The *Journal of Cotton Science 19:250–257 (2015)*
http://journal.cotton.org, © The Cotton Foundation 2015

AGRONOMY AND SOILS

Vertical Blocking in Cotton Cultivar Performance Trials in North Carolina

Daryl T. Bowman* and Vasu Kuraparthy

ABSTRACT

The three principles of experimental design—randomization, replication, and blocking—have been followed routinely in field experiments. This paper addresses issues of blocking in conducting cultivar performance trials. Most agronomists design (block) their trials by considering the slope of the field at most. However, the direction of field variation might not always follow the slope of the field. Blocking in the direction of field operations is advocated. The objective of this study was to compare precision between three years of horizontal blocking with three years of vertical blocking in North Carolina Official Cotton Variety Trials. No significant difference in precision was found between the two blocking arrangements. However, the standard analysis of variance of a randomized complete block design revealed that vertical blocking produced a larger standard error than when the tests were blocked horizontally. Although vertical blocking results in increased spatial distance, the use of a spatial analysis package should mitigate this problem.

The field plot design principles of randomization, replication, and blocking (Cochran and Cox, 1957) have been followed routinely by agronomists for the majority of field research, including crop performance trials. Field plot design for cultivar performance trials has seen changes over the preceding decades. Randomization and blocking have seen the most changes with the advent of row-column designs and alpha designs (generalized lattice) (Patterson et al., 1978; Williams, 1986; Williams et al., 1999).

Most field agronomists and breeders block their crop performance trials based on the slope of the field at most. However, the direction of field variation might not always follow the slope of the field. The actual field variation is not normally known to the researcher/project leader. Even knowing the makeup of soil types in the field might not help because variability is not always associated with soil types. A simple calculation of residuals from a performance trial or distribution of yield in uniformity trials can illustrate field variation for that season. Thus, this knowledge could be used to assign blocking in subsequent seasons if variation is not influenced by season. However, spatial variability has been shown to vary with weather patterns (Ping and Green, 2000), crops (Vieira and Gonzalez, 2003), and between years (Harris and Scofield, 1920). Variability over time was deemed more important than spatial variability in a study by Erghball and Varvel (1997). In cases where crop performance trials are planted on private farms, which are often not ideal for research plot work, a whole new set of issues such as matching row width, equipment used, and crop management could contribute to experimental error.

Blocking field trials for crop performance evaluations is routine with most researchers using three or four replicates per trial. Little thought is given to concerns raised by Pearce (1995) regarding the possibility that blocks might cause more harm than good by sacrificing degrees of freedom when blocking is not effective; this is seldom an issue in crop performance trials because the number of entries found in most trials is large enough where the sacrifice of a few degrees of freedom would make no difference in the F test. However, Pearce made an important point when he stated "… blocks are often chosen so casually that they correspond to nothing in the field …." Mulla et al. (1990) also stressed that blocking is difficult due to the patterns of soil variability and might not be effective.

Most researchers understand that blocking should be used for local control of variation and the variation controlled usually is related to the soil. Patterson et al. (1978) stated that compact areas must be identified in the field that will contain a block and the blocks should be arranged such that operations can be completed within each block. Compact areas are ideal, but the concept is seldom practiced because most plots are not square. Unfortunately,

D. Bowman*, North Carolina Foundation Seed Producers , Inc., 8220 Riley Hill Road, Zebulon, NC 27597 and V. Kuraparthy, Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7620.
Corresponding author: bowman@ncsu.edu

most researchers do not consider blocking to aid in field operations because most researchers block horizontally or perpendicular to the direction of field operations such as planting and harvesting. As mentioned above, slope direction is most often the primary consideration when blocking a field. Most blocking designs assume a clear-cut field variation with the exception of the row-column designs, whereas spatial analyses such as nearest neighbor analysis (Papadakis, 1937) and trend analysis (Kirk et al. 1980) do not assume a clear-cut variation but a smooth transition in variation.

