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Increasing Precision in Agronomic Field Trials Using Latin Square Designs

Marcus Jones,* Richard Woodward, and Jerry Stoller

ABSTRACT

Spatial variation from soil and related factors often affects the outcome of agronomic field experiments. The randomized complete block (RCB) is the most prevalent design despite inefficiencies that can result in inflated error terms. Experimental designs such as the Latin square (LS) allow for bidirectional blocking and offer the potential to account for spatial variability better. The objectives of this research were to investigate the occurrence of two-way gradients in agronomic field trials and compare the estimated relative efficiency (ERE) of a LS to a RCB. Thirty LS trials were evaluated in 10 states during 2013 across the midwestern United States investigating crop yields of corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and sorghum [*Sorghum bicolor* (L.) Moench]. The results show that 47% of the trials exhibited a two-way gradient, indicating this characteristic is widespread across a large geographic region. Overall, the ERE was increased in 70% of the trials by using the LS design. A lower ERE occurred in 7% of the trials conducted using a LS. Multiple gradients appear common in agronomic field plot trials and enough variation existed between the two blocking directions to justify the use of a LS design. Our data indicate the LS offers a low risk, high reward option of experimental design for controlling spatial heterogeneity and increasing precision. When possible, the LS design should be used in field experiments where the trial area appears uniform and gradients to block against are not obvious.

"The elimination or control of the variable factors, so essential in experimentation of any sort, becomes unusually difficult in field experiments where we have to deal with so many uncontrollable conditions (Smith, 1907)." Variability in agronomic field trials has long been recognized as a factor that can influence the outcome of experiments, as noted by Louie Smith in his manuscript. Variation can stem from a number of sources, including parent materials, topography, vegetation, tillage, fertilization, and cropping history. Kravchenko and Bullock (2000) demonstrated that topographic features alone explained between 6 and 54% of the variability in corn and soybean yields in midwestern soils. Soil physical properties have also been highly correlated with landscape position (Ovalles and Collins, 1986). Organic matter (Miller et al., 1988) and water holding capacity (Hanna et al., 1982) have been shown to vary with slope position. Further, Pierce et al. (1994) showed that the coefficient of variation for pH and the concentration of P, K, Ca, and Mg ranged from 6 to 81% in three Michigan soils. As fields possess a number of characteristics that tend to be nonhomogeneous, physical and chemical soil properties introduce variation into the system.

M. Jones, R. Woodward, and J. Stoller, Stoller Enterprises Inc., Houston, TX 77043. Received 6 May 2014. *Corresponding author (mjones@stollerusa.com).

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The impact can be especially detrimental when treatment effects are small relative to experimental error. New product development programs rely on the ability to differentiate between product treatments that are often evaluated in small plot field research. Agronomic field trials are frequently conducted according to long-established principles of experimental design where experimental units are replicated and treatments are randomly assigned to experimental units (Casler, 2013). Replication, along with randomization, permits the estimation of experimental error or the ability to treat the variance among experimental units alike.

Field trials generally use some form of blocking, where the goal is to arrange experimental units into homogeneous groups. The goal of blocking is to reduce the experimental error by controlling the contribution of sources of variation among the experimental units. The RCB is the most used experimental design in agronomic field trials. A review of all research published in Volumes 93 to 95 of *Agronomy Journal* revealed that 96.7% of agronomic field experiments were conducted using a RCB (van Es et al., 2007). From a statistical perspective, the RCB relies on the assumption that variability among plots within a block is small relative to variability among blocks (Clewer and Scarisbrick, 2001). To satisfy this requirement, prior knowledge of the variability occurring within the trial site is helpful when positioning blocks. In practice, however, researchers often arrange blocks based on logistical convenience or in an arbitrary fashion. Incorrect positioning of blocks

Abbreviations: ERE, estimated relative efficiency; LS, Latin square; RCB, randomized complete block.

without prior knowledge of spatial variability can severely limit the efficiency of a RCB.

A common challenge in positioning blocks is that field plot trials are often placed within visually uniform fields. Potential gradients from slopes or tillage operations may be discernible, whereas variability due to local pest problems, residual fertility, and soil characteristics may not be visually apparent. Blocks that are positioned incorrectly have the potential to reduce the precision at which treatments are compared (Lin et al., 1993). This situation could result in an inflated error term, where treatment differences are declared to be nonsignificant despite large numerical differences between treatment means. Increasing the number of replicates will lower the experimental error but the finite availability of land devoted to research may limit the researcher's willingness to do so. Experimental designs that allow for bidirectional blocking can help guard against incorrect block positioning when multiple gradients exist but may not be obvious.

