Increasing Precision in Agronomic Field Trials Using the Latin Square Design

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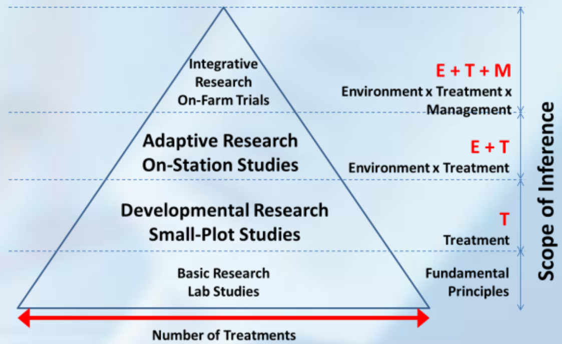
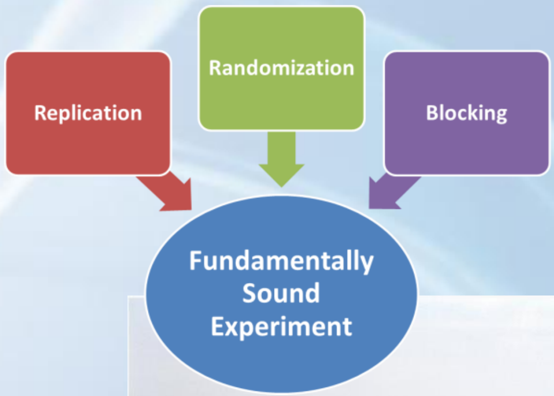
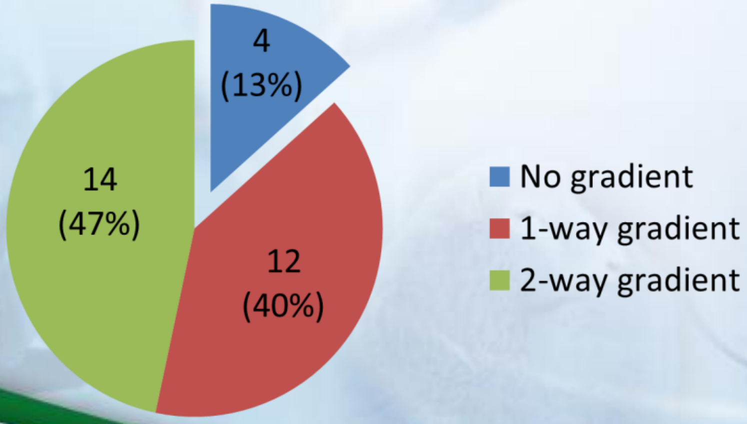
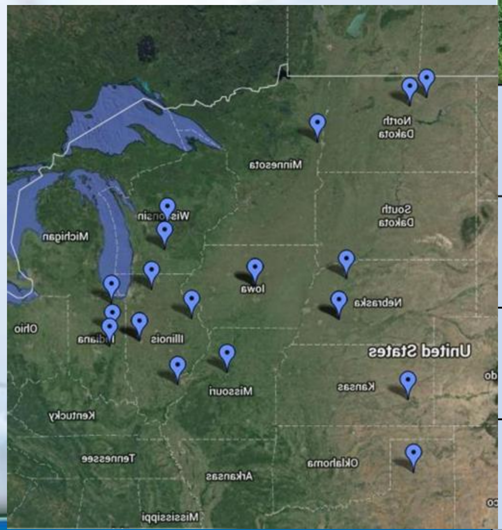
Overview



- Research strategies and principles of design
- The Randomized Complete Block Design (RCBD)
- Sources of variation in the field
- Features of Latin Square Design
- Relative Efficiency RCBD vs LS
- Case study of 30 Midwest trials designed as LS but ANOVA as RCBD and as LS







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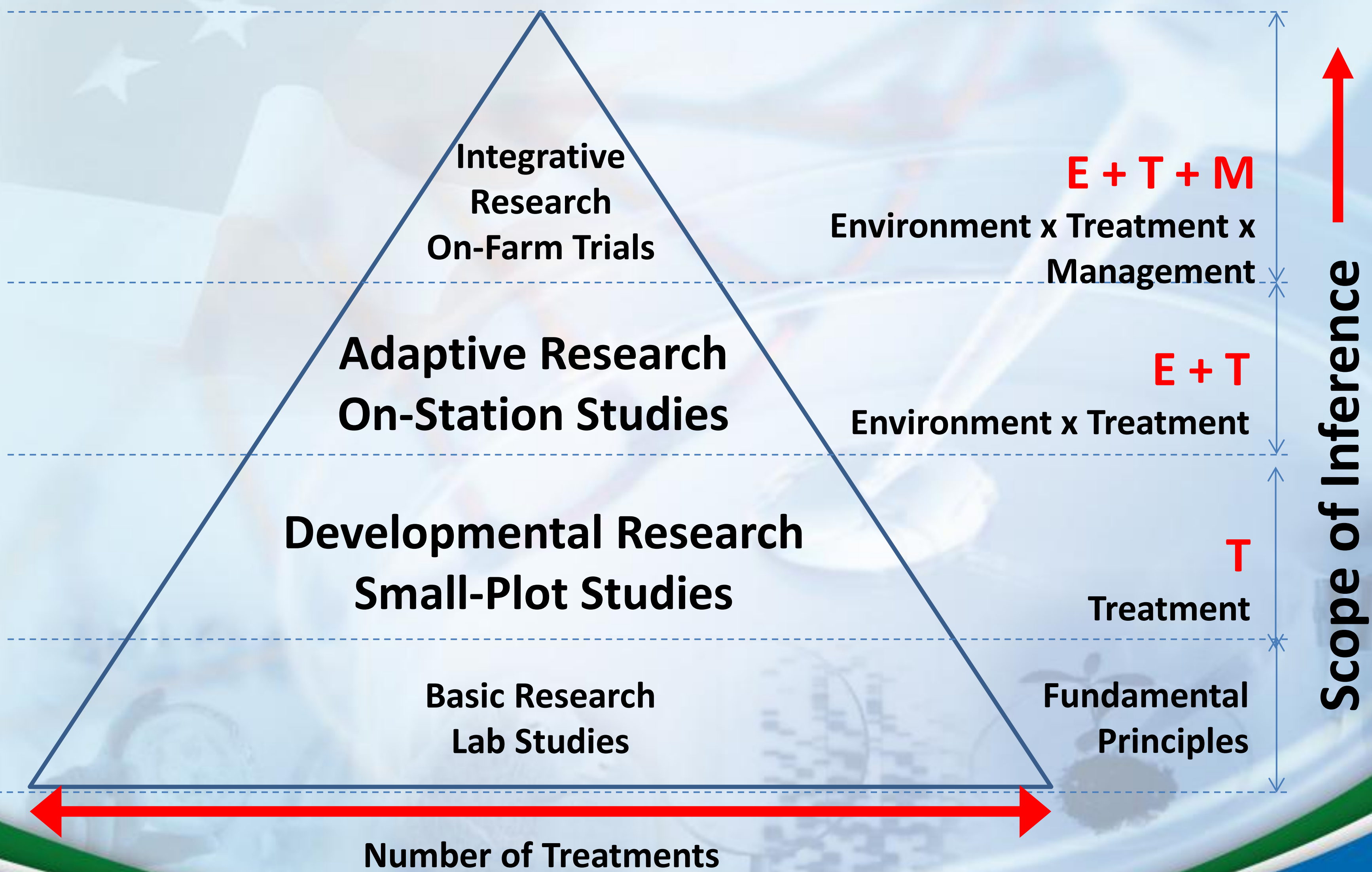
D	A	B	C

Research Strategies for Product Development



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Explanation of Statistical and Experimental Design Terms

