EARLY FIELD PERFORMANCE OF RUST-RESISTANT CLONES OF SLASH PINE: A COMBINATION OF DIRECT AND INDIRECT SELECTION

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Abstract.--Two-month old slash pine (Pinus elliottii Engelm. var. elliottii) seedlings from four known rust-resistant, full-sib families and three known rust-resistant, open-pollinated families were subjected to a standardized fusiform rust inoculation test. Based on rust infection readings taken 4 months post-inoculation, all the families showed greater resistance than the susceptible checklot ((6 SL). Five of the seven families showed greater resistance than the resistant checklot (FA2), based on their Index of Relative Resistance.

In a field study conducted near Bogalusa, Louisiana, all seedlings without galls were cloned by rooted cuttings and outplanted, along with two seedling checklots. After two growing seasons, survival averaged 95% for the rooted cuttings and 98% for the seedlings. Rust infection was 1% for the rooted cuttings compared with 43% and 29% for the resistant and susceptible seedling checklots, respectively. The rooted cuttings were smaller than the seedlings after one growing season (0.83 and 1.07 ft, respectively), but this difference can probably be attributed to differences in initial planting size. During the second growing season, both propagule types grew a similar amount in height (1.51 ft for rooted cuttings and 1.56 ft for seedlings).

Keywords: fusiform rust, Pinus elliottii Engelm. var. elliottii, rooted cuttings, rust resistance, seedlings.

INTRODUCTION

Fusiform rust, caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme, is a serious disease affecting slash pines (Pinus elliotii Engelm. var. elliottii) and other pines over much of the southeastern United States (Powers et al., 1975; Anderson et al., 1986). Tree improvement programs using genetic resistance in slash pine populations along with clonal forestry techniques offer significant promise for reducing losses caused by this devastating fungus in commercial timber plantations.

Clonal forestry is becoming increasingly important as a reforestation tool, with clonal propagation providing a means of exploiting both the additive genetic gain achieved through sexual breeding and the nonadditive genetic gain identified through testing for superior genotypes. Predicted gains from vegetative propagation techniques, however, have been based on the assumption that cuttings will perform comparably with seedlings. If cuttings do not grow as well as seadlings, predicted genetic benefits of rooted cutting programs will not be realized. Comparative field tests of rooted cuttings and seedlings from genetically related populations should provide the information necessary to assess the potential of vegetative propagules in clonal reforestation and tree improvement programs. Used together, artificial

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inoculation procedures and rooted cutting techniques may provide a powerful tool for increasing selection effectiveness and reducing the time required to enhance rust resistance in pine stands significantly.

Foster and Shaw (1987) proposed a program in which known disease-resistant parents (direct selection) of loblolly pine (P inus **teat** L.) would be mated and their seedling offspring subjected to an initial screening **for disease** resistance (indirect selection). The resistant seedlings would then be cloned and the clones planted in field trials. After a few years, the most resistant clones would be selected (direct selection) for further use in a large scale reforestation program. A subsequent study by Foster and Anderson (1989) tested the feasibility of such an approach. The resulting tree improvement program was effective in developing genotypes of loblolly pine that were highly resistant to the fusiform rust fungus, and preliminary evidence suggested that the program could be effective in providing highly rust-resistant clones for reforestation.

The objectives of this study were (1) to test the effectiveness of direct and indirect selection for fusiform rust resistance in slash pine seedlings of which the gall-free survivors were cloned and established in a field trial, and (2) to estimate genetic parameters for height growth from a clonal study with slash pine.

MATERIALS AND METHODS

Slash pine seeds for this experiment were obtainad from the Texas Forest Service and originated from three open-pollinated **and** four full-sib family seedlots (Table 1). The parents for all families were selected for superior resistance to fusiform rust based on field progeny test results. Seedlings were grown and inoculated with fusiform rust spores via a standard rust-screening process used in the U.S. Forest Service Resistance Screening Center (Anderson et al., 1983).

Table 1. Rust data for the slash pine open-pollinated and full-sib families and number of clones per family in the field study.

