COMPARISON OF PLOT DESIGNS FOR SHORT-TERM GENETIC TRIALS

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Abstract.--Consistant reports from retrospective early genetic trials suggest height or height increment measured in closely-spaced trials can be used to screen families by age 3-years. Closely-spaced trials come into intense tree-to-tree competition and competitive stress may bias selection and parameter estimates. In our closely-spaced plot design study, bias from inter-family competition was not apparent, even when families from different seed sources were interplanted. Block plots were found to be unnecessary, row and multiple single-tree plots provided similar mean family rankings and multiple single-tree plots appeared slightly more efficient in short-term genetic trials.

Keywords: Inter-family competition, genetic parameter estimates, family by plot design interactions, Pinus taeda L.

INTRODUCTION

There are increasing reports of high positive correlations between seedling height and mature-tree height (Franklin 1983, Campbell et al. 1985, Magnussen and Yeatman 1986, Williams 1987, Stonecypher pers. comm.). The trend among some species, including loblolly pine, is for juvenile-mature correlations to become increasingly positive as seedling height increases (e.g. Fig. 1). This trend from negative to positive correlations has been attributed to substantial changes in seedling ontogeny 1987).

Most reports come from studies planted on agricultural sites where intensive site preparation has improved progeny testing resolution. The best results have been achieved with a minimum square spacing of 33 to 50 cm; a survey of North Carolina loblolly families indicates age-age correlations are negligible at spacings lower than 10 cm (Wearstler 1979, Fig. 1). Close spacing reduces area required for testing and site heterogeneity (Rehfeldt 1983); it may also aid trait expression in other ways (Franklin 1983).

These closely-spaced tests experience intense tree-to-tree competition early and there is a genetic component to competition which may bias genetic parameter estimates and selection (Adams 1980, Shimizu 1985, Tuskan and van Buijtenen 1986). Competition among genetic entries is likely to increase the risk of making incorrect selections in closely-spaced short-term trials. Gain estimation may also be biased by intense inter-family, inter-genotypic competition.

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Planting families in blocks reduces bias from inter-family competition but at a cost. Block plots require more replications to achieve the same degree of resolution as row or single-tree plots. Increasing the number of replications within a test substantially increases testing costs and may increase site heterogeneity. Can smaller plot designs in closely-spaced short-term genetic trials be used without biasing selections? Our objective is to compare plot designs from a closely-spaced study for similarities in mean family ranks and genetic parameter estimates.

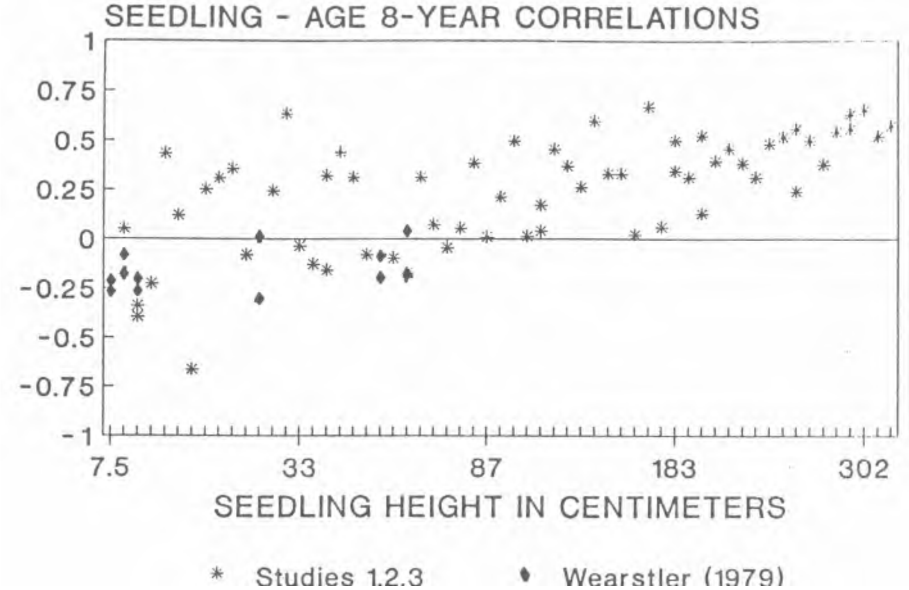

Figure 1. Retrospective early testing studies using Weyerhaeuser's first-generation open-pollinated families. All tests were planted on agricultural sites at close spacings. Data were used from Wearstiler (1979), Franklin (1983), Williams (1987) and an unpublished Weyerhaeuser study. Eighth-year field test data are described in Lambeth and Duke (1981).

