# The Efficacy of Breeding for Brown Spot Disease Resistance in Longleaf Pine

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Abstract:-- The study objective was to determine whether selection for brown spot disease (caused by Scirrhia acicola (Dearn.) Siggers) resistance in longleaf pine (Pinus *palustris* Mill.) is beneficial for areas where brown spot is not present. Two groups of selections, comprising those that performed (survival and growth) well in the presence of brown spot disease and those that performed well in its absence, were selected. These selections were made in tests planted on the Harrison Experimental Forest (HEF) in southeast Mississippi. Within selection groups, the selections were mated in a partial diallel and their progeny were planted in replicated tests on two sites at the HEF. At one site, all trees were sprayed with a fungicide to protect the trees from brown spot disease, while at the other site no protection was provided. Brown spot infection was assessed one year after planting, and survival and height were assessed at years 1, 2, 3, 4, and 7. Overall, survival was significantly lower and disease incidence higher at the unsprayed site. At 7 years, survival at the unsprayed site was 73% for families selected in the presence of brown spot and 59% for the families selected in the absence of brown spot. Brown spot infection was significantly lower in the families selected in the presence of brown spot when planted at the unsprayed site, indicating that selection for brown spot resistance was effective. At 7 years, families selected in the presence of brown spot were significantly taller at the unsprayed site, but were significantly shorter at the sprayed site. Thus, selection for brown spot resistance is beneficial for those areas where brown spot disease is present, but not for areas where brown spot is controlled or absent.

Keywords: Brown spot needle blight, disease resistance, Pinus palustris.

# **INTRODUCTION**

Despite longleaf pine (*Pinus palustris* Mill.) being more resistant to all the major insects and diseases that affect other southern pines, its wide planting and regeneration is limited by brown spot disease caused by *Scirrhia acicola* (Dearn.) Siggers. Brown spot attacks seedlings in the nursery and young seedlings in the field during the grass stage, causing mortality, delaying the initiation of rapid growth and generally leading to reduced growth at maturity (Boyer 1990). Infected seedlings grow slowly and may remain in the grass stage for 10 years or more, while non-infected seedlings remain in the grass stage for only one or two years (Boyer 1990; Phelps and others 1978). Once out of the grass stage, longleaf pine is no longer susceptible to brown-spot and has many desirable traits such as excellent stem form and good wood qualities that make it a highly valued species.

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Brown spot can be controlled by proper silvicultural practices, but breeding for disease resistance offers a more permanent solution and may be economical in controlling the disease. Genetic improvement will not only improve resistance to brown spot but also increase survival and growth (Snyder and others 1977). The limited information on breeding for brown spot resistance in longleaf pine suggests that selection for brown spot resistance can be effective. For example, Snyder and Allen (1968) found that provenances differed in resistance to brown spot disease, and Byram and Lowe (1985) and Snyder and Derr (1972) found high heritability estimates for brown spot disease resistance. The study objective was to determine if selection for resistance is beneficial for those areas where brown spot is not present.

# MATERIALS AND METHODS

# <u>Treatments</u>

Two groups of tress that performed well for survival and height growth were selected in tests planted on the Harrison Experimental Forest (HEF) in southeast Mississippi. Family plus within-family selection was applied to obtain (1) nine unrelated trees that performed well in the absence of brown spot disease and (2) nine unrelated trees that performed well in the presence of brown spot. The first group was selected in tests sprayed with a fungicide to protect the trees from brown spot disease while the second group was selected in unsprayed tests. Both groups were selected from tests on the HEF; however the second group included family information for performance (survival and height) in tests in Alexandria, Louisiana as well as the HEF (see Lott and others 2001 and Synder and Kais unpublished<sup>1</sup> for details).

Each of the nine trees was control pollinated with from one to four different trees (partial diallel) within the two selection groups giving a total of 18 full-sib families per group. The set of 18 families within each group is hereafter referred to as a treatment, where treatment 1 (T1) is families whose parents were selected in the absence of brown spot disease and treatment 2 (T2) is families whose parents were selected in the presence of brown spot. Two open-pollinated families (one known to be resistant and one susceptible to brown spot) were added to each treatment at each site to serve as controls. The susceptible control was similar to the material commonly used for planting in southeast Mississippi.