Pearce (1995) pointed out that the soil at the top of the field is generally less fertile than the soil at the bottom of the slope, but that does not always result in variation following the slope. Bowman (1990) illustrated the use of a spatial analyses (polynomial regression) called trend analysis or Proc Trend on a flue-cured tobacco (*Nicotiana tabacum* L.) trial. The trial was blocked parallel to the direction of the slope (opposite to what one would typically block on a slope); blocking alone was effective in minimizing the field variation for yield. This suggested that blocking based on the direction of slope is not always optimal. Also, effectively blocking for yield did not have the same results for another trait measured, grade index, where variation ran in two different directions and blocking was not effective and would not have been entirely effective in either direction.

Stroup et al. (1994) evaluated wheat (*Triticum aestivum* L.) test data in Nebraska and found that field variability could not be controlled by blocking alone and that standard analysis was insufficient. This was also the conclusion of Gusmão (1986). Stroup et al. (1994) advocated spatial analyses to remove uncontrolled variability. This has been a common theme since Papadakis first introduced this method (Papadakis, 1937) and has been elucidated by Warren and Mendez (1982) and many others (Bartlett, 1978; Campbell and Bauer, 2007; Cullis and Gleeson, 1989; Kempton et al., 1994; Piepho et al., 2008; Williams, 1986).

Warren and Mendez (1982) pointed out that inadequate blocking could result in inflated experimental error and distorted treatment effects, more so with increasing replicates. The authors recognized that there are limits one can accomplish through design alone and they advocated using blocks to control operational variation such as planting, cultivating, and harvesting, i.e., vertical blocking. Finally, they advocated keeping block size as small as possible.

It is generally thought that block shape is critical with the square-shaped block being the most suitable (Patterson et al., 1978). Van Es and Van Es (1993) stressed the need for spatial balance in placement of treatments within a block. Randomization alone will ensure spatial balance only if an infinite or a large number of replicates are used, which is impractical in most field trials. The use of constrained randomization has been advocated (Van Es and Van Es, 1993) such as the balanced nearest neighbor designs (Cressie, 1991). The trend analysis that fits a polynomial response surface to the data also addresses spatial balance (Kirk et al., 1980), but limitations must be placed on the number of terms in the model. Thus, the importance of using a balanced spatial design might not be as critical with trend analysis or with nearest neighbor analysis.

Wilcox and Zhang (1999) demonstrated the possibility of using noncontiguous replicates in soybean (*Glycine max* (L.) Merr.) performance trials to minimize losses due to environmental hazards such as floods. This might not be practical with spatial analyses where residuals are calculated for each plot and there is a greater need for contiguous blocks, but it is acceptable for standard randomized block analyses that assume a clear-cut delineation in variability.

Pearce (1995) stated "It is helpful to have block boundaries where it is safe to stop without damage to the experiment. If the break in operations does lead to a differential effect, it will be absorbed by the blocking system." For most crop performance trials, this means that the trials need to be blocked vertically or in the direction the field operation runs. In other words, sources of variation that the researcher has some control over, such as planting and harvesting, should serve as the basis for blocking, as mentioned by Warren and Mendez (1982).

In lieu of using a row-column design, vertical blocking becomes a viable alternative way to design yield trials. The objectives of this study were to demonstrate the ineffectiveness of blocking and compare precision of three years of horizontal blocking with three years of vertical blocking in the North Carolina Official Cotton Variety Trials.

MATERIALS AND METHODS

Details on plot sizes, dates of planting, soil types, dates of harvest, and general agronomic practices can be found in the North Carolina Crop Science Research Reports (Bowman, 2007, 2008, 2009, 2010, 2011, 2012). Table 1 lists the locations, dates of planting and harvest, and soil types of the various trials. All plots were 12 m in length. Cotton data from 2007 through 2009 were from trials that were blocked horizontally, whereas data from 2010 through 2012 were from trials blocked vertically. The only change was the direction of blocking; most other field plot decisions were the same across years. Differences between locations included dates of planting and harvesting and row width. Some of the trials were in the same field for both horizontal and vertical blocking. The software program TFPlan (Bowman, 2000; Bowman and Kuraparthy, 2012) was used to create all field plans.