MATERIALS AND METHODS

In March 1980 a study was installed in five replications of four plot designs: 60 x 60 cm row, 60 x 60 cm multiple single-tree (a.k.a. non-contiguous), 60 x 60 cm block and 120 x 120 cm row plots; each containing nine measurement trees on an agricultural site located at Magnolia, Arkansas. The nine measurement trees in the block plots were surrounded by two buffer rows which were not measured. The terminal trees at the end of each row plot were not used in the analysis. To obtain equal sample size two trees were randomly discarded from each multiple single-tree and block plot.

Height was measured in December 1981, November 1982 and December 1983. Height was used to detect the onset of competition by comparing growth in the 120 x 120 cm and 60 x 60 cm row plots. The 120 x 120 cm plots were not used in any other analysis. Plot means from the 60 x 60 cm plots were used 1) to estimate Pearson's product-moment correlation coefficients and 2) in family by plot design factorial analysis of variance based on the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{kj} + \alpha\beta\gamma_{ijk} \qquad (1)$$

where, μ = overall mean,
α_i = replication effects, $i = 1,2,\ldots.r$,
β_j = family effects, $j = 1,2,\ldots\ldots.f$, and
γ_k = plot design effects, $k = 1,2,\ldots\ldots p$.

Individual tree values were used to estimate variance-components and intraclass correlations for each plot design based on the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{k(ij)} \qquad (2)$$

where, μ = overall mean,
α_i = replication effects, $i = 1,2,\ldots\ldots r$,
β_j = family effects, $j = 1,2,\ldots.f$, and estimates 1/4 additive genetic variance, and
$\varepsilon_{k(ij)}$ = within plot error, $k = 1,2,\ldots\ldots s$ for each ij, and estimates environmental variance, 3/4 additive genetic variance and dominance genetic variance.

Phenotypic variance was estimated as family variance + within-plot variance + family by replication variance.

Families represented half-sib, open-pollinated female families from five separate seed sources. There were seven families from coastal North Carolina, three from Piedmont, North Carolina, three from north Louisiana/south Arkansas, one from north Mississippi and one from east Texas. These seed sources were intentionally selected to exaggerate differences in growth and inter-family competition.

RESULTS

Inter-family competition began influencing seedling growth between the second and third measurement dates based on height growth of the 120 x 120 cm versus 60 x 60 cm row plots (Table 1). Harms and Langdon (1976) likewise reported height reductions across similar spacings.

Differences among families and among 60 cm square plot designs were significant at each measurement date (Table 2). Thus, differences among families were detectable in all plots and plot design influenced the absolute expression of mean family height. Row plots produced the tallest trees, followed by multiple single-tree and then block plots (Table 1). In contrast, Hart (1986) found no difference in height among various plot designs at conventional spacings. The differential performance of the plot designs tested in the close-spaced study merits further study, yet in reference to early genetic trials, plot design did not affect mean family rank. There were no significant family by plot design interactions for total height or third-year incremental height at the 60 x 60 cm spacings (Table 2).

The absence of family rank changes across plot designs was confirmed by Pearson's product-moment correlation coefficients. Mean family heights were positively correlated among all plot designs at each measurement date (Table 3). In general, the correlation coefficients increased between the first and second measurements, then decreased slightly at the third measurement. The decrease in the correlation coefficients at the third measurement date corresponds to the time when competitional effects were detected for height.

Table 1. Height over time by plot design for multiple single-tree, row and block plots containing 15 open-pollinated families representing 5 seed sources of loblolly pine grown at close spacings on an agricultural site.

Plot Design	Planting Height	1st-Year Height	2nd-Year Height	3rd-Year Height
	– – – – – – – –	meters	– – – – – – – –	
120 x 120 cm Row	0.18 a[1]	0.46 a	1.76 a	3.16 a
60 x 60 cm Row	0.17 a x[2]	0.46 a x y	1.76 a x	3.04 b x
60 x 60 cm Block	0.17 x	0.45 y	1.70 y	2.96
60 x 60 cm Multiple Single-tree	0.17 x	0.47 x	1.73 x Y	2.99 x y

[1] Means within columns for 120 x 120 cm and 60 x 60 cm row plots connected by a similar letter are not significantly different based on a t-test at alpha < 0.05.

[2] Means within columns for 60 x 60 cm plots connected by a similar letter are not significantly different based on Waller-Duncan's test at alpha < 0.05.

Table 2. Partial R^2 from family by plot design factorial analysis of variance for plot mean height over time.