Term	Definition
Experimental Design	the set of rules and procedures by which the treatments are assigned to experimental units
Experimental Unit	the smallest unit to which a treatment is applied
Block	a group of (presumably) homogeneous experimental units (a complete block contains all treatments)
Replication	the practice of applying each treatment to multiple and mutually independent experimental units
Randomization	the practice of assigning treatments to experimental units such that each unit is equally likely to receive each treatment
Experimental Error	the variance among experimental units treated alike, often symbolized as σ^2 or σ_e^2.
Precision	the inverse of experimental error, $1/ \sigma_e^2$

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RCBD used in 96.7% of all known trial designs in Agron. J from 2001-2003

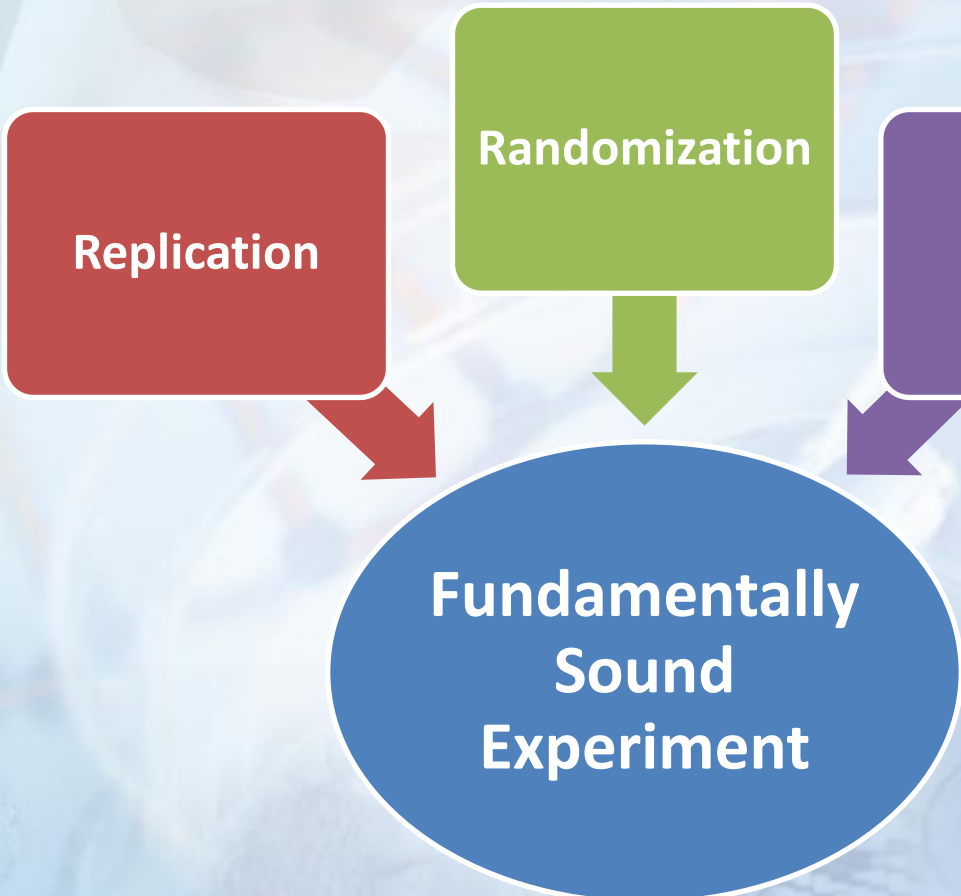
Spatially-Balanced Complete Block designs for field experiments

H.M. van Es[a,*], C.P. Gomes[b,c], M. Sellmann[b], C.L. van Es[c]

Characterization of designs used in field-based experiments reported in Agronomy Journal volumes 93 through 95

Design type	Frequency	Mean	
		# of treatments	# of replicates
Randomized Complete Block	300	8.0	3.8
Completely Randomized	4	17.3	14.7
Randomized Incomplete Block	3	43.6	3.7
Split Block	2	4.0	4.5
Latin Square	1	4	4
Field strips — unknown design	9	4.6	3.2
Split plot — with unknown main-plot arrangement	42	NA	NA
Other	53	NA	NA
Total	414		

Principles of Experimental Design



Randomized Complete Block Design

Each set of treatments occurs once in each replication
Blocks should be perpendicular to the gradient variable

Replication 1	A	D	B	C
Replication 2	D	C	B	A
Replication 3	C	A	D	B
Replication 4	B	C	D	A

Gradient (i)

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Randomized Complete Block Design

Linear Additive Model

$$Y_{ij} = \mu + B_i + T_j + \varepsilon_{(ij)}$$

Where:

Y_{ij} = observation from the ij^{th} experimental unit [dependent variable]

μ = overall mean

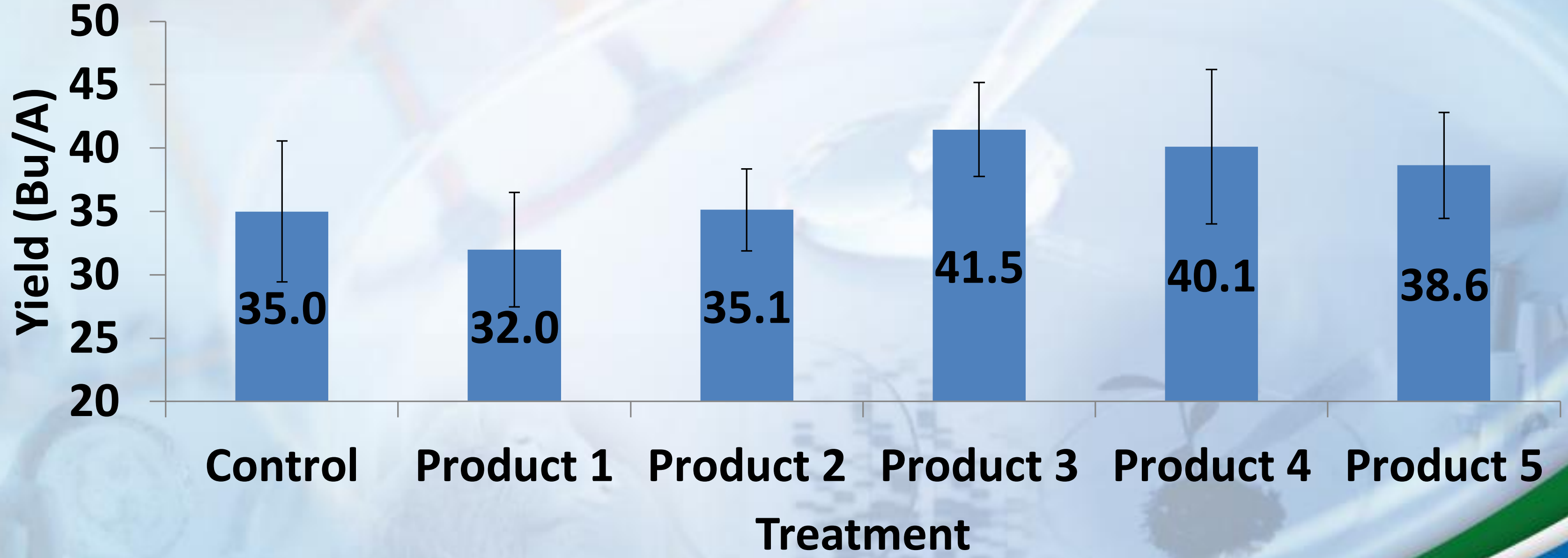
B_i = effect of the i^{th} block

T_j = effect of the j^{th} treatment

$\varepsilon_{(ij)}$ = residual error

Randomized complete block design with 6 replications. Error bars represent standard error of the mean.