Entries changed from year to year with nearly one-third of the cotton entries changing each year (Bowman, 1998). The number of entries changed as well, with average number of entries are shown in Table 2.

Table 1. Agronomic data on North Carolina cotton trials from 2007 to 2012.

Year	Location	Row Width (cm)	Date of Planting	Date of Harvest	Soil Type
2007	Bertie	91	1 May	26 Sep	Norfolk loamy sand
2007	Edgecombe	91	3 May	17 Sep	Norfolk loamy sand
2007	Johnston	91	30 Apr	24 Sep	Norfolk loamy sand
2007	Scotland	97	2 May	19 Sep	Marlboro loamy sand
2007	Washington	97	21 May	8 Oct	Portsmouth fine sandy loam
2008	Bertie	91	5 May	7 Oct	Norfolk loamy sand
2008	Edgecombe	91	6 May	16 Oct	Norfolk loamy sand
2008	Johnston	91	15 May	22 Oct	Norfolk loamy sand
2008	Scotland	97	14 May	23 Oct	Marlboro loamy sand
2008	Washington	97	7 May	15 Oct	Portsmouth fine sandy loam
2009	Edgecombe	91	12 May	23 Oct	Norfolk loamy sand
2009	Johnston	91	13 May	3 Nov	Norfolk loamy sand
2009	Scotland	97	4 May	4 Nov	Marlboro loamy sand
2009	Washington	97	8 May	29 Oct	Portsmouth fine sandy loam
2010	Bertie	91	5 May	22 Sep	Norfolk loamy sand
2010	Halifax	97	11 May	11 Oct	Exum silt loam
2010	Johnston	91	4 May	12 Oct	Norfolk loamy sand
2010	Scotland	97	6 May	13 Oct	Marlboro loamy sand
2010	Washington	97	7 May	14 Oct	Portsmouth fine sandy loam
2011	Bertie	91	5 May	18 Oct	Goldsboro sandy loam
2011	Edgecombe	91	6 May	12 Oct	Norfolk loamy sand
2011	Johnston	91	9 May	21 Oct	Norfolk loamy sand
2011	Scotland	97	2 May	5 Oct	Marlboro loamy sand
2012	Bertie	91	3 May	23 Oct	Goldsboro sandy loam
2012	Edgecombe	91	8 May	31 Oct	Norfolk loamy sand
2012	Johnston	91	2 May	2 Nov	Norfolk loamy sand
2012	Scotland	97	7 May	17 Oct	Marlboro loamy sand

Table 2. Effect of blocking direction on yield and precision.

Blocking Direction	Number of Data Points	Average S.E. (Kg/Ha)^{z}	Average Yield (Mg/ha)	Average Number of Entries
Horizontal	13	38(54)	1.2	30
Vertical	11	44(63)	1.1	35

To make fair comparisons between the data sets, initially we determined if any relationship existed between error variance and productivity level. The natural log of the error variance was regressed on the natural log of the mean yield in a previous study (Bowman et al., 2013). Regression values significantly ($P < 0.05$) different from zero indicate a relationship; i.e., error variances tend to increase with increased mean yields. In the study by Bowman et al. (2013) the b value was 0.85, thus large differences in yield will necessitate adjustments when comparing precision.

Average standard errors were compared between the blocking methods to determine changes in precision (Brownie et al., 1993). The data were analyzed with the standard ANOVA and with Proc Trend; the latter is routinely followed in the NCOVT program (Kirk et al., 1980). To prevent over fitting the model the terms were limited to eight and a significance level of 0.05 was used for adding terms to the model. The data were analyzed using standard ANOVA to see if spatial distance, which we knew would increase with vertical blocking, would lower precision.

Maps of residuals were compared to determine consistency in directions of field variability and probability of direction of blocking impacting precision. This was only performed at locations (Bertie, Scotland, and Washington counties) where the trials were situated in the same field for two or more years. Residuals are plot values minus the average for that particular entry. One iteration of a smoothing technique was used to allow better viewing of the general trend of variation. This smoothing technique entailed averaging the residual values of all plots adjoining the plot in question with that plot to make the adjustment.