Adequately controlling spatial variation may require the use of additional blocking criteria. Experimental designs that use two blocking factors include the LS, Youden squares, and general row–column designs. A review of these designs can be found in Federer (1955) and Mead (1988). The LS is a row–column design that is blocked in two directions and a complete set of treatments occurs once in each row and column. This two-way stratification allows for the reduction of two sources of variation from the experimental error, which may be considerably reduced. Latin square designs are particularly useful in agricultural settings where multiple gradients may occur. The LS requires the number of treatments to be equal to the number of rows and the number of columns. Thus, trials with few treatments (four to eight) lend themselves well to a LS design. When the number of treatments is ≤ 4, a LS with multiple squares can increase the degrees of freedom for the error term. The LS layout can also be used as the whole plot for split-plot randomizations (Casler et al., 2000, 2001) and modified augmented designs (Casler et al., 2000; Lin and Poushinsky, 1985). A LS combined with a modified augmented design can accommodate large-scale trials with many treatments.

The analysis of LS designs is only slightly more complicated than that for a RCB and procedures exist for omitting one or more treatments, rows, or columns if necessary (Yates, 1936). Previous research by Cochran (1940), Ma and Harrington (1948), and Evans and Thompson (1984) illustrate the potential efficiency of the LS compared with the completely randomized and RCB designs. However, in practice, the LS is rarely used by current agricultural scientists (van Es et al., 2007). Much like the RCB, the LS will only increase precision if the variation originating from the second blocking direction is large enough to offset the loss of degrees of freedom used to estimate experimental error.

Casler (2013) noted that the inability to detect differences among treatment means is the result of an error in the experimental design, the treatment design, or the experimental conduct or results from a lack of true differences among treatment means. Intensely managed agronomic landscapes that are continually in production need to be recognized as a significant contributor of variation that, if left unchecked, will favor nonsignificant data. Too often, the process of choosing the experimental and treatment design are overlooked and the underlying principles necessary from a statistical perspective are not considered in the planning process. Researchers and stakeholders involved in product development need confidence in the results of trial data to make precise decisions regarding treatment effects. The purpose of this research was to investigate the occurrence of variation resulting from two-way gradients in agronomic field trials and to compare the relative efficiencies of a LS and a RCB.

MATERIALS AND METHODS

To investigate the occurrence of variability from two-way gradients in agronomic field trials, 30 LS field trials were conducted during 2013 across 10 states primarily located in the Midwest region of the United States (Illinois, Indiana, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, Texas, and Wisconsin). Trials were conducted by land-grant universities and private contract researchers investigating crop yield of corn, soybean, and sorghum. Common to dedicated research facilities, each trial was located on a plot of land that had a rich but often unknown agronomic history. All trial sites appeared visually uniform, with the exception of occasional slopes (Table 1). Hybrids represented a range of seed brands and maturities and hybrid selection at each site was based on plant adaptation to local environmental characteristics.

Experimental units for each trial were arranged as a LS of six rows and six columns and individual experimental plots were comprised of four rows, each 5.3 to 15.2 m in length. Treatments included micronutrient and phytohormone products manufactured by Stoller USA (Stoller USA, Houston, TX). Management of each trial site consisted of pest and fertility practices typical for the region. Each site used a corn–soybean or corn–soybean–sorghum crop rotation. Research plot combines that were capable of recording plot yield, weight, and moisture were used to harvest the middle two rows of each plot.

Within the 30 trials, particular attention was directed toward a sorghum field experiment that illustrated the presence of gradients, the importance of block orientation, and how design control can improve precision in field trials. The trial was conducted on a Ulysses silt-loam (fine-silty, mixed, superactive, mesic Aridic Haplustolls) with 21 mg kg^{-1} P, 647 mg kg^{-1} K, 1.3% organic matter, and a pH of 8.4. Individual experimental

Table 1. Characterization of trial host and gradient type of 30 Latin square field trials conducted in 2013.

