

## Improving the precision of cotton performance trials conducted on highly variable soils of the southeastern USA coastal plain

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With 1 figure and 4 tables

Received December 11, 2006/Accepted March 6, 2007

Communicated by W. E. Weber

### Abstract

Reliable agronomic and fibre quality data generated in Upland cotton (*Gossypium hirsutum* L.) cultivar performance trials are highly valuable. The most common strategy used to generate reliable performance trial data uses experimental design to minimize experimental error resulting from spatial variability. However, an alternative strategy uses *a posteriori* statistical procedures to account for spatial variability. In this study, the efficiency of the randomized complete block (RCB) design and nearest neighbour adjustment (NNA) were compared in a series of cotton performance trials conducted in the southeastern USA to identify the efficiency of each in minimizing experimental error for yield, yield components and fibre quality. In comparison to the RCB, relative efficiency of the NNA procedure varied amongst traits and trials. Results show that experimental analyses, depending on the trait and selection intensity employed, can affect cultivar or experimental line selections. Based on this study, we recommend researchers conducting cotton performance trials on variable soils consider using NNA or other spatial methods to improve trial precision.

**Key words:** *Gossypium hirsutum* — lint yield — fibre quality — experimental design — nearest neighbour analysis — spatial variability

In Upland cotton (*Gossypium hirsutum* L.) and many other crop breeding programmes, replicated field trials are repetitively conducted across environments and years to compare individual cultivars and experimental lines for a range of traits important to maximize crop production (Campbell and Jones 2005). Data from multi-location and multi-year trials are evaluated to make inferences on cultivar or experimental line performance potential. To generate reliable performance data, most often the researcher determines the type of experimental design to be used, with the main objective of minimizing experimental error. Selection of the appropriate experimental design typically involves some *a priori* information regarding spatial trends such as soil variability and/or soil fertility in individual fields.

Soils within the southeastern USA coastal plain are known to be highly variable; production fields typically consist of numerous soil types (Karlen et al. 1990, Sadler et al. 1998, Johnson et al. 2002). Previous research has found some soil chemical and physical properties are correlated with cotton yield and some fibre quality traits (Johnson et al. 2002). Construction of soil maps for individual fields using intensive soil sampling can be used to determine the extent of soil variability within a field; these soil maps can be used to develop appropriate experimental designs that minimize soil

variability to some degree. However, this practice is not always a realistic alternative due to the time, effort, and cost of generating soil maps for a multitude of experimental fields (Posner et al. 1995). Additionally, soil maps of specific fields may not be conducive to any formal type of experimental design that accounts for the soil variability present.

The most common type of experimental design used in cotton performance trials is the randomized complete block (RCB); however, researchers also commonly use additional experimental designs, such as incomplete blocks, and various lattice arrangements (Cochran and Cox 1957). The RCB and other blocking designs assume spatial variability can be accounted for by blocking the experimental units in a linear fashion. This assumption is not met under field conditions when the RCB contains considerable within-block heterogeneity (Gusmao 1986, Lin et al. 1993). Within-block heterogeneity can be evaluated by looking for pattern in a graph of residuals plotted against row position (Stroup and Mulitze 1991, Brownie et al. 1993). A plot's residual is calculated by subtracting the entry mean from the plot value, and a pattern in residuals relative to row position indicates significant heterogeneity.

Incomplete block and lattice designs account for spatial trends in the same fashion as the RCB, but also account for a portion of the spatial variability within blocks by reducing a complete block into smaller incomplete blocks. The alpha-lattice is an example of a flexible incomplete block design that has been shown to account for spatial trends more efficiently than the RCB, especially, in trials with a large number of entries (Patterson and Hunter 1983, Yau 1997). However, unaccounted spatial variation may still persist within incomplete blocks (Yang et al. 2004). There are also examples of experimental designs that take spatial variability into account at the design stage by accounting for spatial autocorrelation (van Es and van Es 1993).