RESULTS AND DISCUSSION

Figures 1a-c show residuals of the trials at Scotland County for years 2007 through 2009. The trials were blocked horizontally although the direction of blocking should not impact residual values. Each replicate/block consisted of approximately 3.5 ranges/tiers on the X axis. The pattern of variation was similar between 2007 and 2008 but differed in 2009. In 2008 and 2009 the ANOVA revealed blocking was not effective, i.e., $F > 0.05$. Although Figs. 1a and 1b appear to show similar patterns, the blocks source of variation was significant in 2007 but not the following year; yields the second year were three times higher (1442 vs 457 kg/ha).

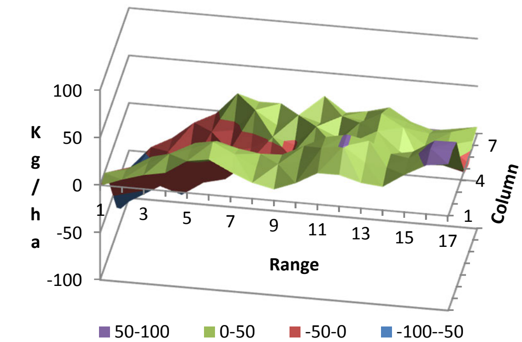

Figure 1a. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2007.

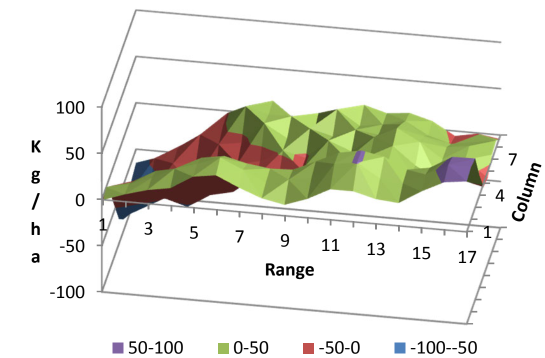

Figure 1b. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2008.

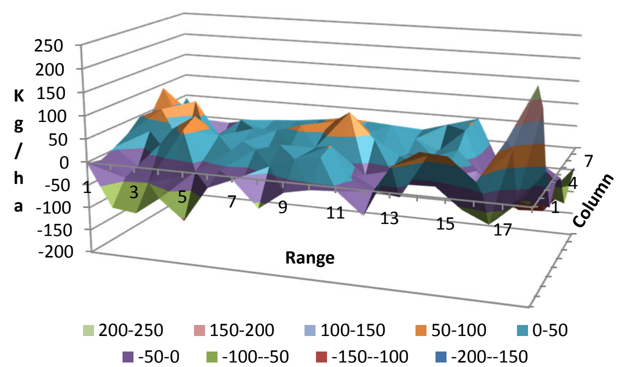

Figure 1c. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2009.

The trial remained in the same field, at the exact location in the field, in Scotland County throughout the 6 yr of this study. For the years 2010 through 2012 the variation continued to change from year to year (Figs. 2a-c), with 2012 showing the greatest variability. During these years, the trials were blocked vertically and each replicate/block consisted of two columns in the graphs as shown on the Z axis. Even though the block effect was significantly greater than 0.05 for 2007, 2011, and 2012, it was not effective in 2008, 2009, and 2010. For years when the trials were blocked vertically (2010-2012), the purpose of blocking was for operational purposes and not for the purpose of removing field variability. Creating field plans with field variability in mind and blocking to remove that variability would be dubious at this location.

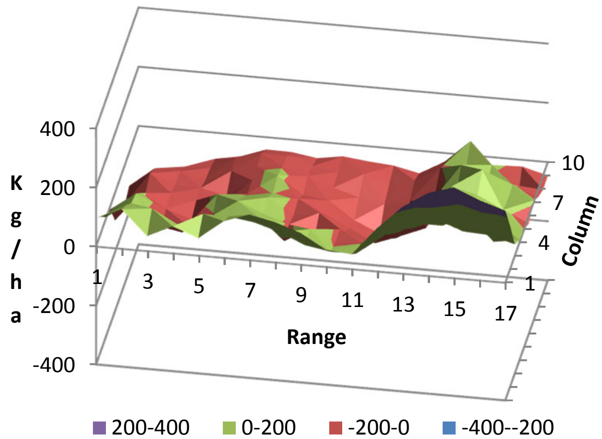

Figure 2a. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2010.