Trial host	Frequency	Frequency by crop type		
		Corn	Soybean	Sorghum
Land-grant university	18	6	11	1
Private contract researcher	12	6	4	2
Total	30†	12	15	3
Gradient type				
Two-way gradient	14	6	6	2
One-way gradient	12	4	8	0
Gradient not present	4	2	1	1
Total	30†	12	15	3

† Trials were conducted in Illinois (8), Indiana (3), Iowa (3), Kansas (1), Minnesota (1), Missouri (2), Nebraska (5), North Dakota (4), Texas (1), and Wisconsin (2).

Table 2. Source of variation, degrees of freedom and expected mean square (EMS) for a Latin square experimental design.

Source	df	EMS
Row	$t - 1$	
Column	$t - 1$	
Treatment	$t - 1$	$\sigma^2 + t\,\Phi\,(T)$
Error	$(t - 1)(t - 2)$	σ^2
Total	$t^2 - 1$	

plots consisted of four rows, 15.2 m in length at 0.76 m spacing, with the two center rows used to collect yield data. Weed control consisted of a pre-emergence application of acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide], atrazine [6-chloro-*N*-ethyl-*N*′(1-methylethyl)-1,3,5-triazine-2,4-diamine], and paraquat dichloride (1,1′-dimethyl-4,4′-bipyridinium dichloride) and postemergence applications of pyrasulfotole [(5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)(2-[methylsulfonyl]-4-[trifluoromethyl]phenyl)methanone], bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), and atrazine, each applied at recommended label rates.

Analysis of variance for all trials was performed using the CAR package and lm, anova, and summary functions within R software (R Development Core Team, 2013) and ANOVA was conducted for yield, with rows, columns, treatment, and error as the sources of variation (Table 2). An α of 0.10 was used as a cutoff to determine the presence or absence of a significant gradient for rows or columns. The mean squares from each analysis were used to calculate the ERE of a LS compared with a RCB according to the following equation by Kuehl (2000):

$$s^2_{\mathrm{RCB}} = \frac{\mathrm{mean\_square\_columns} + (t-1)\,\mathrm{MSE}}{t}$$

where MSE is the mean square error from the current LS ANOVA and t is the number of treatments. A similar equation is used to calculate the relative efficiency for the row criteria of the LS. A correction for estimating σ^2 by s^2 was calculated by:

$$\frac{(f_{\mathrm{LS}}+1)(f_{\mathrm{RCB}}+3)}{(f_{\mathrm{LS}}+3)(f_{\mathrm{RCB}}+1)}$$

where f_{ls} and f_{rcb} are the error degrees of freedom for the LS and RCB, respectively (Kuehl, 2000).

Each ERE value represents the multiple of replications needed in a RCB to achieve the same level of precision in estimating treatment effects as seen in the LS. An ERE value less than 1.0 indicates a RCB is a more efficient design, whereas values greater than 1.0 indicate that the LS provides greater precision.

RESULTS AND DISCUSSION

The potential utility of the LS is illustrated by considering the results of the sorghum dataset. The data indicate that Product 3 increased yield by 406 kg ha^{-1} compared to the control, a difference that could be economically beneficial to a practitioner depending on the product cost (Fig. 1A). Because the LS contains a complete set of treatments in each row and column, the effects from the two-way stratification can indicate the presence of gradients irrespective of treatment effects. Further investigation of the data reveals that yield is strongly influenced by row and column position, indicating the presence of multiple gradients within the trial site. A clear pattern appears, illustrating that sorghum yields increased with increasing row position (Fig. 1B). In contrast, sorghum yields decreased with increasing column position (Fig. 1C). The interpretation of the data changes dramatically depending on the experimental design and the resulting ANOVA (Table 3). When the data are analyzed as a LS accounting for both blocking directions, the model is significant at α = 0.10 (Table 3). Treatment contrasts between the control and Product 3 results in a *P* value of 0.0623. Block orientation greatly influences the results if the data are analyzed as a RCB. Analysis of the data as a RCB with either rows or columns as the blocking factor results in treatment *P* values of 0.7367 or 0.1242, respectively (Table 3). Analysis of the dataset as a RCB regardless of blocking direction results in a Type II experimental error. This single example illustrates the potential effect of gradients in agronomic field trials and the importance of proper block orientation, along with design control, to account for spatial heterogeneity.

Table 3. Analysis of variance table for a dataset analyzed as a Latin square or as a randomized complete block using rows or columns as the blocking factor. Yield (kg ha^{-1}) of micronutrient and phytohormone products was evaluated for yield enhancement of sorghum compared to an untreated control in 2013.

