## NON-REPLICATED TESTING IN THE REAL WORLD D. Steven Calhoun and Jerry B. Riney Stoneville Pedigreed Seed Co. Idalou, TX

#### **Abstract**

The objective of this study was to determine the utility of non-replicated evaluations in predicting line performance. Two sets of progeny rows were evaluated in non-replicated trials at the Stoneville Pedigreed Seed Company research station near Lubbock TX in 1999. A subset of these entries was evaluated in replicated trials at the same location in 2000. Correlation coefficients of yield in 2000 trials vs. visual appeal rating, unadjusted yield, and yield expressed as a percentage of the nearest check in 1999 were generally not significantly different from zero. Correlation coefficients of yield in 2000 vs. adjusted yield calculated from Modified Augmented Design analysis in 1999 resulted in r-values of 0.20 (n=84, p=0.08) and 0.24 (n=132, p<0.01) for progeny row Set 1 and Set 2, respectively. R-values for storm resistance ratings and for fiber length, strength and micronaire in 1999 vs. 2000 were >0.50 (p<0.01) for both progeny row sets. R-values for maturity rating in 1999 vs. 2000 were 0.37 (n=84, p<0.01) and 0.17 (n=132, p=0.06) for Set 1 and Set 2, respectively. In this experiment, visual appeal ratings were worthless for predicting future yield performance. Measured yield was better at predicting future yield, but r-values were still rather low, given the resources required to collect the data. Fiber quality, storm resistance, and to a lesser extent, maturity evaluated in non-replicated trials were fairly accurate predictors of subsequent performance for these traits.

#### **Introduction**

Regardless of how segregating breeding populations are handled in early generations, most breeding programs reach a stage where a large number of relatively uniform progeny rows are available for evaluation. Two primary challenges are encountered at this stage: 1) limited seed supply prevents replicated testing, and 2) the proportion of these strains carried forward for replicated testing must be relatively small due to other resource limitations. In our program, it is a simple matter to generate several thousand progeny rows, but resources dictate that only a few hundred can be carried forward for replicated trials the following year. Most other cotton breeding programs face a similar situation.

The objective of this research was to determine the predictive value of observations in non-replicated tests. Factors studied included yield, storm resistance, maturity, and fiber quality.

# **Materials and Methods**

Experiments were conducted during 1999 and 2000 on the Stoneville Pedigreed Seed Company Texas High Plains Research Station near Lubbock TX. Soil type was a Pullman Clay loam (0-1% slope) and irrigation was supplied through a sub-surface drip system applying approximately 0.17 inches per day. Agronomic practices were common for the area.

Two sets of progeny rows and corresponding check varieties, identified as "Set 1" and "Set 2", were evaluated in 1999. Set 1 consisted of 304 experimental strains from 8 non-transgenic picker x stripper crosses and 48 plots of recurring check varieties. Set 2 consisted of 390 experimental strains from 7 BXN® picker x non-transgenic stripper crosses and 50 plots of recurring check varieties. Each plot consisted of a single 20-foot row with 40 inch spacing between rows. Plots were arranged in a modified augmented design (MAD) (Lin and Pouskiksky, 1983). The MAD uses "main plots" each consisting of a recurring check variety ("central control sub-plot") flanked on either side by a certain number (in this case, 5) of sub-plots containing experimental lines. Main plots are arranged in a grid of rows and columns in the field. The MAD analysis tests for row and/or column effects and adjusts the values of experimental lines for field variability as measured by row and/or column effects.

Prior to harvest, each plot was rated on a 1 to 9 scale for maturity (1=late, 9 = early), storm resistance (1=very loose boll, 9 =very storm proof), and visual appeal (1=poor, 9=good). The visual appeal score was determined primarily by apparent yield potential, but also included consideration of plant architecture and other factors. Each plot was harvested with a brush stripper equipped with a bur extractor and plot yield of seed cotton was determined. Grab samples consisting of 600 to 1200 g of seed cotton from selected entries were ginned to determine lint percentage and fiber quality. Fiber length (upper half mean), strength, and micronaire were determined by high volume instrument at the Louisiana State University Fiber Laboratory.

Data for seed cotton yield, maturity, storm resistance and visual appeal were analyzed as a MAD experiment using Agrobase (Mulitze, 1990) software. Part of the output of the MAD analysis is adjusted yield (and other parameters) based

on field variability as interpreted by the analysis. In addition, yield was calculated as a percent of the nearest central control sub-plot (check).