# Field tests

Seeds were sown in a greenhouse at the HEF in 1982. Two field tests were established in 1984 on the HEF. At one site (S1) all trees were sprayed with Bordeaux fungicide according to label to protect the trees from brown spot disease while at the other site (S2)

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no protection against brown spot was provided. The field test design was a randomized complete block using 24 replications of single-tree plots at each site. Seedlings were evaluated for brown spot infection in the field one year after planting. Brown spot infection was scored visually for each seedling as a proportion of total needle tissue showing signs of brown spot disease. Survival and total height were assessed at ages 1, 2, 3, 4 and 7 years after planting.

#### <u>Analyses</u>

To test the significance of site, treatment and family, and their interactions, data were pooled across sites and analysed using the general linear model (GLM) procedure of SAS version 6.03 (SAS Institute, Inc. 1985). The following linear model was used for the analyses:

$$Y_{ijklm} = \mu + S_i + R_{j(i)} + T_k + ST_{ik} + F_{l(k)} + SF_{il} + \varepsilon_{ijklm}$$
[1]

where:

 $\mathbf{Y}_{ijklm}$  = is the observation on the m<sup>th</sup> tree in the i<sup>th</sup> site in the j<sup>th</sup> replicate in the k<sup>th</sup> treatment and l<sup>th</sup> family,

$$\begin{split} & \mu \ = \text{overall mean}, \\ & S_i \ = \text{random effect of the } i^{th} \text{ site}, \\ & R_{j(i)} = \text{random effect of the } j^{th} \text{ replicate within site}, \\ & T_k = \text{random effect of the } k^{th} \text{ treatment}, \\ & F_{l(k)} \ = \text{random effect of the } l^{th} \text{ family within treatment}, \\ & ST_{ik} = \text{random interaction effect of the site and treatment}, \\ & SF_{il} = \text{random interaction effect of the site and family, and} \\ & \epsilon_{ijklm} = \text{ residual}. \end{split}$$

All means reported are least squares means (SAS Institute, Inc. 1985) adjusted for missing values. Survival data were converted to 0,1 (dead, alive) scale prior to analysis. To determine if early assessments were good predictors of later assessments and to determine relationships between different traits family mean correlations were estimated as product-moment correlations using PROC CORR procedure in SAS (SAS Institute, Inc. 1985).

#### RESULTS

#### <u>Survival</u>

Sites, treatments and families differed significantly in survival at all ages (P < 0.05), except sites at ages 3 and 4 years, and treatments at age 7 (Table 1). Interactions between site and treatment, and site and family were only significant at ages older than 3 years.

Survival at age 1 year was high (> 80%) at both sites. Survival progressively declined with time at the unsprayed site (S2). At 7 years, survival declined to 66% at the

unsprayed site, but was unchanged at the sprayed site (S1), suggesting that spraying against brown spot improves survival especially when considering older ages. At 7 years, survival at the unsprayed site was 73% for families selected in the presence of

brown spot and 59% for the families selected in the absence of brown spot (S2-T2 vs. S2-T1, Figure 1). This represents a 24% improvement in survival due to both selecting parents and planting their progeny in the presence of brown spot disease. In contrast, families derived from parents selected in the absence of brown spot had better survival in the sprayed test, supporting the significant site x treatment interaction. The advantage of planting resistant families in unsprayed test was quite apparent at age 7 years (Figure 1).

### Brown spot infection

Significant differences (P < 0.05) were observed for brown spot infection among sites, treatments and families at age 1 year (Table 1). Brown spot infections were much lower at the sprayed site (S1; 2%) than the unsprayed site (S2; 43%), indicating that fungicide treatment offers effective protection against brown spot disease. The absolute levels of brown spot infections at the unsprayed site were higher in families whose parents were not specifically selected for brown spot resistance (T1; 51%) than in families whose parents were selected for resistance (T2; 34%) (Figure 2). The realized gain in disease resistance at this site is 41%, indicating that breeding for resistance to brown spot disease is effective. Realized gain in resistance estimated at 4 years using data from a previous study (Lott and others 2001) was in close agreement with findings in this study (realized gain was 38% at Alexandria and 47% at the HEF).

# <u>Height growth</u>

Sites, treatments and families differed significantly in height growth at all ages (P < 0.05), except for treatment at ages 2, 3, and 7 years (Table 1). Interactions between site and treatment, and site and family were significant at all ages (P < 0.05). Trees planted at the sprayed site (S1) grew better than those at the unsprayed site (S2) at ages older than 1 year. At 7 years of age, trees at the sprayed site averaged 6 meters in height while those at the unsprayed site were only 3.8 meters in height.