Source of variation	df	Sum of squares	Mean square	*F* value	*P* > *F*
ANOVA results when using rows and columns as the blocking factor					
Rows	5	362.88	72.58	2.2536	0.08856
Columns	5	2896.85	579.37	17.9906	<0.0001
Treatment	5	389.60	77.92	2.4195	0.07181
Error	20	644.08	32.20	–	–
ANOVA results when using rows as the blocking factor					
Rows	5	362.9	72.575	0.5124	0.7643
Treatment	5	389.6	77.919	0.5501	0.7367
Error	25	3540.9	141.637	–	–
ANOVA results when using columns as the blocking factors					
Columns	5	2896.8	579.37	14.3842	<0.0001
Treatment	5	389.6	77.91	1.9345	0.1242
Error	25	1007.0	40.28	–	–

The individual experimental ANOVAs revealed 14 of the 30 trials (47%) exhibited a two-way gradient (Table 1). Twelve of the trials exhibited a one-way gradient and no gradient was present in the four remaining trials. Each gradient type occurred within each crop species, with the exception of sorghum, which did not display a one-way gradient. Bidirectional gradients were observed in 8 of the 10 states included in this evaluation, indicating that these occurrences were not limited to a particular location (data not shown). The data indicate that although blocking is a sound practice, multiple gradients are commonplace in agronomic fields and bidirectional blocking is needed to appropriately account for variability.

Estimated relative efficiency was increased in 21 of the 30 trials (70%) by using the LS design (Fig. 2). Precision was increased in all 14 trials exhibiting a two-way gradient. Of the 12 trials that exhibited a one-way gradient, the precision was increased in seven of those instances regardless of the blocking direction chosen. Of the five remaining trials that exhibited a one-way gradient and two of the trials that did not have a gradient, the RCB would have provided more precision only

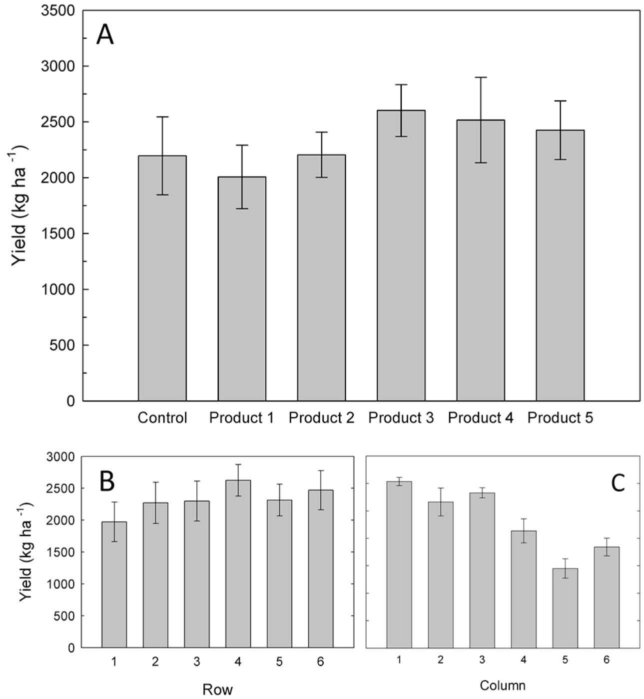

Fig. 1. Yield (kg ha^{-1}) of five products evaluated for increased productivity of sorghum compared to an untreated control evaluated in a Latin square design (A). Yield (kg ha^{-1}) averaged across treatments by row (B) or column (C). Error bars represent the standard error of the mean (n = 6).

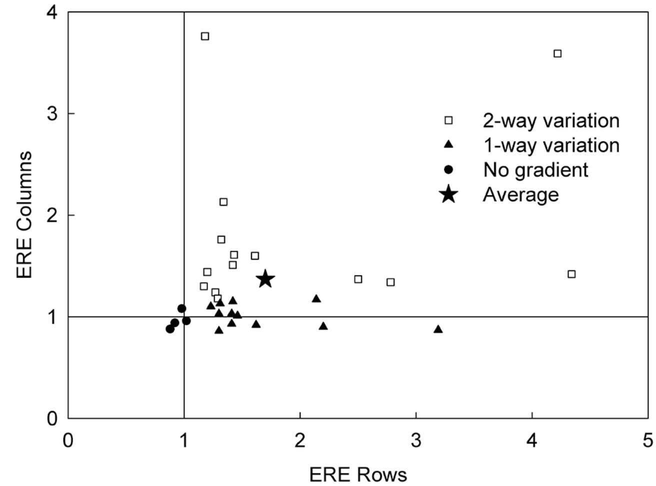

Fig. 2. Gradient type and estimated relative efficiency (ERE) of 30 Latin square (LS) trials conducted in 2013. Each ERE value represents the multiple of replications needed in a randomized complete block (RCB) to achieve the same level of precision in estimating treatment effects as the LS. An ERE value less than 1.0 indicates that a RCB is a more efficient design, whereas values greater than 1.0 indicate that the LS provides greater precision.