Source of Variation	df	1st-Year Height	2nd-Year Height	3rd-Year Height	3rd-Year Increment
Replication (R)	4	.171	.155	.213	.291
Plot Design (P)	2	.025*	.018*	.016*	.007
R x P	8	.103	.081	.098	.110
Female (F)	14	.171**	.221**	.229**	.132**
R x F	56	.135	.154	.115	.097
P x F	28	.075	.054	.069	.073
Error	112	.318	.318	.260	.290

* and ** indicate significance based on F-test results at alpha < 0.05 and 0.01, respectively.

Table 3. Pearson's correlation coefficient (r) among plot designs for family mean height over time.

	1st-Year Height	2nd-Year Height	3rd-Year Height	3rd-Year Increment
Multiple Single-tree vs. Row	.62[1/]	.82	.71	.73
Multiple Single-tree vs. Block	.66	.68	.54	.53
Row vs. Block	.56	.69	.69	.70

[1/] r-values > .51 and .63 are significantly greater than zero at alpha < 0.05 and 0.01, respectively.

The magnitude of the variance estimates for additive genetic, error (within-plot) and total phenotypic variances varied among plot designs. At the first measurement date, additive variance estimates were largest for the multiple single-tree plots, and at the third measurement date additive variance estimates were largest for the block plots. The multiple single-tree plot had the largest error estimates of the three plot designs at each measurement date. Estimates of error variances for the row and block plots were similar. The difference in the size of the estimates of error variances between multiple single-tree and row or block plots were less at close-spacings than that reported by Loo-Dinkins and Tauer (1987) at conventional spacings.

Changes over time in parameter estimates were generally similar among the three plot designs (Table 4). For example, estimates of phenotypic variances increased over time in all plot designs. These results differ from Franklin (1979) in that additive variance estimates did not decrease after competition began affecting growth.

As a consequence of plot differences in variance estimates, intraclass correlation estimates also varied. Intraclass correlation estimates were initially highest for the multiple single-tree plot, yet at the third measurement date intraclass correlation estimates were similar among plot designs (Table 4).

The changes in means and variances suggested the error variances may not be homogeneous among plot designs, and that a logarithmic transformation may be necessary. The partitioning of inter-family competition variance among the sources of variation also varied among plot designs (McCutchan pers. comm.), and further supported the hypothesis that error variances may not be homogeneous. Using Bartlett's test, the probability of heterogeneous variances was greater than .05 but less than .10; thus, transformations were not necessary.

Table 4. Estimates of genetic parameters for height over time derived from three plot designs at close spacing on an agricultural site.

Plot Design	1st-Year Height	2nd-Year Height	3rd-Year Height
Multiple Single-tree			
Error Variance	.014	.088	.133
Additive Variance	.006	.023	.060
Phenotypic Variance	.018	.117	.201
Intraclass Correlation	.083	.049	.075
Row			
Error Variance	.012	.072	.123
Additive Variance	.002	.018	.048
Phenotypic Variance	.014	.087	.156
Intraclass Correlation	.036	.052	.077
Block			
Error Variance	.012	.077	.126
Additive Variance	.001	.030	.062
Phenotypic Variance	.061	.107	.174
Intraclass Correlation	.004	.070	.089

DISCUSSION

Varying degrees of inter-family competition across plot designs had no effect on mean family performance. Results are limited to three years of data from a closely-spaced trial, and consequently the prolonged, cumulative effects of inter-family competition were absent. Differential height measurements between 120 cm and 60 cm plots were detected only in third-year height.

Plot design had no effect on the family mean ranking. Morrow (1983) likewise found no significant seed source by plot design interactions for row and block plots at conventional spacings. Conversely, Hart (1986) found mean family ranks were not correlated between block and row plots or block and multiple single-tree plots, but were correlated between row and multiple single-tree plots. There were minor changes in family ranks among plot designs that did not influence the significance of the correlations in our study. These changes in family rank may be due to measurement precision, measurement scale or an artificial designation of a culling level.

Loo-Dinkins and Tauer (1987) reported higher statistical efficiencies with multiple single-tree plots compared to row or block plots, even on uniform sites. Lambeth et al. (1983) concluded fewer replications are required for multiple single-tree plots because of these higher efficiencies. The statistical efficiency gained from multiple single-tree plots in long-term genetic trials is partially negated by planting early genetic trials on agricultural sites at close spacing for a short period of time. Site uniformity, close spacing and short duration studies diminish some of the advantages of multiple single-tree plots. Therefore, the difference in the number of replications required for similar efficiencies between multiple single-tree and row or block plots is less in short-term genetic trials than in tests planted at conventional spacings.

In conclusion, competition adversely affected growth, yet there were no appreciable changes in family ranks across plot designs at 60 cm square spacings. Selection based on third-year height or third-year incremental height will not be biased by inter-family competition. Block plots appear unnecessary in closely-spaced, short-term trials and should be superceded by row or multiple single-tree plots.

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