A subset of strains from 1999 progeny rows was evaluated in replicated tests in 2000. From progeny row Set 1, 88 strains were chosen and assigned to one of two trials. From Set 2, 132 strains were chosen and assigned to one of three trials. Replicated trials consisted of 48 entries each (including checks) and 4 replications arranged in randomized complete blocks. Plots were 2 rows wide, 40 feet long with 40 inch spacing between rows. Plots were rated for storm resistance and maturity and were harvested as described above. Grab samples from two replications were used for determination of lint percentage and fiber quality.

Frequency distributions for yield, fiber quality and agronomic traits were determined for the total populations (i.e. all 1999 progeny rows) and for the population subsets (i.e. strains included in 2000 replicated trials). Simple correlation coefficients among traits of interest were calculated.

#### **Results**

Analysis of yield data from MAD experiments conducted in 1999 showed a significant column effect in progeny row Set 1 and significant row and column effects in Set 2 (Table 1). Of the adjustments for field variability offered by MAD analysis, "Method 1" is preferred if only row or column effects are significant (e.g. Set 1). "Method 3" is preferred if both row and column effects are significant (e.g. Set 2). Experimental error in replicated tests during 2000 was considered low (coefficients of variance for yield in these tests ranged from 6.5 to 9.9%, data not shown). Data from both years were considered reliable for this environment.

Correlation coefficients for yield-related parameters in 1999 (non-replicated tests) vs. lint yield in 2000 (replicated tests) are shown in Table 2. The correlation between visual rating in 1999 and lint yield in 2000 was not statistically significant for either set of progeny rows. Unadjusted yield in 1999 and yield expressed as a percentage of the nearest check in 1999 were significantly (p<0.05) correlated with yield in 2000 only for Set 2. Non-replicated yield of Set 1, adjusted by MAD Method 1 (as suggested by ANOVA), was significantly correlated with replicated yield in 2000 (p = 0.08). For Set 2, non-replicated yield adjusted by Method 3 (as suggested by ANOVA), as well as by Method 1, were significantly correlated with replicated yield.

Even r-values for yield-related parameters that were statistically greater than zero, were, nonetheless rather small (<0.25). This can be explained by at least two possible factors. First, a low level of selection for strains toward the upper end of the 1999 yield distribution was practiced to determine which strains were carried forward to replicated testing (Fig. 1 and 2). Had a larger portion of lower yielding strains been carried forward, it is possible that the correlations between replicated and non-replicated results would have been higher. However the shift in distribution was relatively small, and, in the real world, breeders will carry forward only the upper end of the yield distribution as much as possible.

A second factor that could explain the relatively small yield-related r-values is the interaction between year and genotype. Replicated trials of 24 commercial varieties were conducted in the same field and years as the present study. The correlation coefficient between 1999 and 2000 yields in this variety trial was also small (r = 0.15, n = 24, p = 0.49). In the real world, environment and the interaction between environment and genotype can be expected to mask genotypic effects and alter the relative performance of genotypes—and based on the years of this study, non-replicated evaluation appears to be no exception.

Visual evaluation of storm resistance in both years was much more highly correlated than yield-related parameters (Table 3). Correlation coefficients between replicated (2000) and non-replicated (1999) storm resistance scores were >0.5 (p<0.01) in both progeny row sets. These relatively high r-values were observed even after selecting rather intensely in the desirable tail of the original distribution (Fig. 3).

Visual evaluations of relative maturity in the two years were significantly correlated in both progeny row sets, although the r value for Set 1 was inexplicably larger than for Set 2 (Table 3). Correlation coefficients for maturity scores were smaller than for storm resistance scores. Maturity was not a major criterion for advancing strains to replicated yield trials. Therefore, only a modest shift in distribution frequencies from selected to un-selected populations was observed (Fig. 4).

Fiber length measurements in replicated and non-replicated trials were highly correlated in both progeny row sets (Table 4). Fiber properties were determined only for a portion of the progeny rows. Only the shortest-fiber entries were dropped from further testing, so the shift in frequency distribution from selected to un-selected populations was minor (Fig. 5). Fiber strength measurements were also highly correlated, although the r-value in Set 2 was smaller than in Set 1. Micronaire in replicated trials was significantly correlated in both progeny row sets. No selection was made among progeny rows for strength or micronaire.

### **Conclusions**

Visual rating of progeny rows was a poor indicator of subsequent yield performance in replicated yield trials. Although in this case, visual ratings included factors other than yield, perceived yield was the primary determinant of visual rating scores. The wide variation in boll type, from very loose to very storm proof, likely affected visual yield estimates.