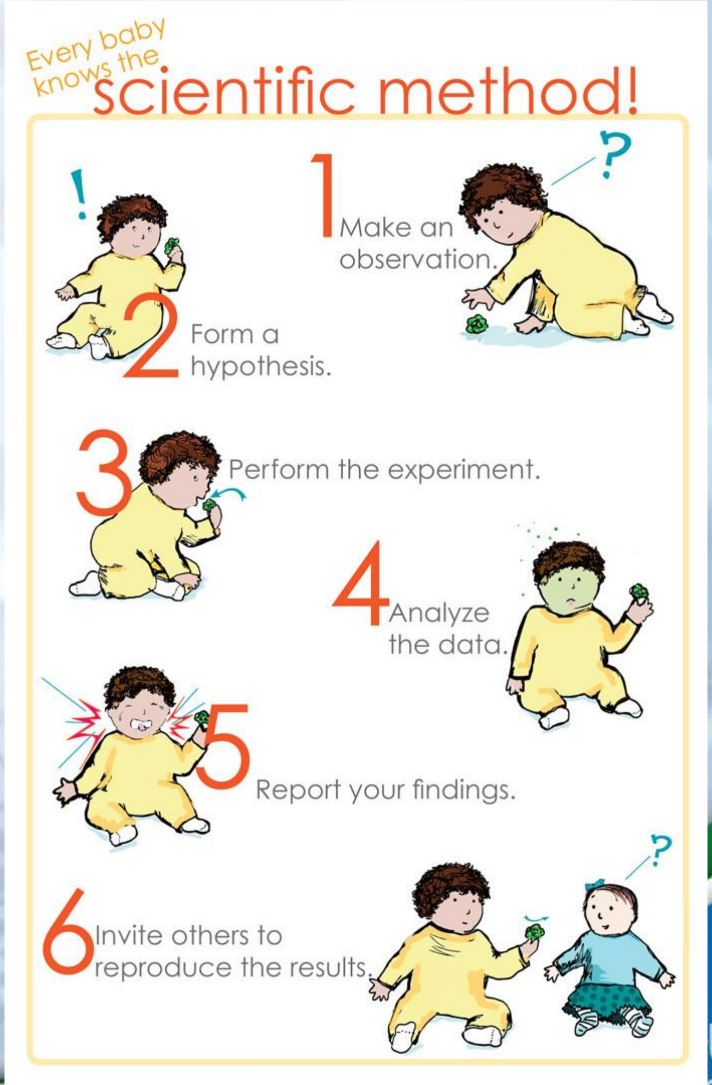
Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
Rep	5	362.9	72.575	0.5124	0.7643
Treatment	5	389.6	77.919	0.5501	0.7367
Error	25	3540.9	141.637	-	-



How does this happen?

Casler, M.D. 2013. Fundamentals of Experimental Design: Guidelines for Designing Successful Experiments. Agron. J. 105:1-14.

1. **A poorly designed experiment with insufficient power to detect differences between treatment means.**
2. **Poorly designed treatments that didn't reflect the initial hypothesis; positive controls / negative controls.**
3. **An improperly conducted experiment without proper oversight over treatment and data collection.**
4. **Lack of true differences between the treatment means.**



Principles of Blocking

Textbook

- Plot-to-plot variation within blocks is smaller than block-to-block variation

- Blocks are oriented perpendicular to a gradient

Assumption

- Prior knowledge of site variation is required

- Variation follows a gradient

Ground view of 30 inch row-spaced corn.
While you can see the plants,
soil shows through the crop.

Photo courtesy of the Iowa Soybean Association

Aerial view of the same field taken on the same day. Notice how you see mostly soil differences compared to plant differences.