In recent years, statistical theory has produced *a posteriori* statistical procedures that account for plot-to-plot variation within a block to account for spatial variability in crop performance trials. One of the most common analysis tools used in cotton performance trials and other crop trials is the nearest neighbour adjustment (NNA) (Papadakis 1937, Wilkinson et al. 1983, Besag and Kempton 1986, Stroup and Mulitze 1991). Although there are several variations of the NNA procedure, the major principle involves removing heterogeneity amongst experimental units from experimental

error. The NNA procedure uses an adaptation of analysis of covariance based on the residual between the mean of a single experimental unit and the mean of neighbouring experimental units.

The efficiency of NNA and other methods to account for spatial variability has been investigated for a number of different crops that include lentil (*Lens culinaris* ssp. *culinaris*) (Sarker et al. 2001), perennial cool-season forage grass species (Casler 1999, Smith and Casler 2004), alfalfa (*Medicago sativa* L.) (Casler and Undersander 2000), wheat (*Triticum aestivum* L.) (Bhatti et al. 1991, Stroup et al. 1994, Wu et al. 1998, Qiao et al. 2000, Durban et al. 2001), soybeans (*Glycine max* L.) (Brownie et al. 1993, Vollmann et al. 1996) and maize (*Zea mays* L.) (Brownie et al. 1993). These investigations found spatial analysis improves the efficiency of trial analyses by comparing the error variance between spatially adjusted and unadjusted analyses. In these studies, the relative efficiency of NNA and other spatial methods was calculated using several methods based on differences in the size of the error variance between different experimental designs and spatial methods. The complexity of the variance structure of a specific design or spatial method necessitates the use of particular error variance comparisons (Brownie et al. 1993). These comparisons include the average variance of difference between entry means, the average standard error of the difference between entry means, and the error mean square (Qiao et al. 2000). However, for RCB, trend and Papadakis analyses, Brownie et al. (1993) concluded that comparing error mean squares provided a valid estimate of relative efficiency.

Investigations of spatial analyses in cotton performance trials are generally lacking and have not examined the impact on fibre quality. A single study conducted in Pakistan accounted for lint yield spatial variability in one cotton performance trial by decreasing experimental error variance by 14% (Bhatti et al. 1991). To date, the impact of spatial variability on cultivar fibre quality estimates in cotton performance trials has not been reported. Fibre quality is increasingly important because new machinery used by the textile industry requires higher quality fibre to maximize efficiency and product quality (Meredith 2005). Hence, the objective of this study was to evaluate the efficiency of NNA to adjust for spatial trends impacting cultivar estimates of lint yield, yield components and fibre quality.

## Materials and Methods

Yield and fibre quality performance data were collected on cotton performance trials conducted from 2000 to 2005. Each year, the trial was conducted at the Pee Dee Research and Education Center near Florence, SC, USA. Planting and harvest dates varied depending upon weather conditions in a given year and trials contained 18–24 entries. The trial in 2000 was conducted on a Norfolk loamy sand soil, whereas the 2001 trial was conducted on a Nobocco loamy sand soil. Trials conducted from 2002 to 2005 were evaluated on a Goldsboro loamy sand soil. Cultivars were arranged in a RCB design with four replications. In each of the trials, a single replication was planted to a linear range in the east-west direction, with four ranges in the north-south direction. Each cultivar was grown in a two-row plot 10.7-m long with 96.5-cm spacing between rows and 3-m alleys between ranges. Two to four border rows were planted on both sides of the trial to minimize border effects. Plots were managed conventionally and followed the established local practices.

In this study, data for four agronomic and five fibre quality traits were used for analysis. Agronomic traits were boll weight, lint weight

per boll, seed index and lint yield. Fibre quality traits were fibre length, strength, elongation, uniformity index and micronaire. Prior to harvest, a 50-boll sample was randomly collected from each plot and ginned using a 10-saw laboratory gin. The sample was weighed prior to ginning to calculate boll weight, and the weight of the lint after ginning was used to calculate lint weight per boll. The weight of 100 fuzzy seeds was recorded as the seed index. A sample of lint from the 50-boll samples of the first two reps was used to measure fibre properties using the High Volume Instrument (HVI). Each plot was harvested using a 2-row, mechanical spindle picker and total seed cotton was weighed for each plot. Lint yield was calculated by multiplying total seed cotton by the per cent lint determined after ginning the 50-boll sample.

