

## Expected Genetic Gains and Development Plans for Two Longleaf Pine Third-Generation Seedling Seed Orchards

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**Abstract:** Selection and thinning plans were developed for two longleaf pine (*Pinus palustris* Mill.), third-generation seedling seed orchards located in southeastern Mississippi and central Louisiana. The two orchards were part of several long-term experimental field tests designed to investigate genetic variation in height growth and brown spot needle blight (caused by *Scirrhia acicola* (Dearn.) Siggers) resistance in a longleaf pine population. Phil Wakeley identified the original population in the 1920s in southeastern Louisiana and E. B. Snyder and H. J. Derr continued to advance the population through selection and breeding for early height growth and brown spot resistance. Our current results suggest that both traits can be improved by another round of selection and deployment through these third-generation seedling seed orchards. Operationally expected genetic gains range from 4.7% to 9.1% for height at age 9 years and 3.6% to 4.3% for brown spot resistance through age 4 years. These expected gains represent an approximate tripling in early height growth rate and doubling of brown spot resistance compared to the second generation.

**Keywords:** longleaf pine, grass-stage, brown spot needle blight, disease resistance.

### INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) genetics and breeding research has been ongoing since the 1950s at the Southern Institute of Forest Genetics (USDA Forest Service, Southern Research Station) near Gulfport, Mississippi. As part of this work E. B. Snyder and co-workers (Snyder 1969; Snyder and Derr 1972; Snyder et al. 1977; Snyder and Bey 1978) selected trees in local forests, made crosses among the selections to produce control- and open-pollinated progeny, evaluated the progeny in replicated field trials, and in some instances made second- and third-generation selections for advanced generation breeding and seed orchard development. One such series of experiments produced a second-generation seedling seed orchard (Study 3.10) and field tests designed for the establishment of two third-generation seedling seed orchards (Study 3.45, Part A1). These materials were derived from selections made by Wakeley (1970) in the 1920s in southeastern Louisiana. The current study continues this work with an analysis of the third-generation field tests planted at two locations. Heritabilities and expected genetic gains for the seedling seed orchards are computed and presented for both traits (height growth and brown spot resistance) based on a selection scheme that maintains a high level of genetic diversity while capturing genetic gain using within-family, within-block selection.

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## MATERIALS AND METHODS

Four 9-parent partial diallel tests were established at two field locations— Saucier, MS (Harrison Experimental Forest) and Alexandria, LA (Palustris Experimental Forest, Johnson Tract). The 36 parents were selected from replicated open-pollinated family tests growing at the same two locations (Study 3.10). Combined family and within-family selection was practiced for both brown spot resistance and early height growth. Each parent was crossed with four other parents, resulting in 18 crosses per diallel or 72 crosses for the four diallels. The field design was a reps-in-sets design, where families were replicated (i.e., blocked) within diallels (i.e., sets). Single-tree plots (one tree per family per block) were used in 24 blocks per diallel per location. Spacing was 0.91 m (3 ft) within the rows by 3.0 m (10 ft) between rows. In addition two check lots were included in each block— a bulk local source and an open-pollinated family from a known brown spot resistant selection (Wash 1-77). Trees were evaluated for brown spot infection (% leaf surface symptomatic) in years 1-4 and height in years 2-5, 9, and 18. Additional details can be found in Lott et al. (2001), except that they considered the 9 year-old trees to be 10 years old and the field design to be a randomized complete block with 4 treatments (i.e., diallels) and 24 blocks (i.e., reps) of single-tree plots.