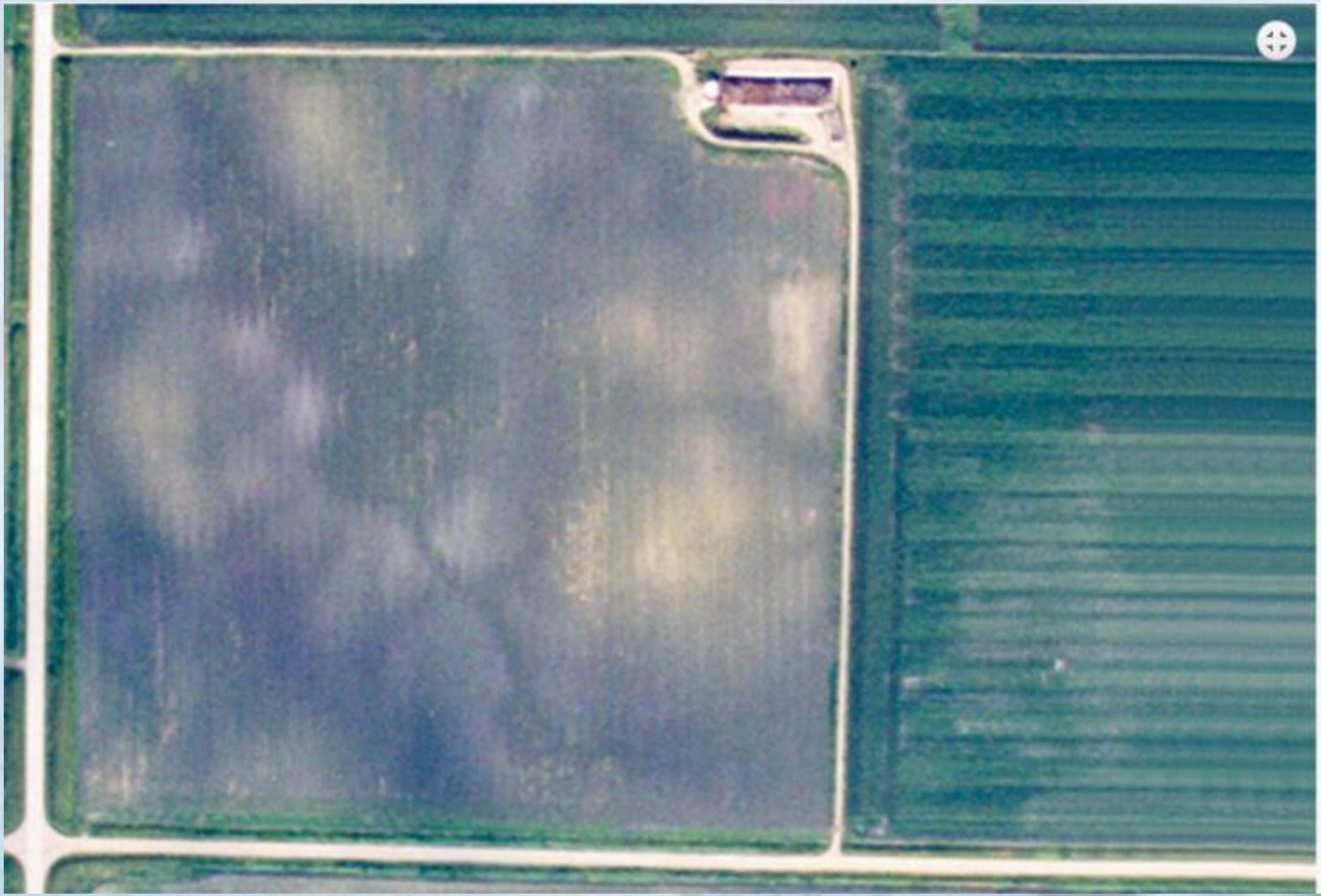


Photo courtesy of the Iowa Soybean Association

Tile Lines Visible within a Field

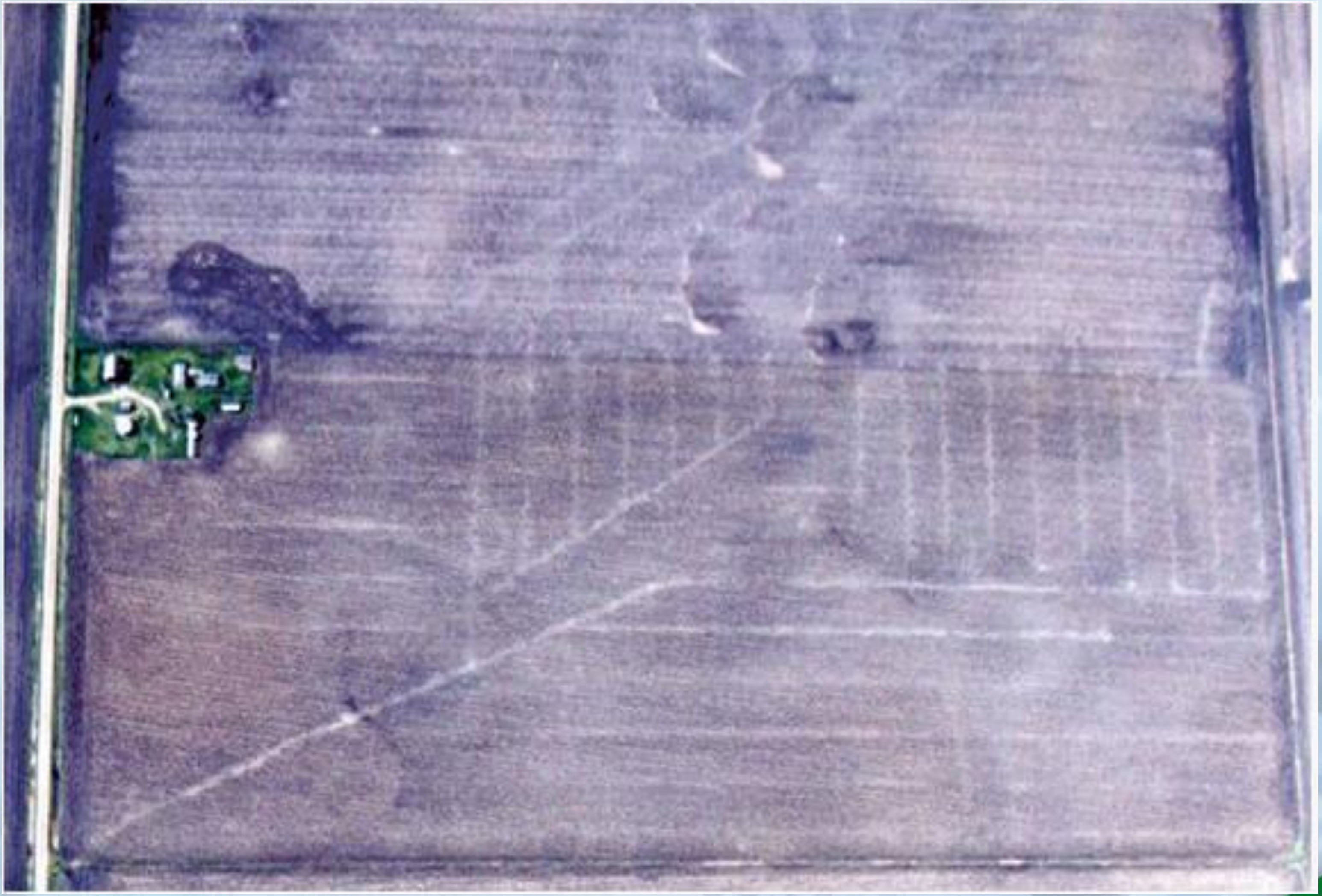


Photo courtesy of the Iowa Soybean Association

Equipment Patterns

Photo courtesy of the Iowa Soybean Association

Anhydrous Ammonia Skips

Photo courtesy of the Iowa Soybean Association

Uneven Distribution of Residue

Photo courtesy of the Iowa Soybean Association

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Features of Latin Square Design

- Reduce experimental error by blocking on two perpendicular sources of variation [simultaneously capture two sources of nuisance variability]
- Each treatment appears only once in each row and each column
- Treatments = Replications [4 x 4, 5 x 5, 6 x 6]
- Low degrees of freedom for small squares

Gradient (i)

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

Gradient (j)

Latin Square Design

Linear Additive Model

$$Y_{ijk} = \mu + R_i + C_j + T_k + \varepsilon_{(ijk)}$$

Where:

Y_{ijk} = variable to be analyzed from i^{th} row and j^{th} column and the k^{th} treatment [dependent variable]