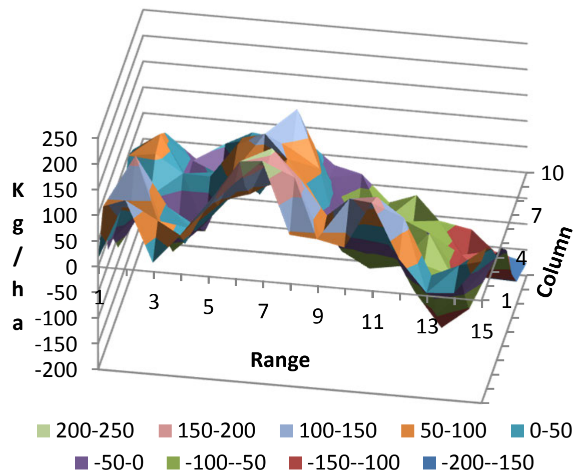

Figure 2b. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2011.

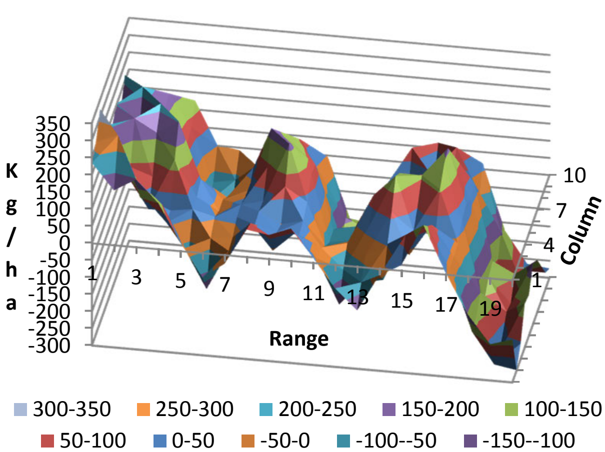

Figure 2c. Residual values (kg/ha) for lint yield in a cotton variety trial in Scotland County 2012.

The trial was in the same field in 2007 and 2009 in Washington County (Figs. 3a and 3b) and blocked horizontally both years. Again, each replicate/block consisted of approximately 3.5 ranges/tiers as shown on the X axis. The field was less productive in the front of the trial (left side of the graph) in both years but also had variation from side to side. Uniformity within blocks was not achieved and would not have been possible in any direction.

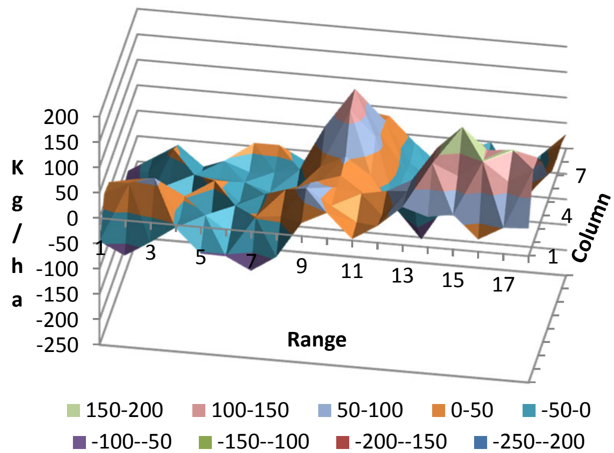

Figure 3a. Residual values (kg/ha) for lint yield in a cotton variety trial in Washington County 2007.