Trait data from each trial were analysed as a RCB design and also subjected to NNA using AGROBASE Generation II (Mulltze 2004). The RCB model partitioned sources of variation according to the model

$$Y_{ij} = \mu + v_i + r_j + \varepsilon_{ij},$$

where  $Y_{ij}$  is the mean value of the  $i$ th entry of the  $j$ th block,  $\mu$  is the overall mean,  $r_j$  is the effect of the  $j$ th block,  $v_i$  is the effect of the  $i$ th entry, and  $\varepsilon_{ij}$  is the random error. The  $\varepsilon_{ij}$ s are assumed to be independently distributed with constant variance, thus implying no spatial trend.

The NNA covariate was computed based on Papadakis (1937) using adjacent residuals to correct for within-block variability amongst plots in the east-west direction according to

$$t_{kl} = 1/2(e_{k,l-1} + e_{k,l+1}),$$

where  $t_{kl}$  is the covariate corresponding to the plot located in row  $k$  and column  $l$  of each trial and the two values  $e_{kl}$  are the residuals to the left and right of the  $k$ th plot. For plots at either end of a range,  $t_{kl}$  was calculated as the residual for the one neighbour. The analysis of covariance incorporating the NNA covariate to adjust for east-west spatial trends was based on Stroup et al. (1994) and followed the model

$$Y_{ij} = \mu + v_i + r_j + bt_{ij} + \varepsilon_{ij},$$

where  $b$  is the regression coefficient associated with the NNA covariate and  $\varepsilon_{ij}$  is the residual error. The analysis of covariance was repeated until the difference between adjusted cultivar means was negligible in successive iterations (Wilkinson et al. 1983).

Arithmetic and adjusted cultivar means were computed and rankings compared for the RCB design and the NNA analysis. Pearson and Spearman rank correlations were calculated to compare cultivar means and rankings between the RCB design and NNA analysis. A measure of relative efficiency was computed by comparing the error mean square for the RCB design relative to that of a completely randomized design (CRD) and the error mean square for the NNA analysis relative to the RCB design similar to methods described by Casler (1999). To calculate the relative efficiency of the RCB, the RCB error mean square was divided by the CRD error mean square and multiplied by 100. The residual error mean square was estimated for a CRD following the model

$$E_{\text{CRD}} = \frac{n_b E_b + (n_t + n_e) E_e}{n_b + n_t + n_e},$$

where  $E_b$  and  $E_e$  are the block and error mean squares and  $n_b$ ,  $n_t$  and  $n_e$  the block, treatment and error degrees of freedom as described by Cochran and Cox (1957). In addition, the relative efficiency of the NNA analysis relative to the RCB was calculated by dividing the NNA error mean square by the RCB error mean square and multiplying by 100.

To compare RCB arithmetic means to NNA adjusted cultivar means for a specific trait, a similarity measurement was calculated at a number of fixed selection intensities following the methods described by Qiao et al. (2000). For all traits in each trial, cultivars were selected at intensities of 5, 10, 20, 30 and 40% and the Czekanowski coefficient ( $D$ ) (Snijders et al. 1990, Everitt 1993) was calculated following

$$D = \frac{a}{a + c},$$

where  $a$  is the number of cultivars selected by the NNA and RCB analysis and  $c$  is the number of cultivars selected by the RCB analysis only.