μ = overall mean

R_i = effect of the i^{th} row

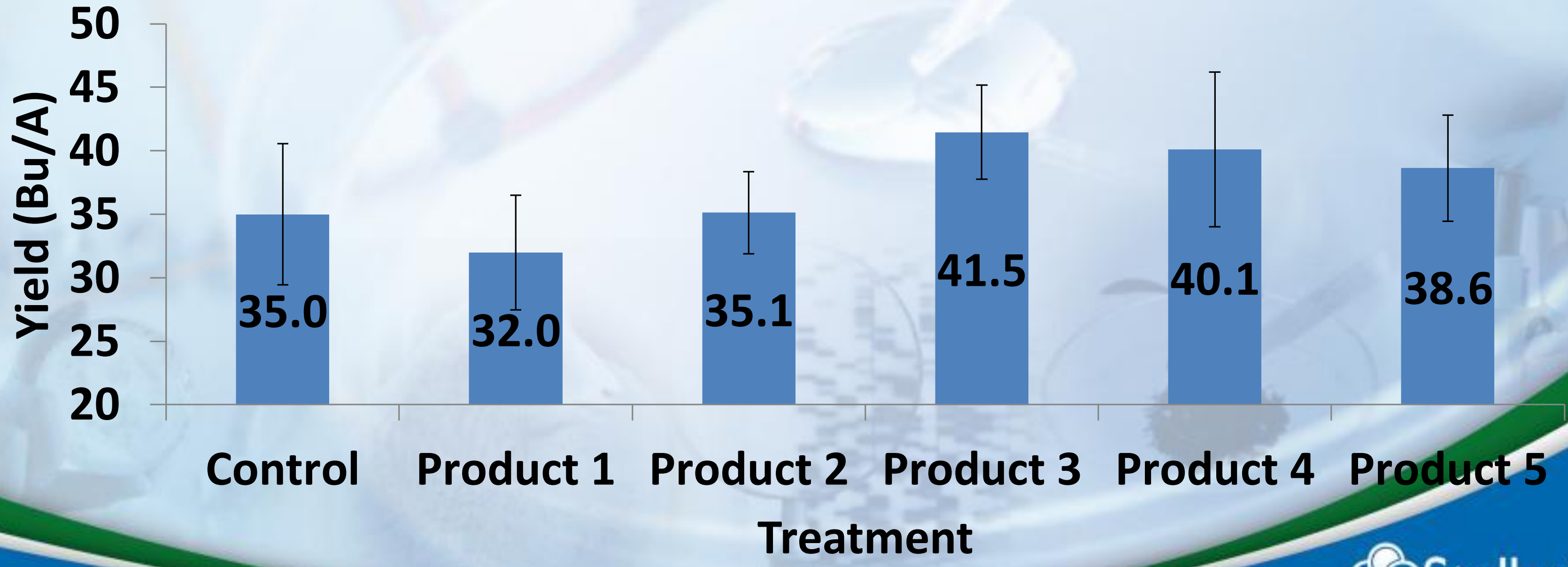
C_j = effect of the j^{th} column

T_k = effect of the k^{th} treatment

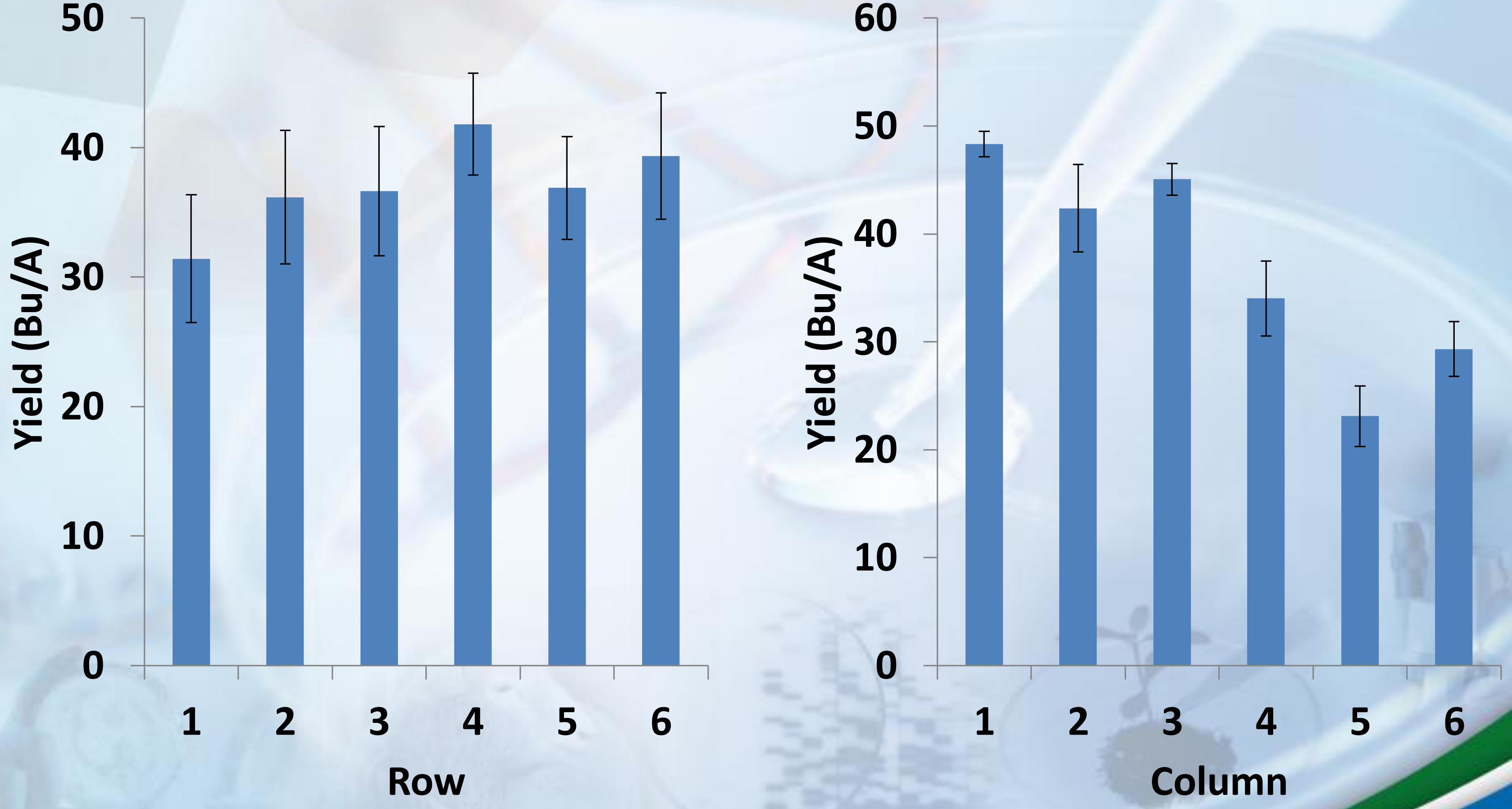
$\varepsilon_{(ijk)}$ = residual error

Original Example
Randomized complete block design with 6 replications. Error bars represent standard error of the mean.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
Rep	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	-	-



Yield Charted by Rows & Columns



Rows as block

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
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	-	-

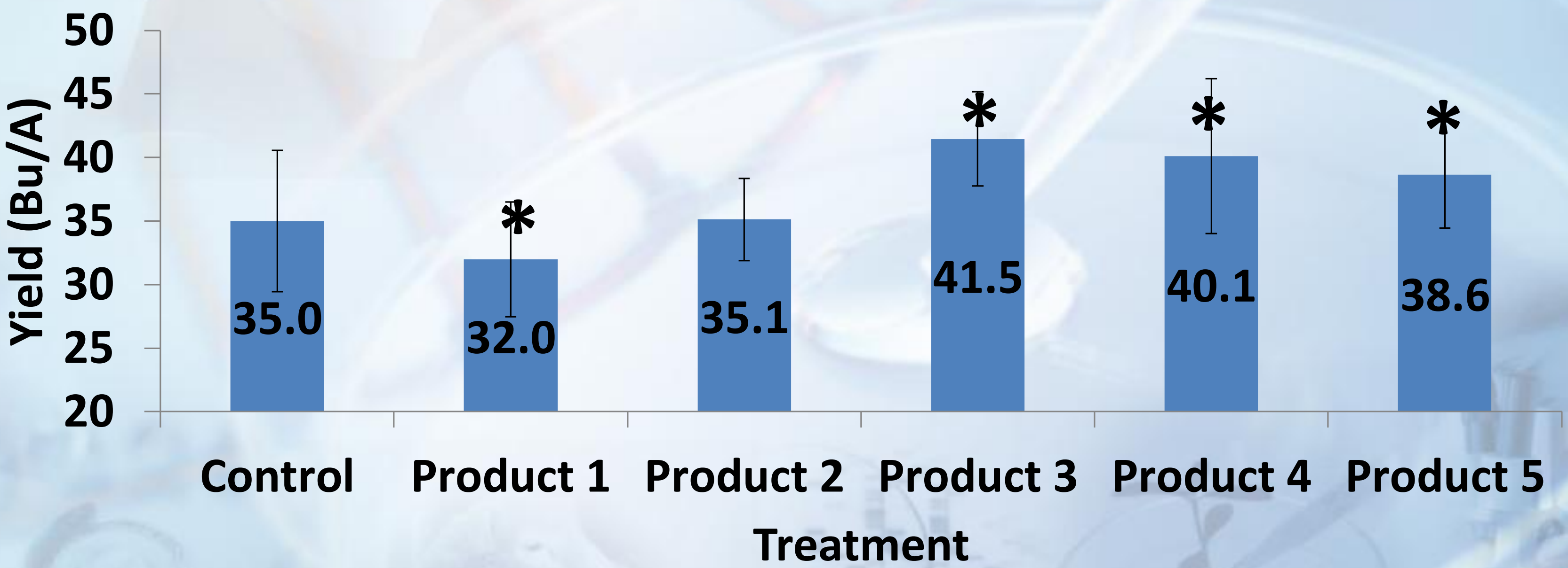
Columns as block

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
Columns	5	2896.8	579.37	14.3842	1.122e-06
Treatment	5	389.6	77.91	1.9345	0.1242
Error	25	1007.0	40.28	-	-

Rows and Columns as blocks

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
Rows	5	362.88	72.58	2.2536	0.08856
Columns	5	2896.85	579.37	17.9906	8.578e-07
Treatment	5	389.60	77.92	2.4195	0.07181
Error	20	644.08	32.20	-	-

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob > F
Rows	5	362.88	72.58	2.2536	0.08856
Columns	5	2896.85	579.37	17.9906	8.578e-07
Treatment	5	389.60	77.92	2.4195	0.07181
Error	20	644.08	32.20	-	-



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Relative Efficiency Compared to RCBD

Robert O. Kuehl, 2000, Design of Experiments: Statistical Principles of Research Design and Analysis, 2nd Edition.