For the purposes of this study we chose to combine the brown spot data over all 4 years providing a brown spot resistance score (BSR1-4) calculated as  $100 - (\text{mean brown spot infection over years 1-4})$ . For height growth we chose to analyze the data collected at age 9 years (HT9). This time point provided ample time for the trees to emerge from the grass stage, yet not so much time that above-ground, tree-to-tree competition would affect individual tree growth. Both traits were analyzed with a linear model using SAS Proc GLM (SAS Institute, Inc. 1990). The full model was

$$Y = L + D + L \times D + B(D) + F(D) + L \times (F(D)) + E,$$

where Y is the observed data for a tree, L is location (MS or LA), D is diallel (1-4), B(D) is block (1-24) within diallel, F(D) is full-sib family (1-18, as check lots were not included) within diallel, L x (F(D)) is location x family within diallel interaction, and E is residual error. All factors were considered random, with appropriate tests of significance constructed using Satterthwaite's approximate F-tests (Proc GLM). Variance components (V) and heritabilities ( $h^2$ ) were calculated as follows, assuming no non-additive variance (Falconer 1981)

$$V_{F(D)} = (MS_{F(D)} - MS_{L \times F(D)}) / t_2$$

$$V_{L \times F(D)} = (MS_{L \times F(D)} - MS_E) / t_1$$

$$V_E = ME_E, \text{ where}$$

$MS_i$  = mean square for the  $i^{\text{th}}$  variance source from the Proc GLM analysis

$t_2 = V_{F(D)}$  expected mean squares coefficient provided by Proc GLM, essentially number of trees per family across locations

$t_1 = V_{L \times F(D)}$  expected mean squares coefficient provided by Proc GLM, essentially number of trees per family per location

$$V_{\text{additive}} = 2 \times V_{F(D)}$$

$$V_{\text{phenotypic}} = V_{F(D)} + V_{L \times F(D)} + V_E$$

$$h^2 = V_{\text{additive}} / V_{\text{phenotypic}}$$

A reduced model, specific to the individual locations, was also used to analyze data from each location separately.

For each trait the residuals from the fitted model were studentized (mean=0, variance=1) and then added together to produce a composite variable for evaluating the trees for selection. This variable provides equal weight for selection on brown spot resistance (BSR1-4) and height growth (HT9). Expected gains from selection for each trait were calculated as

$$G = (\text{mean of selected trees} - \text{location mean}) * h^2.$$

Expected gains were calculated on individual location and over-location bases using the respective means and heritabilities (Falconer 1981). For each block, we evaluated within-family selection using various selection intensities with and without restrictions based on relatedness or tree-to-tree spacing. Finally, the data provided an opportunity to track the mean performance of this population in the previous (second), current (third), and next (fourth) generation, thus we calculated those means (observed and expected) using height and brown spot resistance at age 3 years (HT3, BSR3).

## RESULTS AND DISCUSSION

Trees at the Mississippi location (MS) grew taller and suffered less brown spot disease than those at the Louisiana (LA) location. The check lots performed as expected, with the resistant family showing less brown spot infection in year 3 than the susceptible source at both locations (44% vs. 55% infection at MS and 44% vs. 50% at LA). These results also indicate that both field sites provided a good test of brown spot resistance. Full-sib family differences were highly significant ( $p \leq 0.001$ ) for both traits (BSR1-4 and HT9) as were Location\*Family interactions, suggesting that families should be selected for deployment based on their performance at the individual locations.

Individual-tree heritabilities for both traits by and over locations are shown in Table 1. The heritabilities for HT9 by locations are more than twice as large as the heritability over locations, again indicating the significance of the Location\*Family interaction. Over-locations heritability of BSR1-4 was slightly larger than that for HT9 (0.16 vs. 0.12). The BSR1-4 heritabilities were different between sites, 0.37 at MS vs. 0.29 at LA. These results (age 3 growth and brown spot infection levels and heritabilities at the respective locations and the Location\*Family interaction for both traits) were very similar to Synder and Derr's (1972) results with this population at these two test locations in the previous generation.

Expected gains for each trait from selection on the combined trait index (HT9 + BSR1-4) are shown in Table 2. The observed number of families and the maximum number of trees per family selected at each location are given in Table 3. Selection intensities from 1/18 (6%) to 3/18 (17%) on a per-block basis were considered with and without restriction on tree-to-tree spacing and relatedness. The spacing consideration consisted of not taking an otherwise selected tree if it was within the same row and within two positions (i.e.,  $\leq 1.92$  m or 6 ft) of a previously selected tree. The relatedness consideration eliminated an otherwise selected tree if it shared a parent with a previously selected tree. In all cases selections were made and restrictions were applied within the 18-tree blocks (check lots were not considered), keeping the tree(s) with the largest residual value(s).