Family	Entry code	Resistance Screening		Clones in field test
		Index[1]		
D5PC98	1	194.00	52.3	3
D5PC286	2	136.66	56.3	7
S4PC1	3	160.08	73.2	3
D4PC13 x D5PC268	4	246.46	37.7	5
D4PC13 x S5PC3	5	166.02	41.8	4
D4PC40 x D5PC286	6	229.24	33.8	5
S4PC5 x S5PC5	7	140.26	62.2	2
6 SL [2]		83.14	76.3	
FA2 [3]		143.10	68.3	

[1] **Index** from Walkinshaw et al. (1980).
[2] Resistance Screening Center susceptible standard checklot.
[3] Resistance Screening Center resistant standard checklot.

Gall-free seedlings were cloned at 6 months of age for use in a field trial. The seedlings were shipped to Bogalusa, Louisiana and uppotted to 3-gallon pots in September 1984. Terminal buds were removed from each seedling shortly after uppotting to enhance shoot production and delay further height growth. The trees were maintained outside in a shadeframe. In May 1985, 4-inch long cuttings were collected from each seedling and were set for rooting. The cuttings were rooted by the International Forest Seed Company of Odenville, Alabama using their standard procedures (Hughes, 1987).

The rooted cuttings were received from International Forest Seed Company in late fall 1985 and were prepared for field planting. Since slash pine has little or no chilling requirement, the rooted cuttings were placed immediately in a greenhouse and grown using normal watering and fertilization procedures. The rooted cuttings were grown in 10-in^3 Leach Super Cells^{TM}. Seedlings representing two checklots were also grown in the greenhouse in similar containers. One seedlot (BRCK) came from a first-generation seed orchard owned by the Brunswick Pulp Land Company of Brunswick, Georgia and included genetically superior trees selected for growth and rust resistance. The parent trees originated in south Georgia and north Florida. The other seedlot (WOCK) was a commercial checklot from a seed production area in southeast Texas and developed by the Western Gulf Forest Tree Improvement Program for use in their progeny tests, and could be expected to show some genetic improvement.

By spring 1986 the rooted cuttings and seedlings were ready for field planting. They were planted near Bogalusa, Louisiana on May 12, 1986 at an 8 x 8-ft spacing, Two to four (mainly three) ramets per clone and twelve seedlings per seedling checklot were planted in each of two replications in a randomized complete block design. This yielded a total of four to seven (mainly six) ramets per clone and 24 seedlings per checklot. The ramets per clone or seedlings per checklot were randomized within each replication in a noncontiguous fashion.

Measurements were recorded at the end of the first and second growing seasons, and then the study was accidently burned, thereby destroying it. Total height was measured at years one and two, and presence of a rust gall was observed at year two. All galls were located on the main stem; therefore gall location data were unnecessary.

Analysis of variance of height data was computed using a model that included clones, replications, clone x replication interaction, and within-plot sources of variation (Table 2). The two seedling check lots were not included in this genetic analysis. Variation by family source was not measured because there were only two family types (full-sib and open-pollinated) and there was a small number of families per type. With family types combined, a total of 29 clones were included in the analysis. All sources of variation were considered to be random. Variance components were calculated by equating the mean squares and expected mean squares (type III , PROC GLM; SAS Institute Inc. , 1985).

Table 2. Form of the analysis of variance for analyzing height growth data of 29 slash pine clones.

Source of variation	Degrees of freedom	Expected mean squares[1]
Clones (C)	28	$\sigma^2 + 2.7\,\sigma^2_{CR} + 5.3\,\sigma^2_C$
Replications (R)	1	$\sigma^2 + 2.5\,\sigma^2_{CR} + 72.6\,\sigma^2_R$
C x R	28	$\sigma^2 + 2.7\,\sigma^2_{CR}$
Within-plot	98	σ^2
Total	155	

[1] Symbols explained in text.

Broad-sense heritabilities were calculated on an individual-ramet basis (H^2) and on a clone-mean basis (H^2_x). Selection of clones is generally based on their average performance, hence the second heritability is most appropriate.

$$H^2 = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2_{RC} + \sigma^2} \qquad (1)$$

$$H^2_x = \frac{\sigma^2_C}{\sigma^2_C + \frac{\sigma^2_{RC}}{r} + \frac{\sigma^2}{nr}} \qquad (2)$$

where

σ^2_C = variance among clones,
σ^2_{RC} = variance due to interaction between clones and replications,
σ^2 = variance among ramets within a clone and replication plot,
r = number of replications,
n = number of ramets per clone and replication.