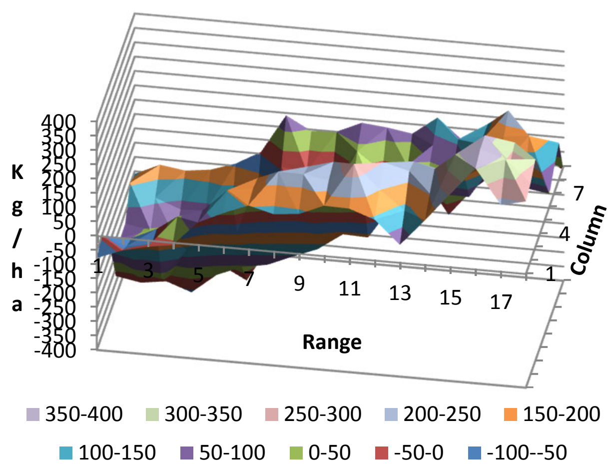

Figure 3b. Residual values (kg/ha) for lint yield in a cotton variety trial in Washington County 2009.

The trial was in the same field for 2007 and 2010 in Bertie County (Figs. 4a and 4b). The trial was blocked horizontally in 2007 with each replicate/block consisting of 6 tiers/ranges and blocked vertically in 2010 with each replicate/block consisting of two columns. Figures 5a and 5b show residual patterns in the same field in Bertie County in 2008 and 2011, which was blocked horizontally the first year with 3.5 tiers/ranges composing each replicate/block and vertically the second year with each replicate/block composed of two columns. Field variation as shown in residual patterns varies from year to year.

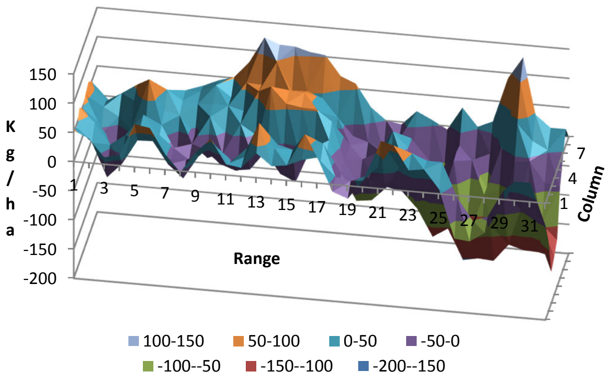

Figure 4a. Residual values (kg/ha) for lint yield in a cotton variety trial in Bertie County 2007.

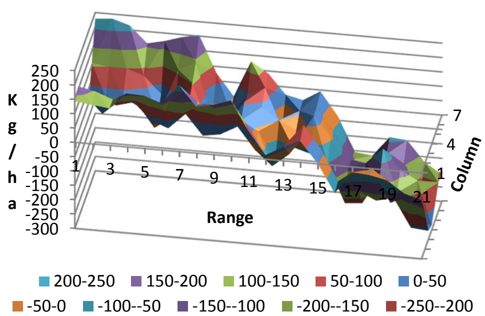

Figure 4b. Residual values (kg/ha) for lint yield in a cotton variety trial in Bertie County 2010.

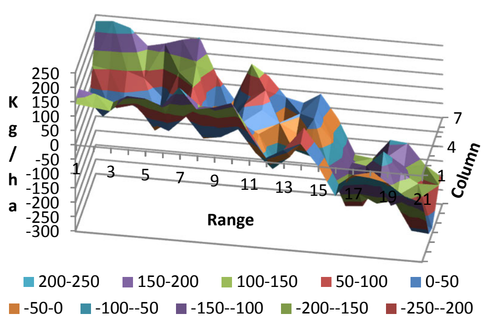

Figure 5a. Residual values (kg/ha) for lint yield in a cotton variety trial in Bertie County 2008.

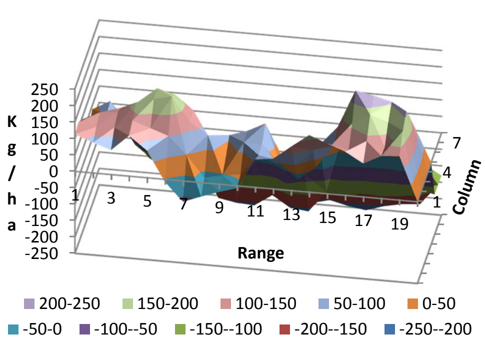

Figure 5b. Residual values (kg/ha) for lint yield in a cotton variety trial in Bertie County 2011.