## Results

### Efficiency of RCB design and NNA analysis

In all of the trials, the RCB analysis indicated significant block effects for all traits. For each of the agronomic and fibre quality traits, NNA analysis identified putative field trends in at least two of the 6 years based on residual scatter plots. Residual scatter plots, where a plot's residual is plotted against plot position, allow the detection of pattern and putative field trends. Significant pattern in the residuals of the unadjusted data suggests the presence of significant field trends not accounted for by the RCB. After NNA analysis, observing a similar residual scatter plot, based on NNA adjusted plot data, should reveal no pattern in the residuals. Hence, the NNA analysis was effective in removing additional spatial trends not accounted for by the RCB experimental design, thus improving the estimates of cultivar performance. An example of residual scatter plots for unadjusted and NNA adjusted plot data for lint yield in the 2003 trial is shown in Fig. 1. Overall,

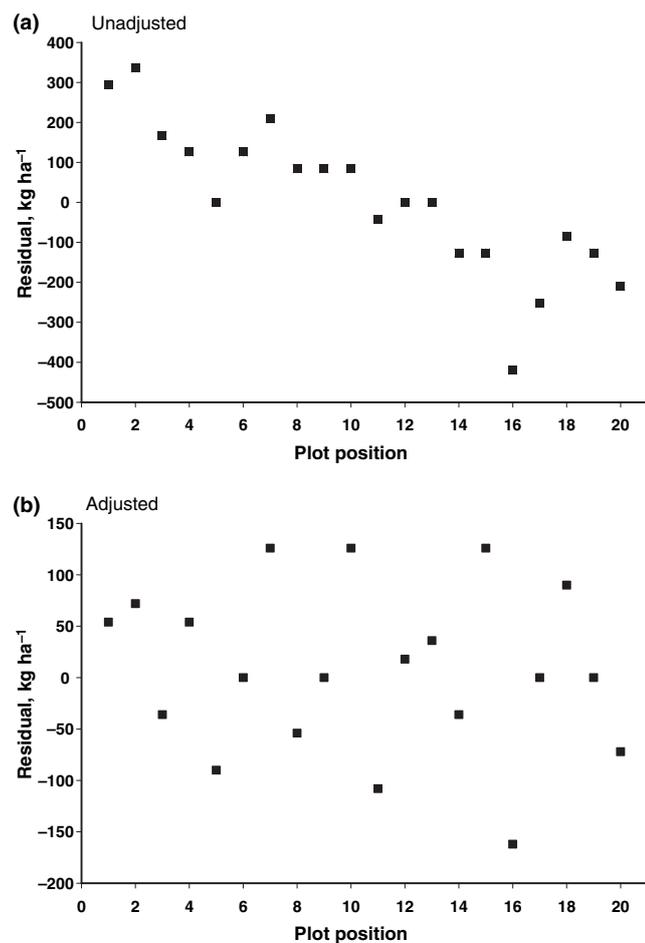


Fig. 1: Lint yield residuals (Plot lint yield - Entry mean) plotted against plot position for the 2003 trial conducted at Florence, SC, USA calculated (a) prior to NNA adjustment and (b) after NNA adjustment

all six trials showed significant field trends for micronaire, five for fibre length, four for lint weight per boll and lint yield, three for fibre strength, fibre elongation and uniformity index, and two for boll weight and seed index.

### Yield and yield component traits

Table 1 presents a summary of the RCB and NNA relative efficiencies calculated for yield and yield component traits. Overall, RCB and NNA analyses both reduced experimental error for yield and yield component traits relative to the CRD and RCB, respectively. On average the relative efficiency of the RCB analysis relative to the CRD was highest for lint weight per boll (146%) and lint yield (118%). The relative efficiency of the RCB relative to the CRD for lint weight per boll and lint yield ranged from 97% to 368% and 98% to 140%, respectively. The single largest relative efficiency for the RCB analysis was found in the 2000 trial for lint weight per boll (368%).

The average relative efficiency of the NNA analysis relative to the RCB was highest for lint yield (147%) and boll weight (116%). For lint yield, relative efficiencies ranged from 101% to 267%. For boll weight, relative efficiencies ranged from 107% to 126%. The single largest relative efficiency for the NNA analysis was found in the 2003 trial for lint yield (267%). For this trial, the efficiency of the RCB to the CRD was only 98%. Hence, the NNA analysis provided a considerable improvement over the CRD and RCB. Lint yield NNA relative efficiencies reported here are similar to the 14% increase in efficiency reported by Bhatti et al. (1991).