if the proper blocking direction was chosen. Otherwise, the LS would be a more efficient design. A lower ERE would have resulted in 2 of the 30 trials (7%) with the LS design.

The average ERE from the 21 trials where precision increased was 1.67 (range: 1.01–4.34) by including a second blocking direction. Based on the average gain in efficiency from either adding rows or columns, a RCB would require 1.67(6) = 10 replications of each treatment to have an estimated variance of the treatment equal to that from the LS design in these examples. In contrast, the average loss in efficiency from the two trials where precision decreased was 0.91 (range: 0.88–0.94). The potential change in precision from the seven remaining LS trials would depend on the blocking direction being perceived (or not) before the experiment. The average ERE value of the seven LS trials in question was 1.3 when both blocking directions were included. In comparison, the average ERE value would be 1.69 if the blocks were oriented correctly or 0.92 if oriented incorrectly. Overall, the average ERE value of all 30 LS trials was 1.54 (1.7 for rows and 1.37 for columns).

CONCLUSIONS

Field plot trials are subject to a myriad of factors that could result in the occurrence of one or multiple gradients (On Farm Network, 2013). Uncontrolled variation has the potential to inflate the error term and distort comparisons among treatments. Arranging experimental units into homogenous groups, known as blocking, is a fundamental design principle with the goal of increasing precision by controlling the adverse effects of soil heterogeneity (Clewer and Scarisbrick, 2001). Blocking is indeed justified, as only 13% of the trials in this evaluation did not exhibit a significant gradient. Single direction blocking is most effective when a gradient is unidirectional and apparent and when blocks are positioned perpendicular to the gradient. In practice, however, gradients are not always obvious and trials may be placed into fields that appear homogenous. Blocks are often arranged according to logistical constraints resulting in improper block orientation which limits the efficiency of the RCB (Lin et al., 1993). The bidirectional blocking of LS designs is added insurance to control variation when gradients may not be apparent. Ideally, possible gradients from topography, tile lines, equipment patterns resulting from tillage or fertilizer applications, and information relating to soil characteristics would all be considered when orienting an LS trial.

Our data indicate that bidirectional gradients are commonplace in agronomic field trials, with nearly half the experiments exhibiting this characteristic. Despite the regularity of two-way gradients and increase in ERE for over two-thirds of the trials included in this evaluation, the LS design is uncommon in current field research. Of 310 known trial designs published in *Agronomy Journal* from 2001 to 2003, only one made use of the LS (van Es et al., 2007). It is likely that the requirement that the number of rows and columns equals the number of treatments limits the use of the LS. In these cases, a modified augmented design using a split-plot randomization of a LS layout could be used as an alternative (Casler et al., 2000). Incomplete block designs (Casler, 2013) or some form of nearest neighbor adjustment (Stroup et al., 1994) would provide an alternative to the RCB to improve the accuracy and precision of the data. Current agronomic literature, however, indicates that the RCB is the overwhelming design of choice and researchers apparently are comfortable with the RCB and consider it convenient and sufficient. Accounting for bidirectional gradients does not appear to be a priority or is not recognized among agronomic researchers. The use of a RCB in these trials would have resulted in artificially inflated error terms, increasing the difficulty of differentiating differences among treatments for the majority of trials.

These findings indicate that multiple gradients are common in agronomic field plot trials and enough variation existed between the two blocking directions to justify the use of a LS design. Latin square designs offer the capability for bidirectional blocking and can be an excellent safeguard against unrecognizable gradients. The results from these trials also indicate that the LS was more efficient than the RCB across a majority of field trials covering a large geographic area. A LS design should be used in field experiments where the trial area appears to be uniform and gradients to block against are not obvious. Furthermore, our data indicate that the LS offers a low risk, high reward option of experimental design for controlling spatial heterogeneity and increasing precision.

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