The rust data were not subjected to statistical analysis since virtually no rooted cuttings were infected by the rust, whereas substantial numbers of both seedling checklots were infected. Most of the clones would therefore have zero values for percentage of trees with galls, while the seedlings displayed more traditional distributions of rust data values. We concluded that assumptions for analysis of variance (normal distribution of errors and equality of variances among treatments) could not be met even with data transformation.

RESULTS AND DISCUSSION

The late spring (May 12, 1986) planting of the rooted cuttings and seedlings resulted in a decrease in average height at the end of the first growing season (0.83 ft for rooted cuttings and 1.07 ft for seedlings) compared with potential first-year growth of slash pine when planted during the winter. The difference in height between propagule types was more an effect of initial planting size (i.e., rooted cuttings were shorter than seedlings) than differential growth. Although height growth during the first season was low for both propagule types, survival of the trees was very high, with 95% of the rooted cuttings and 98% of the seedlings surviving. First-year average height of the 29 clones ranged from 0.58 to 1.06 ft, while the two seedling checklots averaged 1.17 ft (BRCK) **and** 0.97 ft (WGCK).

Analysis of variance of first-year height (HT 1) of rooted cuttings revealed no significant difference among clones (Table 3). The clonal source of variation accounted for 8% of the total variation. Broad-sense heritability on an individual basis was low (H^2 = 0.08), indicating low genetic control of the trait. However, once heritability was expressed on a clone-mean basis, it increased fourfold to H_2 = 0.31.

Micro-site effects or random propagation effects were probably the cause of low heritabilities.

Table 3. Mean squares end variance components for height at ages one and two in a clonal test of slash pine.

Source of variation	Height 1			Height 2		
	Mean squares	Variance components value	Variance components X total	Mean squares	Variance components value	Variance components % total
Clones (C)	.0700*	.0047	8	.6538+	.0598	12
Replications (R)	.0237*	<0	0	.4844*	.0020	1
C x R	.0449*	<0	0	.3369*	<0	0
Within-plot	.0564	.0564	92	.4275	.4275	87

* Not significant at p = 0.05.
\+ Significant at p = 0.05.

The rooted cuttings and seedlings appeared to have become acclimated to their environment by the second growing season. Second-year height (HT2) averaged 2.34 ft for rooted cuttings and 2.63 ft for the seedlings; hence the growth increment during the second growing season was almost identical for rooted cuttings (1.51 ft) and seedlings (1.56 ft). The HT2 ranged from 1.53 to 2.91 ft for the clones and averaged 2.73 ft for BRCK and 2.53 ft for WOCK. The slightly inferior HT2 of the clones versus the seedling checklots is somewhat disappointing, especially compared with the WGCK checklot; however, the fact that the WGCK checklot is slightly genetically improved would lessen the expected difference in growth. We hypothesize that smaller planting size of the rooted cuttings continues to influence size differences at 2 years of age. The genetically superior rooted cuttings might have overtaken the WOCK checklot seedlings if the trees had survived to an older age, but there was no opportunity to test this hypothesis in the current study since they were destroyed.

The genetic analysis of height growth of the clonal portion of this study at age two is one of the largest contributions of this study. There is a distinct lack of such information in the scientific literature for slash pine. The clonal variance is significant for HT2, and this source accounts for 12% of the total variation (Table 3). Genetic control of HT2 increases, compared with HT 1, as evidenced by a larger broad-sense heritability (H^2 = 0.12). Clonal selection should be more effective at **age** two than at age one since H< = 0.43, which is substantially greeter than mean HT 1.

Even though the mean HT2 of the clones was somewhat lower than that of either of the two checklots, selection of the superior clones will lead to a reasonable level of realized genetic gain on similar sites. The **average** HT2 of the **10%** of the clones (2.87 ft) **exceeds** the HT2 of the WOCK checklot by **13%** (2.87 vs 2.53 ft) and of the BRCK checklot by **5%** (2.87 vs 2.73 ft).

The enhanced resistance to fusiform rust of the rooted cuttings versus the checklots is unequivocal. The select clones averaged only 1% rust infection at age two compared with 43% for BRCK and 29% for WOCK (Table 4). Of the 29 clones in the study, only two became infected; and in both cases a single ramet out of five or six ramets per clone was affected.