### Fibre quality

Table 2 presents a summary of the RCB and NNA relative efficiencies calculated for fibre quality traits. On average, the relative efficiency of the RCB analysis relative to the CRD ranged from 100% for uniformity index to 105% for fibre elongation. The single largest relative efficiency for the RCB analysis was found in the 2003 trial for fibre elongation (129%). The average relative efficiency of the NNA analysis relative to the RCB was highest for micronaire (141%) and fibre length (141%). The relative efficiency of the NNA relative to the RCB for micronaire and fibre length ranged from 102% to 207% and 103% to 159%, respectively. The single largest relative efficiency for the NNA analysis was found in the 2003 trial for fibre length (207%). Overall, NNA analysis resulted in considerable improvements in efficiency over the RCB and CRD for fibre length, fibre strength, uniformity index and micronaire in the 2003 trial. Fibre elongation was the only fibre quality trait not showing improved efficiency with the NNA analysis.

### Mean comparisons

Comparing means estimated from the RCB and NNA analyses indicates cultivar rankings can differ amongst the two analyses. Table 3 provides a summary of Pearson and Spearman rank correlations between RCB and NNA means for agronomic and fibre quality traits. For the agronomic traits, mean values for Pearson and Spearman rank correlations were highest for seed index (0.9773, 0.9527) and lowest for lint weight per boll (0.9491, 0.9141). In terms of individual trials, Spearman rank correlations were lowest for lint yield in 2003 (0.8752) and for lint weight per boll in 2004 (0.8658) and 2005 (0.8301). For the

Table 1: Relative efficiencies (%) comparing the randomized complete block (RCB) to the completely randomized design, and the nearest neighbour analysis (NNA) to the randomized complete block design for boll weight, lint weight per boll, seed index and lint yield in six cotton performance trials conducted at Florence, SC, USA from 2000–2005

Year	Boll weight		Lint weight per boll		Seed index		Lint yield	
	RCB	NNA	RCB	NNA	RCB	NNA	RCB	NNA
2000	98	–	368	102	103	–	119	106
2001	108	–	97	–	102	–	117	114
2002	98	–	98	–	102	108	140	101
2003	112	126	101	104	115	107	98	267
2004	119	–	115	103	105	–	107	–
2005	102	107	99	113	114	–	128	–
Mean	106	116	146	105	107	107	118	147

Table 2: Relative efficiencies (%) comparing the randomized complete block (RCB) to the completely randomized design, and the nearest neighbour analysis (NNA) to the randomized complete block design for fibre length, strength, elongation, uniformity index and micronaire in six cotton performance trials conducted at Florence, SC, USA from 2000–2005

Year	Length		Strength		Elongation		Uniformity index		Micronaire	
	RCB	NNA	RCB	NNA	RCB	NNA	RCB	NNA	RCB	NNA
2000	98	132	97	132	98	–	98	–	115	118
2001	98	102	124	119	98	129	98	–	98	145
2002	99	124	100	–	98	102	98	114	98	122
2003	109	207	103	124	103	–	104	142	99	159
2004	99	–	101	–	129	117	99	113	97	103
2005	108	143	99	–	103	–	100	–	98	197
Mean	102	141	104	125	105	116	100	123	101	141

Table 3: Summary of Pearson and Spearman rank correlations for means estimated using the nearest neighbour adjustment (NNA) and randomized complete block (RCB) analyses for boll weight, lint weight per boll, seed index, lint yield, length, strength, elongation, uniformity index and micronaire in six cotton performance trials conducted at Florence, SC, USA from 2000–2005