Table 1. Individual-tree heritabilities for tree height at age 9 years (HT9) and brown spot resistance over years 1 to 4 (BSR1-4) at the Mississippi and Louisiana field locations and both over locations.

| Trait  | Location    |           |         |
|--------|-------------|-----------|---------|
|        | Mississippi | Louisiana | Overall |
| HT9    | 0.31        | 0.29      | 0.12    |
| BSR1-4 | 0.37        | 0.29      | 0.16    |

Note: Heritability estimates assume no non-additive variance.

Table 2. Expected gains in height growth (HT9: %, cm) and brown spot resistance (BSR1-4: %, points) from selection at the Mississippi and Louisiana field locations and over both locations.

| Trait                        | Location                 |            |                        |            |                      |            |
|------------------------------|--------------------------|------------|------------------------|------------|----------------------|------------|
|                              | Mississippi <sup>a</sup> |            | Louisiana <sup>a</sup> |            | Overall <sup>b</sup> |            |
| No. trees selected per block | Unrestricted             | Restricted | Unrestricted           | Restricted | Unrestricted         | Restricted |
| 1                            | 14.3, 64                 | --,--      | 29.2, 89               | --,--      | 8.1, 31              | --,--      |
| 2                            | 13.1, 59                 | 12.7, 57   | 26.4, 81               | 23.3, 71   | 7.3, 28              | 6.9, 26    |
| 3                            | 12.8, 58                 | 12.2, 55   | 25.2, 77               | 22.2, 68   | 7.1, 27              | 6.6, 25    |

  

| BSR1-4                       | Mississippi <sup>a</sup> |            | Louisiana <sup>a</sup> |            | Overall <sup>b</sup> |            |
|------------------------------|--------------------------|------------|------------------------|------------|----------------------|------------|
|                              | Unrestricted             | Restricted | Unrestricted           | Restricted | Unrestricted         | Restricted |
| No. trees selected per block | Unrestricted             | Restricted | Unrestricted           | Restricted | Unrestricted         | Restricted |
| 1                            | 9.3, 6.8                 | --,--      | 9.4, 6.3               | --,--      | 4.8, 3.4             | --,--      |
| 2                            | 8.9, 6.5                 | 8.5, 6.2   | 8.9, 5.9               | 8.0, 5.3   | 4.5, 3.2             | 4.2, 3.0   |
| 3                            | 8.6, 6.3                 | 8.2, 6.0   | 8.4, 5.6               | 7.6, 5.1   | 4.4, 3.1             | 4.1, 2.9   |

Notes: <sup>a</sup> Gains for individual locations are based on the assumption that seeds collected at a location are planted on sites that represent that location. <sup>b</sup> Overall gains are based on the assumption that seeds collected at both locations are planted on sites that represent both locations.

Advancing from 2 to 3 selected trees per block had a similar effect on expected gain as going from unrestricted to restricted selection of 2 trees per block. In either case expected gain reductions were not large. A similar trend is noted for number of families selected and maximum number of trees selected per family. Selecting at least 2 trees per block substantially increases the number of families represented in the selected populations (i.e., seedling seed orchards), but has a relatively small effect on expected genetic gain. Given the current development of the stand to age 18 years, we intend to complete the first thinning leaving 2-3 trees per block. Table 4 provides the operational expected gains based on selecting 3 trees per block with the tree-to-tree spacing and relatedness restrictions employed. These gains apply to planting at both sites while collecting seed from one site or the other. As noted above, additional gains can be made by deploying seedlings to sites that are most similar to the test location where the seed is collected.