Actual measurement of yield in non-replicated progeny rows was better than visual rating in predicting subsequent yield performance in replicated trials, and adjustments for field variability based on repeating checks generally improved the predictive value of non-replicated data. MAD analysis was effective in determining the type of adjustment needed and yield values so adjusted were the best predictors of subsequent yield performance in this study.

Storm resistance and relative maturity scores, as well as fiber quality measurements, in non-replicated trials had better predictive value than did yield measurements. Correlation coefficients for these traits were generally >0.50. In most cases, effective selection for these traits could be practiced in non-replicated plots.

Even with the best yield adjustment, r-values for non-replicated yield vs. replicated yield were still relatively low (<0.25). Since yield determination is typically the most resource-intensive trait measured, careful consideration needs to be given to the cost (time, money, and logistic) of non-replicated yield determination vs. the benefits in an applied breeding program.

#### References

Lin, C.S. and G. Pouskinsky. 1983. A modified augmented design (type 2) for an early stage of plant selection including a large number of test lines without replication. Biometrics 39:553-561.

Mulitze, D.K. 1990. AGROBASE/4: A microcomputer database management and analysis system for plant breeding and agronomy. Agron. J. 82:1016-1021.

Table	1.	Modif	ïed	aug	ment	ed	design	analys	is o	f
varianc	e for	yield	in	two	sets	of	non-rep	licated	yiel	d
trials a	t Idalo	ou TX i	in 1	999.						

Source	Df	Mean Square	Pr>F	
<u>Set 1</u>				
Total	30			
Rows	7	36338	0.40	
Columns	3	117913	0.03	
Residual	20	33158		
Grand mean	= 1223	B, R-squared = 0.97,	CV = 14.9%	
<u>Set 2</u>				
Total	39			
Rows	9	139719	< 0.01	
Columns	3	72206	< 0.01	

	Grand mean = 1228, R-squared = 0.99, CV = 10.9%		
Table 2.	Correlation coefficients (and probability values) of various estimate	ates of	yield i

Residual

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Table 2. Correlation coefficients (and probability values) of various estimates of yield in two sets of non-replicated tests during 1999 versus yield measured in replicated tests in 2000 at Idalou, TX.

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	Non-replicated Test Results, 1999				
Replicated	Raw	Yield as	Yield	Yield	Visual
Tests, 2000	yield	% check	Method 1	Method 3	rating
Set 1 (n=84)	0.11 (0.33)	0.17 (0.13)	0.20 (0.08)	0.15 (0.16)	0.01 (0.90)
Set 2 (n=132)	0.18 (0.04)	0.18 (0.04)	0.23 (0<.01)	0.24 (<0.01)	0.14 (0.13)

Table 3. Correlation coefficients (and probability values) between storm resistance and relative maturity scores in two sets of non-replicated tests during 1999 vs. the same parameters in replicated tests in 2000 at Idalou, TX

	Non-replicated Test Results, 1999		
<b>Replicated Test, 2000</b>	Storm resistance	Maturity	
Set 1 (n=84)	0.56 (<0.01)	0.37 (<0.01)	
Set 2 (n=132)	0.62 (<0.01)	0.17 (0.06)	

Table 4. Correlation coefficients (and probability values) between fiber quality parameters in two sets of non-replicated tests during 1999 and the same parameters in replicated tests in 2000 at Idalou, TX.

	Non-replicated Test Results, 1999				
<b>Replicated Test, 2000</b>	Length (UHM)	Strength (HVI)	Micronaire		
Set 1 (n=84)	0.68 (<0.01)	0.78 (<0.01)	0.53 (<0.01)		
Set 2 (n=132)	0.56 (<0.01)	0.35 (<0.01)	0.52 (<0.01)		



Unadjusted Lint Yield (lbs/A)





Figure 2. Frequency distribution for unadjusted lint yield of the total population of progeny rows in Set 2 at Idalou, TX, 1999 and the subset of that population used in further testing.

# Frequency Distribution for Storm Resistance Score



Figure 3. Frequency distribution for storm resistance scores of the total population of progeny rows in Set 2 at Idalou, TX, 1999 and the subset of that population used in further testing.

**Frequency of Distribution for Maturity** 



Figure 4. Frequency distribution for maturity scores of the total population of progeny rows in Set 2 at Idalou, TX, 1999 and the subset of that population used in further testing.



Figure 5. Frequency distribution for fiber length of the total population of progeny rows in Set 2 at Idalou, TX, 1999 and the subset of that population used in further testing.