These graphs reveal the futility in designing cotton yield trials with the goal of removing spatial variability in North Carolina. This agrees with the conclusions of Stroup et al. (1994) and Gusmão (1986).

Relative to the impact of direction of blocking on precision, the difference in mean yield between years of horizontal blocking and vertical blocking (1.2 versus 1.1 Mg/ha) was not large enough to impact the results (Table 2). The difference in standard error between the two blocking arrangements (38 versus 44 kg/ha) was so small as to infer no significance ($P > 0.15$). A slightly larger difference was detected between horizontal blocking and vertical blocking when spatial analyses were not used (54 vs. 63 kg/ha) but, again, this difference was not significant ($P > 0.20$). Also, there were more entries in the years of vertical blocking, which should have resulted in a larger standard error.

In conclusion, patterns of spatial variability revealed no consistency and vertical blocking did not reduce precision when using spatial analyses. Additionally, the number of entries in these trials actually increased in the years 2010 to 2012 compared to the previous three years (Table 2). A natural consequence of these larger tests should have been less precision, but the precision did not decline with vertical blocking and spatial analysis. Again, precision was not compromised by vertical blocking when spatial analysis (Proc Trend) was used.

To emphasize the practical importance of blocking in the direction of field operations the following incidents occurred: (1)Washington 2010—during harvest operations of the cotton OVT, rain began to fall after harvesting the fourth replicate, vertical blocking allowed the test to be salvaged; and (2) Bertie 2009—residual herbicides from the previous year damaged one side of the test. Because it was blocked horizontally the entire test had to be discarded. The Washington County test would not have resulted in valid data without considerable effort if it had been blocked horizontally; the harvested test would have been lost entirely or use of the data would have been unreliable.

SUMMARY

Blocking should be designed to control variation within the power of the researcher because it has not been shown that blocks can be uniformly arranged. When blocking is inadequate and experimental designs assume clear-cut delineations in variation, blocking should be used to assist in field operations, and a spatial analysis should be followed to account for variation. Thus, vertical blocking according to the direction of operations, e.g., the row direction where planting and harvesting can be started and stopped without compromising the data, is advocated. This is best accompanied by use of spatial analyses, such as nearest neighbor analysis or trend analysis, because no direction of blocking will be effective all the time. We propose that the use of vertical blocking in conjunction with spatial analyses or the use of row-column designs will prove beneficial. For those incidents where there is a possibility of variation in the horizontal direction resulting in loss of plots then the row-column designs can be used because replicates can be discarded in either direction. Row-column designs can be obtained from John and Mitchell (1977) or the software CycDesigN (Whitaker et al., 2006).

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance in conducting the trials in this paper by Johnny Denton, Phil Johnson, and Dwight Parrish.

REFERENCES

Bartlett, M.S. 1978. Nearest neighbor models in the analysis of field experiments. J.R. Stat. Soc., Ser. B. 40:147–174.

Bowman, D.T. 1990. Trend analysis to improve efficiency of agronomic trials in flue-cured tobacco. Agron. J. 82:499–501.

Bowman, D.T. 1998. Using crop performance data to select hybrids and varieties. J. Prod. Agric. 11:256–259.

Bowman, D.T. 2000. TFPlan: Software for restricted randomization in field plot design. Agron. J. 92:1276–1278.

Bowman, D.T. 2007. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 222. Raleigh, NC.

Bowman, D.T. 2008. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 225. Raleigh, NC.

Bowman, D.T. 2009. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 228. Raleigh, NC.

Bowman, D.T. 2010. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 231. Raleigh, NC.

Bowman, D.T. 2011. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 234. Raleigh, NC.

Bowman, D.T. 2012. North Carolina Measured Crop Performance—Cotton and Soybean. N.C. Agric. Res. Serv. Rept. No. 238. Raleigh, NC.

Bowman, D.T., and V. Kuraparthy. 2012. TFPlan. Version 3.0. Software for restricted randomization in field plot design. North Carolina State Univ. Res. Rep. 235. Raleigh, NC.