Table 3. Number of families represented among selected trees at the Mississippi and Louisiana field locations and the maximum number of trees per family selected.

|                              | Location     |            |              |            |              |            |
|------------------------------|--------------|------------|--------------|------------|--------------|------------|
|                              | Mississippi  |            | Louisiana    |            | Overall      |            |
| No. trees selected per block | Unrestricted | Restricted | Unrestricted | Restricted | Unrestricted | Restricted |
| 1                            | 44, 6        | --,--      | 53, 4        | --,--      | 61, 10       | --,--      |
| 2                            | 58, 8        | 64, 7      | 64, 7        | 62, 6      | 70, 12       | 70, 12     |
| 3                            | 66, 9        | 66, 10     | 68, 8        | 66, 12     | 70, 16       | 69, 18     |

Table 4. Operational expected gains in height growth (HT9: %, cm) and brown spot resistance (BSR1-4: %, points) from selection of 3 trees per block, and the number of families and maximum number of trees per family represented in the selected populations.

| Trait                        | Location / Selection Scheme |                        |
|------------------------------|-----------------------------|------------------------|
|                              | Mississippi / Restricted    | Louisiana / Restricted |
| HT9                          | 4.7, 21.2                   | 9.1, 27.7              |
| BSR1-4                       | 3.6, 2.6                    | 4.3, 2.9               |
| No. Families                 | 66                          | 66                     |
| Max. No. of Trees per Family | 10                          | 12                     |

Data provided by Snyder and Derr (1972) for the second generation of this population allow us to evaluate the effectiveness of selection and to develop a progression of observed and expected mean performances over generations (Table 5). The first-generation parents were for the most part selected in the natural forests of southeastern Louisiana (Lott et al. 2001). Open-pollinated progeny of these selections were field tested at Saucier, MS and Alexandria, LA and they represent the second generation (Study 3.10). Selections made in these tests and mated in the diallel tests described here (Study 3.45 Part A1) produced the third generation that was also evaluated at Saucier and Alexandria. Projecting the fourth generation's performance (output of the new seedling seed orchards) on representative sites using our expected gain calculations suggests that early height growth and brown spot resistance (both through year 3) will be tripled (HT3 = 40 cm vs. 12 cm) and doubled (BSR3 = 73% vs. 37%), respectively, compared to the observed performance in the second generation.

Table 5. Four generations of improvement in the southeast Louisiana longleaf pine population: Observed generation means (age 3) for generations 2 and 3 at Saucier, Mississippi, and Alexandria, Louisiana, and the expected means at both locations for generation 4.

| Location    | Trait                            | Generation               |                          |                          |
|-------------|----------------------------------|--------------------------|--------------------------|--------------------------|
|             |                                  | Observed 2 <sup>nd</sup> | Observed 3 <sup>rd</sup> | Expected 4 <sup>th</sup> |
| Mississippi | HT3 (cm)                         | 16                       | 40                       | 47                       |
|             | BSR3 (% needles non-symptomatic) | 29                       | 70                       | 76                       |
| Louisiana   | HT3 (cm)                         | 8                        | 26                       | 34                       |
|             | BSR3 (% needles non-symptomatic) | 45                       | 64                       | 70                       |

## CONCLUSION

For seedling seed orchard development, we decided to emphasize genetic diversity over gain, and thus used within-family, within-block selection such that no families were purposely culled based on low mean performance. Two to three trees were selected per test block. The first tree had the highest performance (brown spot resistance and height growth) relative to its expected family mean. The second and third trees were the next highest, unrelated to the first tree, and separated by at least two within-row planting positions.

Most (66 of 72) of the full-sib families were represented in the selected population at each location, with an average of about 4 or 5 trees per family. Heritabilities (individual tree basis, across locations) for the two traits were 16% for brown spot resistance (over years 1 to 4) and 12% for height at age 9 years. Operationally, the expected genetic gains for resistance are 3.6% and 4.3% and for height are 4.7% and 9.1% at the Mississippi and Louisiana locations, respectively. The expected levels of brown spot resistance in the next generation exceed 70% (i.e., less than 30% symptomatic leaf area), which is nearly a doubling of resistance over the initial tested generation.

## ACKNOWLEDGMENTS

We thank A. G. Kais for his work with Snyder in establishing Study 3.45 and Jim Hamaker for his efforts in producing the plant materials. We also thank J. H. Roberds and M. Stine for useful discussion and comments. Added post-SFTIC, while in review: In honor of those affected by Katrina and Rita we dedicate our efforts and the new seed orchards to restoring the great longleaf pine forests of southern Mississippi and Louisiana.

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