- To compare with a RCBD using rows as the blocks

$$RE = \frac{MS\ Rows + (t-1)MSE}{t}$$

- To compare with a RCBD using columns as the blocks

$$RE = \frac{MS\ Columns + (t-1)MSE}{t}$$

MS = Mean Square
MSE = Mean Square Error
t = treatments

Relative Efficiency compared to RCBD

- **To compare with a RCBD using rows as the blocks**

$$RE = 3.76 \text{ gain by adding columns}$$

- **To compare with a RCBD using columns as the blocks**

$$RE = 1.18 \text{ gain by adding rows}$$

Research Objective

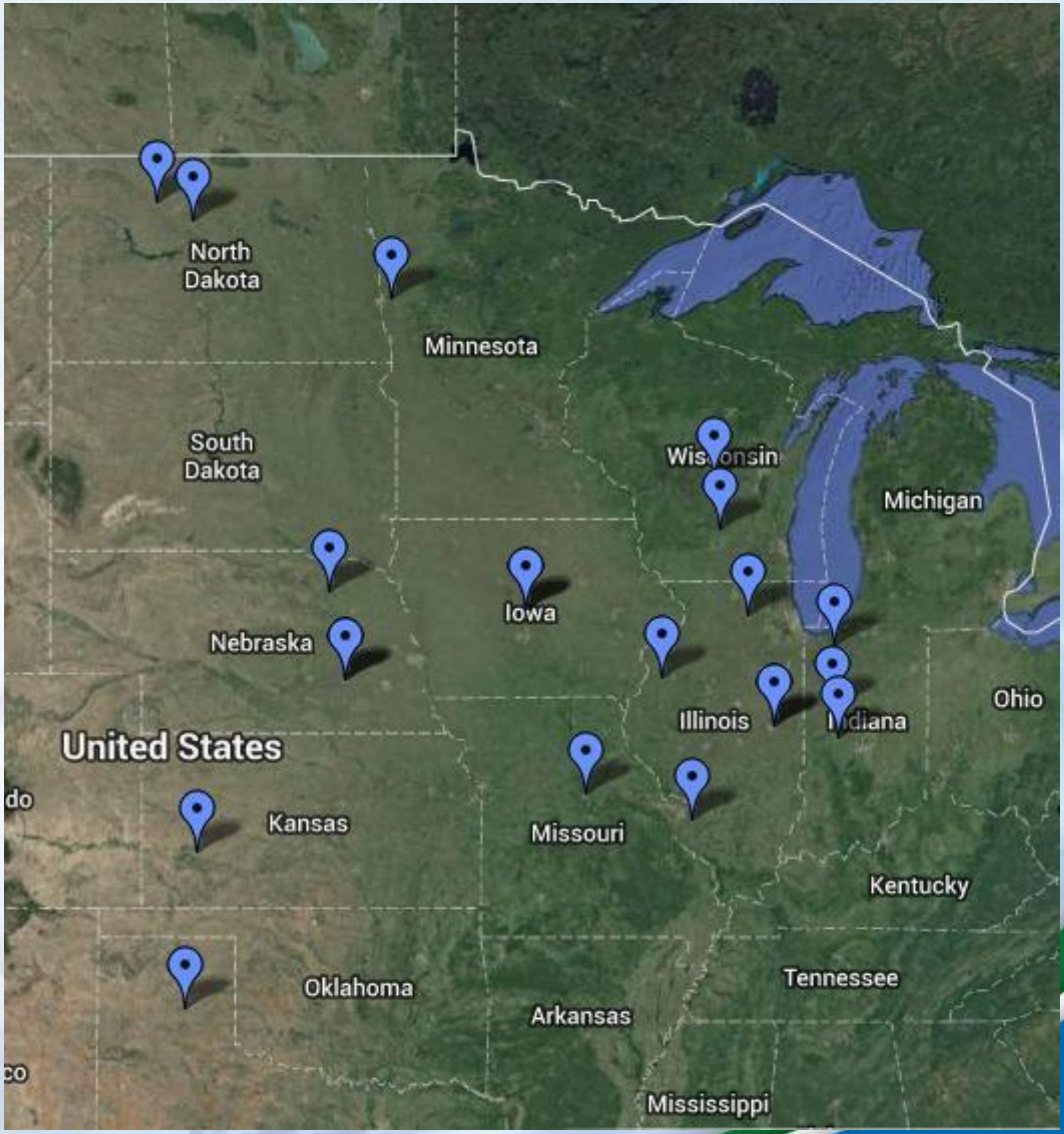
- **Hypothesis**
  - Soil heterogeneity is more prevalent than is apparent.
- **Objective**
  - Evaluate the use of a Latin Square design to control soil heterogeneity and compare the relative efficiency versus the RCBD.