Table 4. Field rust infection data at age 2 years for 29 clones and two seedling check lots expressed as percent galled (either ramets per clone or seedlings per check lot).

Entry[1] code	Percent galled							
	Clones within family							Seedlot
	1	2	3	4	5	6	7	
1	0	0	0	0	0	NA	NA	
2	0	0	NA	NA	NA	NA	NA	
3	0	0	0	NA	NA	NA	NA	
4	0	17	0	0	NA	NA	NA	
5	0	0	0	20	0	NA	NA	
6	0	0	0	NA	NA	NA	NA	
7	0	0	0	0	0	0	0	
BRCK								43
WOCK								29

[1] From Table 1 and text (for checklots).
NA not applicable.

At least two hypotheses exist for the high level of rust resistance in the select clones. First, the combination of direct selection of superior parents and indirect selection of seedlings followed by cloning may provide a highly effective genetic improvement strategy for fusiform rust resistance. Alternatively, the selection system may be strongly augmented by rust resistance due to maturation.

Support for the efficient genetic selection alternative comes from other genetic studies with slash pine. Unfortunately, results from other clonal studies regarding rust resistance of slash pine are not available in the scientific literature; hence seedling studies must be cited. Fusiform rust resistance in slash pine appears to be under moderate additive genetic control (Layton, 1985), and there is evidence of non-additive genetic control (Layton, 1985; Sluder, 1989). In a loblolly pine study, Foster and Anderson (1989) had results very similar (almost complete resistance of rooted cuttings) to those of this study. One difference in the studies was that the loblolly pine test was conducted under artificial inoculation at the Resistance Screening Center as compared with the field test in the current study. Researchers have found the Resistance Screening Center's artificial inoculation system to be particularly effective with slash pine (Walkinshaw et al., 1980), possibly more so than with loblolly pine (Sluder and Powers, 1986). We are concerned, however, that the narrow-sense heritabilities from other studies are not high enough to explain our results. Rust resistance would have to be conditioned largely by non-additive genes in order to confirm our results under the efficient genetic selection alternative, and the few supporting studies do not confirm this.

Some evidence lends credence to the second alternative that genetic resistance is strongly augmented by maturation-induced resistance due to the propagation system. McKeand (1985) reported an unexpected increase in apparent level of maturation in a study with tissue-culture plantlets of loblolly pine even though the source tissue (cotyledons) was very juvenile. Rust resistance, for example, seemed to be enhanced in the plantlets versus seedlings from the same families. Similar evidence for either loblolly or slash pine rooted cuttings is lacking in the scientific literature. As clearly shown by Franklin (1969), Greenwood (1984), and Sweet (1973), among others, trees do mature; and trait expression changes as level of maturation changes. It has been recognized that resistance of white pines (Pinus monticola) to blister rust (Cronartium ribicola J. C. Fisch. ex Rabenh.) increases directly with host age, at least up to the age of 4 years (Bingham, 1969). The source of the slash pine cuttings in the current study was 17-month old seedlings. In a study with rooted cuttings from similar-aged loblolly pine, Foster (1988) showed no difference between growth and morphology of rooted cuttings and that of seedlings from the same families. Rust resistance data were not reported. In the current study, increased maturation of the rooted cuttings is one possible explanation for the slightly depressed growth of the rooted cuttings compared with seedlings; however, the two propagule types were not closely matched for initial size or genetic source. Therefore, close comparisons should not be made.

CONCLUSIONS

Enhanced maturation is one explanation for the large difference in rust resistance for the two propagule sources, although related studies do not provide much support for this hypothesis. A study should be conducted specifically to address this issue for rooted cuttings.

Rooted cuttings of slash pine appear to have promise as planting stock for reforestation. The selection system, combining direct and indirect selection, for choosing superior clones resulted in planting stock with superior field rust resistance. The exact mechanism, genetic versus maturation, behind this resistance is unknown; yet as long as the rooted cuttings grow normally otherwise, knowing the exact mechanism is mainly of academic interest. Early height growth is moderately controlled by genetic factors, thus leading to the possibility of reasonable levels of genetic gain by selecting superior clones.

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