Bowman, D.T., V. Kuraparthy, T. Wallace, and D.C. Jones. 2013. Statistical evaluation of the cotton regional breeders testing network. J. Cotton Sci. 17:279–284.

Brownie, C., D.T. Bowman, and J.W. Burton. 1993. Estimating spatial variation in analysis of data from yield trials: A comparison of methods. Agron. J. 85:1244–1253.

Campbell, B.T., and P.J. Bauer. 2007. Improving the precision of cotton performance trials conducted on highly variable soils of the southeastern USA coastal plain. Plant Breeding 126:622–627.

Cochran, W.G., and G.M. Cox. 1957. Experimental Designs. Wiley & Sons. New York, NY.

Cressie, N.A.C. 1991. Statistics for spatial data. John Wiley and Sons, Inc. New York, NY.

Cullis, B.R., and A.C. Gleeson. 1989. Efficiency of neighbor analysis for replicated variety trials in Australia. J. Agric. Sci., Camb. 113:233–239.

Erghball, B., and G.E. Varvel. 1997. Fractal analysis of temporal yield variability of crop sequence: Implication for site-specific management. Agron. J. 89:851–855.

Gusmão, I. 1986. Inadequacy of blocking in cultivar yield trials. Theor. Appl. Genet. 72:98–104.

Harris, J.A., and C.S. Scofield. 1920. Permanence of differences in the plots of an experimental field. J. Agr. Res. 20:335-356.

John, J.A., and T.J. Mitchell. 1977. Optimal incomplete block designs. J. Royal Stat. Soc. B. 39:39–43.

Kempton, R.A., J.C. Seraphin, and A.M. Sword. 1994. Statistical analysis of two-dimensional variation in variety yield trials. J. Agric. Sci., Camb. 122:335–342.

Kirk, H.J., F.L. Haynes, and R.J. Monroe. 1980. Application of trend analysis to horticultural field trials. J. Am. Hort. Soc. 105:189–193.

Mulla, D.J., A.U. Bhatti, and R. Kunkel. 1990. Methods of removing spatial variability from field research trials. Adv. Soil Sci. 13: 201–213. Papadakis, J.S. 1937. Methode statistique pur des experiences sur champ. Bull. Inst. Amel. Plantes a Salonique. No. 23. Thessalonike, Greece.

Patterson, H.D., E.R. Williams, and E.A. Hunter. 1978. Block designs for variety trials. J. Agric. Sci., Camb. 90:395–400.

Pearce, S.C. 1995. Some design problems in crop experimentation: The use of blocks. Exp. Agric. 31:191–203.

Piepho, H.P., C. Richter, and E. Williams. 2008. Nearest neighbor adjustment and linear variance models in plant breeding trials. Biometrical J. 50:164–189.

Ping, J., and C.J. Green. 2000. Factors affecting yield variability in irrigated cotton. p. 1404–1407 *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 4-8 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN..

Stroup,W.W., P.S. Baenziger, and D.K. Mulitze. 1994. Removing spatial variation from wheat yield trials: a comparison of methods. Crop Sci. 86:62–66.

Van Es, H.M., and C.L. Van Es. 1993. Spatial nature of randomization and its effect on the outcome of field experiments. Agron. J. 85:420–428.

Vieira, S.R., and A.P. Gonzalez. 2003. Analysis of spatial variability of crop yield and soil properties in small agricultural plots. Bragantia 62:127–138.

Warren, J.A., and I. Mendez. 1982. Methods of estimating background variation in field experiments. Agron. J. 74: 1004–1009.

Whitaker, D.E.R., E.R. Williams, and J.A. John. 2006. CycDesigN: A package for the computer generation of experimental designs. Available at: http://www.vsni.co.uk/software/cycdesign/(verified 7 May 2015).

Wilcox, J.R., and G. Zhang. 1999. Noncontiguous replications in soybean performance trials. Agron. J. 91:72–75.

Williams, E.R. 1986. A neighbor model for field experiments. Biometrika 73:279–287.

Williams, E.R., J.A. John, and D. Whitaker. 1999. Example of block designs for plant and tree breeding trials. Austral. New Zealand J. Statist. 41(2):277–284.