Latin Square Case Study

30 total squares
- **Corn – 12**
- **Sorghum – 3**
- **Soybean – 15**

18 locations
- **10 States**

2 Cooperator Types
- **18 Land Grant Univ.**
- **12 Private Contractor**



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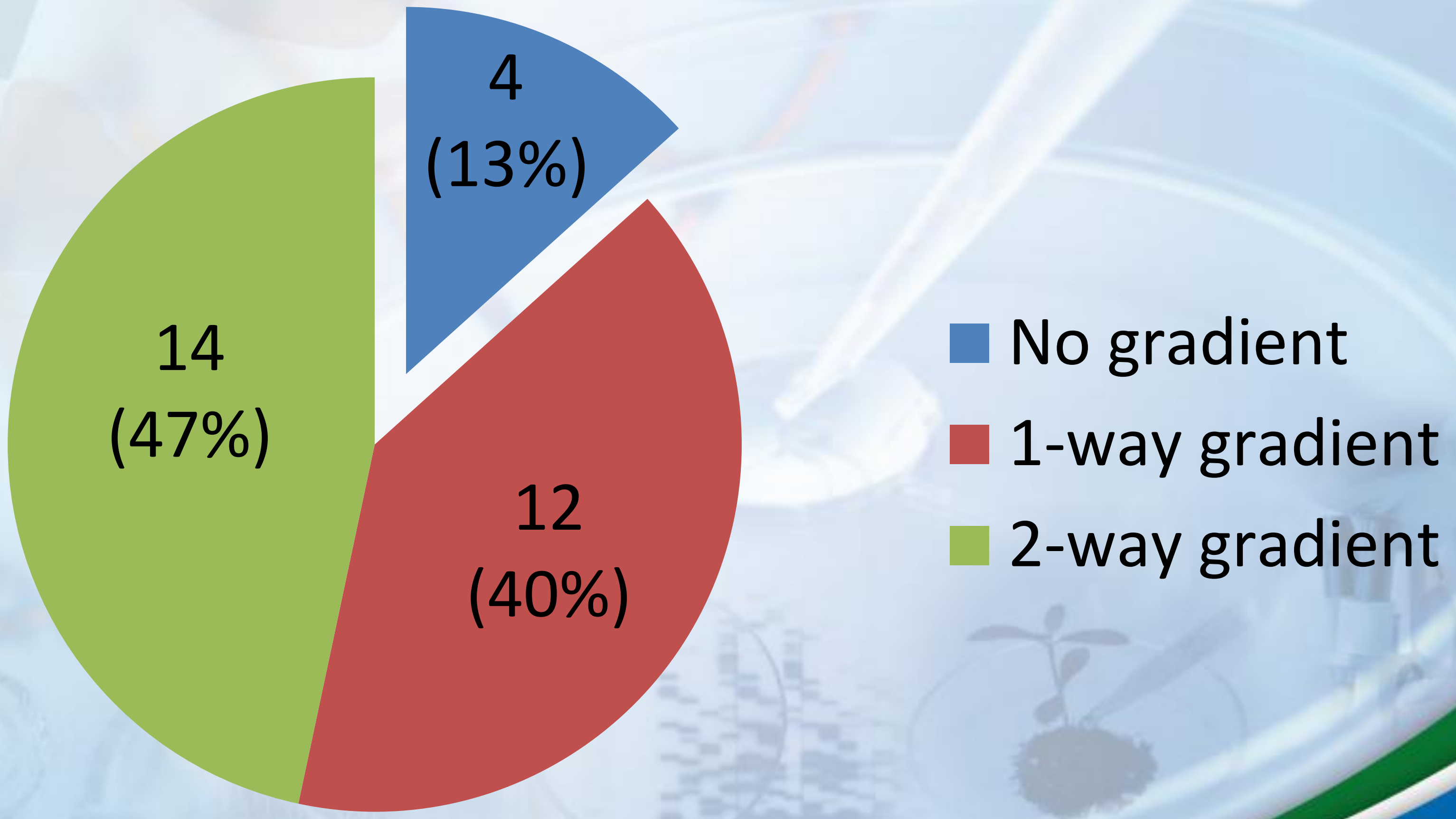
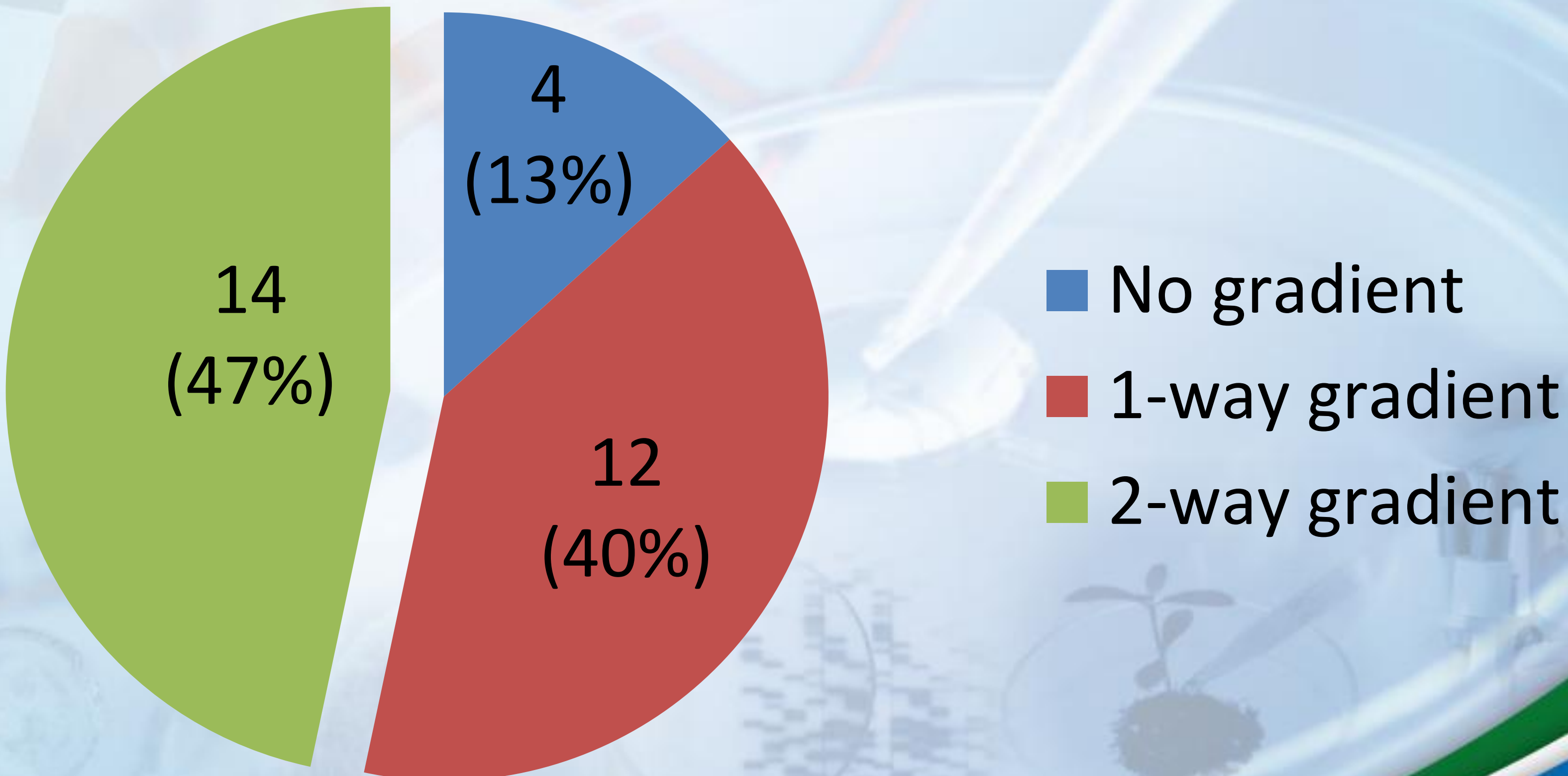
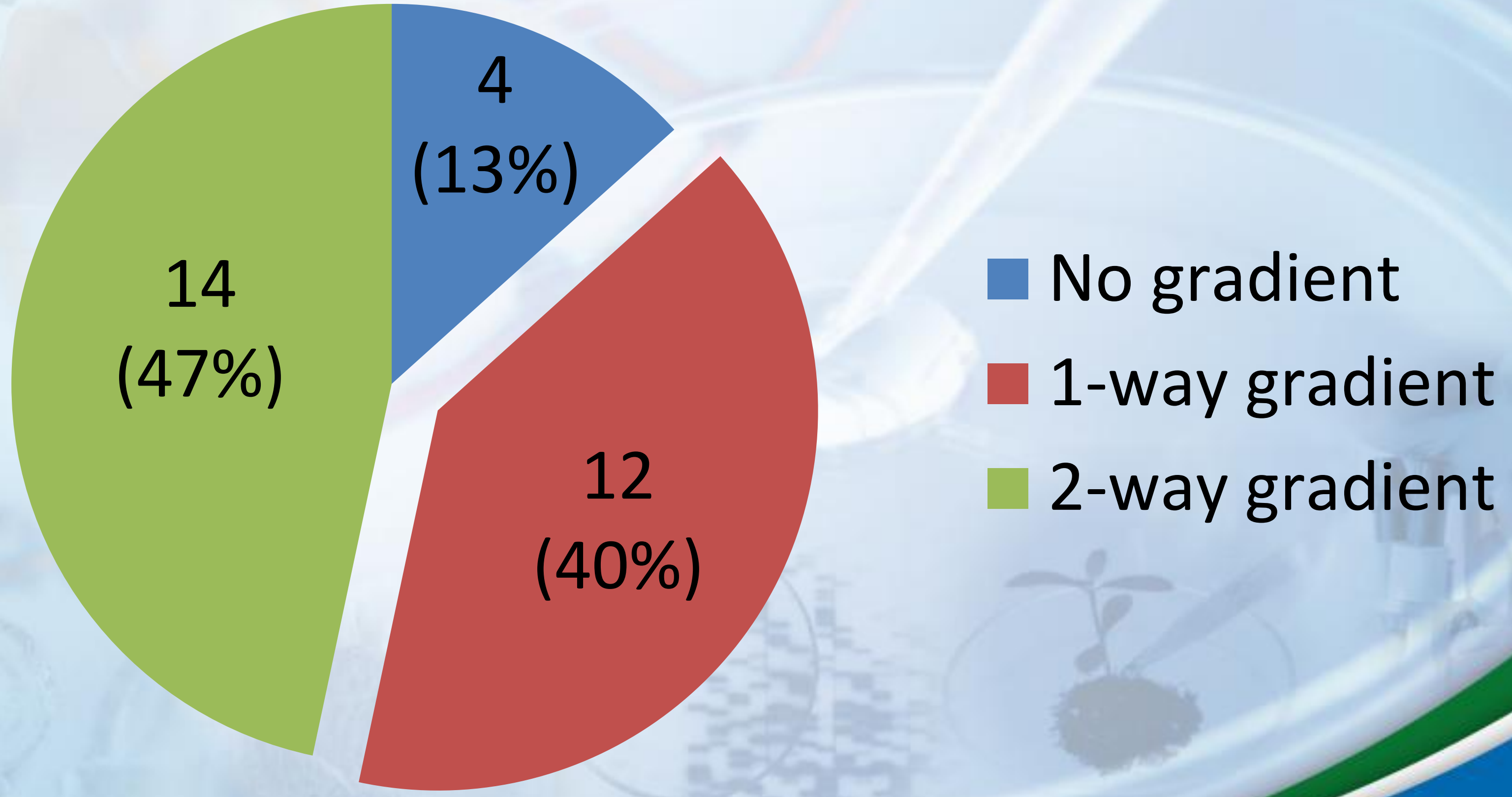
Presence of gradients exhibited in 30 Latin Square trials conducted across 18 locations in 10 states across the upper Midwest. Only four of the trials (13) did not exhibit a significant gradient indicating blocking is a sound practice to help control field variation