At 7 years, families from parents selected in the presence of brown spot (T2) were significantly taller at the unsprayed site (S2), but were significantly shorter at the sprayed site (S1) than families selected in the absence of brown spot (T1) (Figure 3). At 7 years of age, families selected in the presence of brown spot averaged 4.4 meters in height while those selected in the absence of brown spot were 3.4 meters in height at the unsprayed site, indicating a realized gain of 29.4%. In contrast, at the sprayed site, families selected in the presence of brown spot averaged 5.6 meters in height while those selected in the presence of brown spot averaged 5.6 meters in height while those selected in the absence of brown spot averaged 5.6 meters in height while those selected in the absence of brown spot averaged 5.6 meters in height while those selected in the absence of brown spot averaged 5.6 meters in height at the unsprayed site, families selected in the presence of brown spot averaged 5.6 meters in height while those selected in the absence of brown spot were 6.4 meters in height at 7 years. This indicates that there is a 12.5% cost for breeding for resistance if planting on a site where brown spot is not present. Thus, selection for brown spot resistance is beneficial for those areas where brown spot disease is present, but not for areas where brown spot is controlled or absent. Our findings support those of Snyder and Derr (1972) and Snyder and Bey (1978) who reported a poor relationship between family height growth on sprayed and

Age	Source of	DF	Survival	HT (x10 <sup>-2</sup> )	BS (x10 <sup>-3</sup> )
-	variance				
1 year	Site (S)	1	1.69***	33.36***	542.88***
	Rep (R)	46	0.23***	0.49**	0.48*
	Treatment (T)	1	1.67***	5.41***	25.4***
	Family (F(T))	35	0.35***	1.01***	2.23***
	S*T	1	0.23ns	17.75***	24.27***
	S*F(T)	35	0.10ns	0.58**	1.71***
	Residual	1447	0.11	0.27	0.36
2 years	Site (S)	1	0.89**	224.06***	-
5	Rep (R)	46	0.27***	15.50**	-
	Treatment (T)	1	2.14***	1.12ns	_
	Family (F(T))	35	0.33***	39.43***	_
	S*T	1	0.13ns	552.53***	_
	S*F(T)	35	0.11ns	20.60***	_
	Residual	1447	0.12	7.92	-
3 vears	Site (S)	1	0.28ns	65.58***	_
- <b>J</b>	Rep (R)	46	0.30***	27.79**	_
	Treatment (T)	1	1.45***	0.43ns	_
	Family (F(T))	35	0.37***	58.19***	_
	S* T	1	0.25ns	20.49***	_
	S*F(T)	35	0.15ns	40.34***	-
	Residual	1447	0.13	0.33	-
4 vears	Site (S)	1	0.12ns	238.61***	-
<b>J</b>	Rep (R)	46	0.39***	1.38**	_
	Treatment (T)	1	1.44**	6.44**	-
	Family (F(T))	35	0.38***	4.31***	_
	S* T	1	0.57*	80.58***	_
	S*F(T)	35	0.21*	3.04***	_
	Residual	1447	0.14	0.86	-
7 years	Site (S)	1	5.19***	925.84***	_
	Rep (R)	46	0.33**	4.54**	_
	Treatment (T)	1	0.17ns	4.67ns	_
	Family (F(T))	35	0.48***	16.89***	-
	S* T	1	4.90***	166.11***	-
	S*F(T)	35	0.36***	12.82***	_
	Residual	1447	0.17	2.82	-

unsprayed sites. The realized gain in height (57%) was lower than that predicted using data from a previous study (178%) reported by Lott and others (2001).

**Table 1.** Mean squares for analysis of variance for survival (0,1 scale), brown spot (% infected needles), height (HT, cm for ages 1 and 2 and m for ages 3, 4 and 7) assessed at two sites on the Harrison Experimental Forest. Note: ns, \*, \*\*, \*\*\* = not significant at P < 0.05, significant at P < 0.05, P < 0.01, and P < 0.001, respectively.