Trial	Correlation	Boll weight	Lint weight per boll	Seed index	Lint yield	Length	Strength	Elongation	Uniformity index	Micronaire
2000	Pearson	–	0.9936	–	0.9738	0.9936	0.9506	–	–	0.9774
	Spearman	–	0.9876	–	0.9629	0.9794	0.9236	–	–	0.9752
2001	Pearson	–	–	–	0.9837	0.9547	0.9913	0.9851	–	0.9687
	Spearman	–	–	–	0.9571	0.9108	0.9831	0.9814	–	0.9354
2002	Pearson	–	–	0.9949	0.9592	0.9656	–	0.9878	0.9071	0.9764
	Spearman	–	–	0.9896	0.9209	0.9426	–	0.9522	0.8896	0.9356
2003	Pearson	0.9760	0.9618	0.9597	0.9577	0.9287	0.8693	–	0.9339	0.9496
	Spearman	0.9474	0.9729	0.9158	0.8752	0.9218	0.8451	–	0.9413	0.9278
2004	Pearson	–	0.9447	–	–	–	–	0.9845	0.9616	0.9918
	Spearman	–	0.8658	–	–	–	–	0.9670	0.9263	0.9668
2005	Pearson	0.9371	0.8963	–	–	0.9705	–	–	–	0.9709
	Spearman	0.9158	0.8301	–	–	0.9489	–	–	–	0.9429

fibre quality traits, mean Pearson and Spearman rank correlations were highest for fibre elongation (0.9858, 0.9669). The lowest mean Pearson correlation was for uniformity index (0.9342), and the lowest mean Spearman rank correlation was for fibre strength (0.9173). In terms of individual trials, Spearman rank correlations were lowest for fibre strength in 2003 (0.8451) and uniformity index in 2002 (0.8896).

Differences in cultivar ranks between the RCB and NNA analyses present a challenge in selecting the best performing cultivars for a specific trait. To better understand this challenge, Qiao et al. (2000) recommended comparing the selection of the best performing lines from the RCB and NNA mean estimates at a number of fixed selection intensities using similarity coefficients. Hence, five selection intensities of 5, 10, 20, 30 and 40% were applied, and the Czekanowski similarity coefficient was calculated from the selections based on the RCB and NNA means. Table 4 shows similarities at selection intensities of 5 and 10% are more consistent than similarities at selection intensities of 20, 30 and 40%. For example, 28 of

the trait-year combinations presented in Table 4 for selection intensities of 20, 30 and 40% show similarities of 75% or below. Considering the agronomic traits, the average similarity in selection over the 20, 30 and 40% levels for boll weight was 79%, 81% for lint weight per boll, 86% for seed index and 84% for lint yield. Considering the fibre quality traits, the average similarity in selection over the 20, 30 and 40% levels for fibre length was 91%, 87% for fibre strength, 92% for fibre elongation, 79% for uniformity index and 88% for micronaire. These findings suggest differences in the cultivars selected based on the RCB and NNA means would more likely be expected for boll weight, lint weight per boll and uniformity index. The fewest differences in rank would be expected for fibre length and fibre elongation.

## Discussion

This study demonstrates the importance of using experimental design and spatial analyses to account for spatial variability in

Table 4: Percentage agreement (%) in selection of the top genotypes based on the Czekanowski coefficient for comparison of the nearest neighbour analysis with the randomized complete block analysis for boll weight, lint weight per boll, seed index, lint yield, fibre length, strength, elongation, uniformity and micronaire for five selection intensities in trials conducted in Florence, SC, USA from 2000–2005

Selection intensity (%)	Boll weight		Lint weight per boll				Seed index		Lint yield				Length				
	2003	2005	2000	2003	2004	2005	2002	2003	2000	2001	2002	2003	2000	2001	2002	2003	2005
5	100	100	0	0	100	100	100	100	0	100	100	0	0	100	100	100	100
10	100	100	50	100	100	50	100	50	100	50	100	100	100	100	100	50	100
20	75	75	100	100	75	75	80	75	75	75	80	75	100	75	80	100	100
30	67	83	83	86	60	67	86	83	80	86	100	83	100	86	86	86	83
40	87	87	100	90	71	62	100	87	100	89	90	75	100	100	80	87	100

