
429	6	31cd	29
413	6	39d	**28**
429	3	58e	42
413	2	78f	73

[a] Each 413 is a different hybrid tree with north GA shortleaf, while each 429 is the same hybrid tree with southeast AR shortleaf (see text for details).

[b] Means followed by the same letter are not significantly different ($p<.05$).

The geographic source of shortleaf pine in the hybrid parents had a significant effect on 7-year mortality and growth, but not on rust incidence. Backcross progenies with north Georgia shortleaf pine germplasm (source 413) were larger in stem volume than those of southeast Arkansas (source 429), but they also suffered greater mortality. The larger volumes were mostly due to increased height, as source 413 backcrosses averaged 17.3 ft versus **16.6 ft** for source 429. Difference in stem diameter was not significant--3.87 inches versus 3.70 inches for sources 413 and 429, respectively. Although statistically significant for volume and mortality, the applicability of these results is limited because of the small genetic sample sizes from the two sources--six parents from source 413 and only one from source 429.

Rust incidence was primarily conditioned by the slash pine parents, and very little improvement over resistant slash was observed in the backcrosses per se. However, the incidence level in the backcross progeny of one slash clone (male 5) was markedly improved compared with its CFGRP resistance breeding value. Unfortunately, this clone's backcross progeny showed a substantial decrease in growth relative to its breeding value for volume growth. The performance of the backcross progenies of clone 1 is also worth noting. CFGRP breeding values ranked clone 1 first of the six tested in resistance and sixth in growth, yet its backcross progeny performance ranked first in growth and second in rust resistance.

The results of this study appear to be consistent with those for rust incidence in an artificial inoculation test of (shortleaf X loblolly) X loblolly backcrosses conducted by Kraus et al. (1982). In their study, backcrosses with parents of unknown resistance were not significantly less infected than nonselected loblolly pine. In contrast, La Farge and Kraus (1980) and Kraus (1986) found (shortleaf X loblolly) X loblolly backcrosses per se to be significantly less infected than loblolly pine, and in Kraus (1986) these backcrosses were not significantly more infected than shortleaf pine. As in the shortleaf-loblolly pine studies, the potential for completely recovering the height growth of the faster growing species seems promising in the BC1 generation. Thus, in theory, clonal propagules of fast-growing, resistant **BC1** individuals would constitute a desirable clonal variety of 3/4 slash:1/4 shortleaf pine. In practice, however, clonal testing would be required to ensure superior growth and resistance performances of the selected

BC1 clones. Seed-propagated hybrid varieties, such as F1-op, may have some potential (Hyun 1974); however, careful testing and parental selection would also be required to ensure consistent, superior performance.

True backcross varieties, propagated by seed or as clones, normally require several additional generations of selection and, ideally, would retain resistance genes only from shortleaf pine. The likelihood of obtaining quality parental material in a BC4 or BC5 is small due to the time required to advance a population of forest trees to this stage. However, with the help of DNA marker-assisted selection and accelerated breeding, it is likely that both the number of generations and the time to achieve one generation will be substantially reduced.

Currently, two forms of marker-assisted selection should be applicable to southern pine backcross breeding. Genomic selection (Hillel et al. 1990) uses species-specific DNA fingerprints to estimate the proportion of nuclear DNA in backcross individuals. Presuming that a qualitatively inherited trait is under selection, individuals expressing this trait are first selected, and then their DNA fingerprint is analyzed and used to select those most similar to the recurrent parent (or dissimilar to the donor). Alternatively, the DNA is first analyzed and then only trees with desirable DNA fingerprints are tested for trait expression. In either case, such selections in the BC1 are likely to contain 5% to 10% more recurrent DNA than expected. Hillel's formulae suggest that genomic selection in species such as the southern pines will identify BC2 individuals equivalent in recurrent parent DNA to a BC4 generation produced without the assistance of DNA markers.

A second DNA marker-assisted approach requires considerably more molecular genetic development and screening work but promises much greater precision. A saturated linkage map and a mapped trait are required. This approach allows marker-based selection for recurrent DNA, as in genomic selection, but additionally, it provides a means for selecting against donor DNA in the region of the selected trait loci (Young and Tanksley 1989). This added selection opportunity is very beneficial, because donor DNA adjacent to the trait loci is very difficult to select against in the absence of linked markers (Hanson 1959). 0bviously with accelerated breeding, the time for each generation is greatly reduced and the gain in time is multiplicative --the reduced number of generations X the reduced number of years per generation. This kind of time savings should increase the likelihood of successfully implementing a multigenerational backcross breeding program.

Research efforts in recurrent selection and interspecies or intraspecies breeding for fusiform rust resistance should focus on the genetic mechanisms conditioning the interaction of the host and pathogen. Although backcrosses per se offered no improvement over resistant slash pine in this study, pathogen populations may possibly adapt to the resistance factor(s) present in the slash pine. Breeders must be concerned with the rate of this adaptation, which depends to a large extent on the genetics of the host and the pathogen. Interorganismal genetic studies have shown that incompatible interactions (i.e., "resistance") may be conditioned by only one gene in the host and one in the pathogen, while several loci in each may be prevented from expression by an epistatic type of gene action (Loegering 1978). A change from avirulence to virulence at one pathogen locus would spread rapidly in the pathogen population by rendering the corresponding resistance gene in the host ineffective. In the future, breeders will likely require a diverse collection of resistance factors to maintain genetic protection from fusiform rust. Thus, incorporating and maintaining shortleaf pine resistance factors in slash pine genetic backgrounds will be a useful, if not necessary, breeding activity.

In summary, interspecies backcross breeding may prove useful in the southern pines, but several pieces of knowledge and technology, including a rigorous understanding of the inheritance of important traits, the development and practical application of DNA marker systems, and a continuing commitment to accelerated, multigenerational breeding, must be integrated.

ACKNOWLEDGMENTS

I appreciate the efforts of P.C. Wakely and 0.0. Wells for initiating and continuing this breeding experiment, R.L. Doudrick, G.R. Hodge and T. La Farge for reviewing this manuscript, and N.H. Bond and J.M. Hamaker for technical assistance and plot maintenance.

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