**Figure 1.** Average survival of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed test S1 and unsprayed test S2).



**Figure 2.** Brown spot infection of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed S1 or unsprayed S2).



**Figure 3.** Height growth of two treatments (parents selected in sprayed tests T1 or unsprayed tests T2) at two sites (sprayed S1 or unsprayed S2).

### Family mean correlations

Survival assessments at different ages were moderate to highly correlated at the phenotypic level ( $r_p \ge 0.68$ ) (Table 3), indicating that the families that had good survival at the early ages had good survival in the field at older ages. Brown spot infection at one year and survival were negatively correlated, and as age difference between brown spot and survival assessments increased so did the negative correlation.

Height assessments at ages 2 years and older were also highly correlated at the phenotypic level ( $r_p \ge 0.69$ ) (Table 4), but correlation between ages 1 and 7 year heights was low ( $r_p = 0.34$ ) (Table 4). Thus, early assessments of height at ages greater than 1 year may be good indicators of height at older ages. Our results are consistent with those of Lott and others (2001) who found a high correlation between 2 and 10-year heights ( $r_p = 0.76$ ).

	Surv1*	Surv2	Surv3	Surv4	Surv7	
Surv2	0.95					
Surv3	0.90	0.95				
Surv4	0.82	0.86	0.90			
Surv7	0.68	0.72	0.74	0.79		
BS1	-0.07	-0.15	-0.25	-0.33	-0.58	

**Table 3.** Phenotypic correlations between survival and brown spot infection \*Surv = survival, BS = brown spot, HT = height, number is age in years.

	HT1*	HT2	HT3	HT4	HT7
HT2	0.69				
HT3	0.53	0.91			
HT4	0.47	0.84	0.95		
HT7	0.34	0.69	0.84	0.92	
BS1	-0.08	-0.49	-0.67	-0.74	-0.79

**Table 4.** Phenotypic correlations between brown spot infection and height \*HT = height, BS = brown spot, HT = height, number is age in years.

### CONCLUSION

Survival, resistance to brown spot infection and height growth at the unsprayed site were higher in the families selected in the presence of brown spot than those selected in the absence of brown spot. Conversely, when families were planted at the fungicide-sprayed site, height growth was higher in the families selected in the absence of brown spot than those selected in its presence. Thus, selection for resistance is not beneficial for those areas where brown spot is not present, but is beneficial for those areas at which brown spot is present. For effective breeding, selections should therefore be made in a representative environment because selections made in one selection environment are not necessarily the best in an alternative environment.

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#### LITERATURE CITED

- Bey, C. F. and E. B. Snyder. 1978. Genetic gains through testing and crossing longleaf pine plus trees. USDA Forest Service, Res. Note SO-241, 5 p.
- Boyer, W. D. 1990. *Pinus palustris* Mill. Longleaf Pine. Silvics of North America: I. Conifers. Technical Coordinators R. M. Burns and B. H. Honkala. USDA Forest Service, Agric. Handbook 654, pp. 405-412.
- Byram, T. D. and W. J. Lowe. 1985. Longleaf pine tree improvement in the western gulf region. Proc. 18<sup>th</sup> South. Forest Tree Improv. Conf., pp. 78-87.
- Lott, L., D. P. Gwaze and F. E. Bridgwater. 2001. Selection for height growth of longleaf pine in the presence of brown spot disease. Proc. 26<sup>th</sup> South. Forest Tree Improv. Conf., pp. 125-133.
- Phelps, W. R., A. G. Kais and T. H. Nicholls. 1978. Brown-spot needle blight of pines. USDA Forest Service, Forest Insect and Disease Leaflet No. 44, 7 p.
- SAS Institute Inc. 1985. SAS Language Guide for Personal Computers, Release 6.03 Edition. SAS Institute, Inc., Cary, NC, 558 p.
- Snyder, E. B. and R. M. Allen. 1968. Mountain longleaf pine excels only in local plantings. USDA Forest Service, Res. Note SO-83, 4 p.

- Snyder, E. B. and C. F. Bey. 1978. Progeny testing longleaf pine at two locations. USDA Forest Service, Res. Note SO-240, 4 p.
- Snyder, E. B. and H. J. Derr. 1972. Breeding longleaf pines for resistance to brown spot needle blight. Phytopathology 62: 325-329.
- Snyder, E. B., R.J. Dinus and H. J. Derr. 1977. Genetics of longleaf pine. USDA Forest Service, Res. Paper WO-33, 24 p.