Selection intensity (%)	Strength			Elongation			Uniformity index			Micronaire					
	2000	2001	2003	2001	2002	2004	2002	2003	2004	2000	2001	2002	2003	2004	2005
5	100	100	100	0	100	0	100	100	0	100	100	100	0	100	100
10	50	100	50	100	50	100	50	100	100	100	50	100	100	100	100
20	100	100	75	75	75	100	60	100	75	100	75	60	75	100	100
30	100	86	67	100	86	100	86	67	80	100	86	100	67	100	100
40	86	100	75	100	90	100	70	87	86	86	89	80	87	100	87

southeastern USA cotton performance trials. To our knowledge, this study serves as the first to report the impact of spatial variability on the estimation of a cultivar's fibre quality potential. Clearly, the extent spatial variability affects the estimation of cultivar performance differs for each trait and amongst individual trials. The 2003 trial demonstrated the largest gains in relative efficiency from NNA analyses for boll weight (126%), lint yield (267%), fibre length (207%), fibre strength (124%), uniformity index (142%) and micronaire (159%). Overall, the effectiveness of the NNA analyses at reducing experimental error was most evident in a portion of the trials for lint yield, fibre length and micronaire. Detection of significant field trends using NNA analyses supports the notion that unaccounted field heterogeneity often exists within a complete block of the RCB design. Johnson et al. (2002) reported the existence of spatial correlations in a southeastern USA production field amongst a single cultivar's lint yield and soil properties, such as soil pH, soil P and soil organic matter. It is probable that localized soil variability present (i.e. soil pH, soil P, soil organic matter, etc.) in the current study accounts for a portion of the spatial variability accounted for by the NNA analysis. However, this study also illustrates that NNA analyses are not always effective in reducing residual error variance. For example, the overall effectiveness of the NNA analysis is least evident in a portion of the trials for lint weight per boll and seed index. In addition, the overall mean relative efficiency of the NNA was only 116% for both boll weight and fibre elongation.

The current study also illustrates that rank differences for NNA and RCB estimated means can impact the selection of superior cultivars for a given trait, especially, at selection intensities of 20, 30 and 40%. This impact was most evident for boll weight, lint weight per boll and uniformity index, whereas least evident for fibre length and fibre elongation. For the 2003 trial, the 267% relative efficiency of the NNA analysis for lint yield corresponded to a 67% selection similarity averaged over all selection intensities and 78% averaged over the 20, 30 and 40% selection intensities. On the contrary, the 207% relative efficiency of the NNA analysis for fibre length in the 2003 trial corresponded to an 85% selection similarity averaged overall selection intensities and 91% averaged over the 20, 30 and 40% selection intensities. Hence, relative efficiency improvements resulting from NNA analyses do not always correspond

to low selection similarities at 5, 10, 20, 30 and 40%. Moreover, although relative efficiencies of NNA analysis for micronaire ranged from 103% to 159% across all six trials, the overall mean selection similarity was 88%.

Overall, this study provides evidence to recommend the use of NNA analyses or similar spatial methods to improve the precision of cotton performance trials conducted in the southeastern USA. This study also highlights the inefficiency of the commonly used RCB design. Although this study illustrates that cultivar means calculated from RCB and NNA analyses sometime differ in rank for both agronomic and fibre quality traits, we support the conclusions of Stroup et al. (1994), Vollmann et al. (1996), Wu et al. (1998) and Qiao et al. (2000) that recommend using NNA adjusted means for selection purposes when significant spatial trends are present. The presence of significant spatial trends not accounted for by the RCB alone is easily identifiable when relative efficiencies of the NNA analyses are large. NNA adjusted means should be utilized when the error mean square is reduced relative to the error mean square of the RCB.

#### Acknowledgements

Cultivar performance data used in this study are part of the National Cotton Variety Test coordinated by Dr W. R. Meredith and partially funded by CRIS No. 6657-21000-005-00D of the U.S. Department of Agriculture. Special thanks to Bobby Fisher for technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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