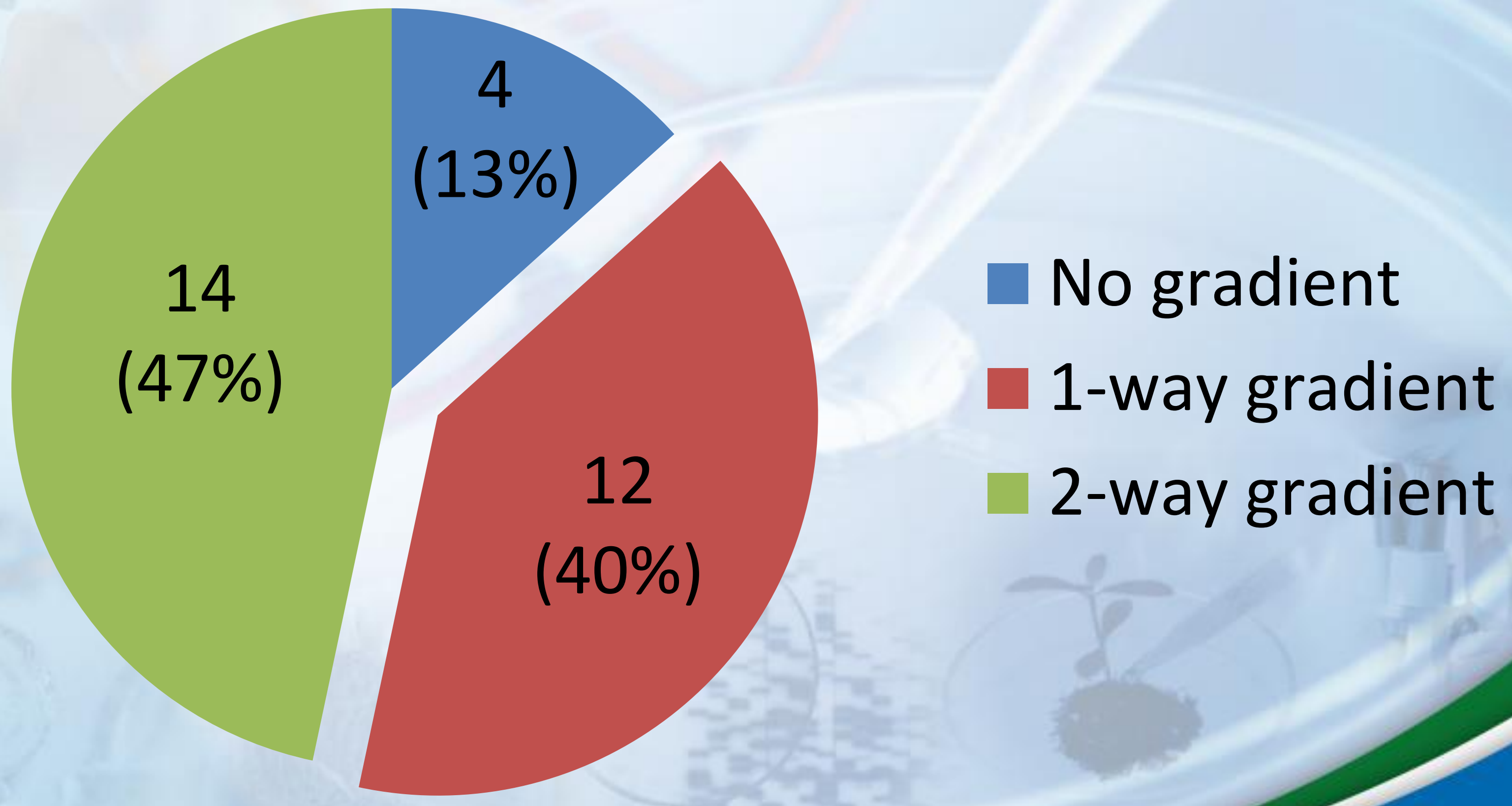
Presence of gradients exhibited in 30 Latin Square trials conducted across 18 locations in 10 states across the upper Midwest. Fourteen of the trials (47%) exhibited two way variation and the precision was increased (lower MSE) by using the Latin Square design.



Of the 12 trials that exhibited one-way variation, the precision was increased (lower MSE) in 7 of those instances regardless of the blocking direction chosen. The precision was increased in 70% (21/30) trials by using the Latin Square design.



Of the 5/12 remaining trials exhibiting one-way variation, the use of the RCBD would only have more precision if the proper blocking direction is chosen. Otherwise the design would be less efficient.

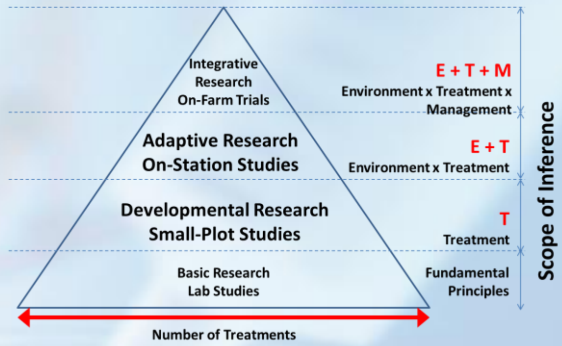


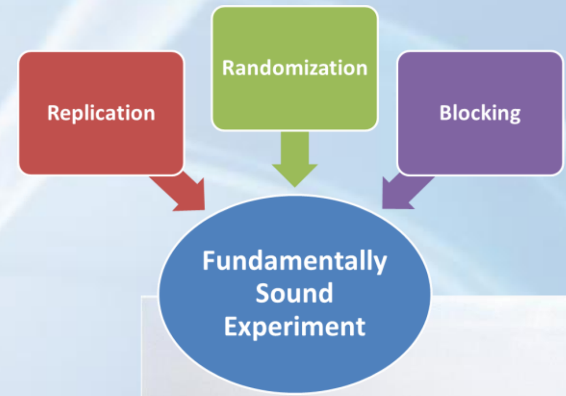
Interpretation and Conclusions

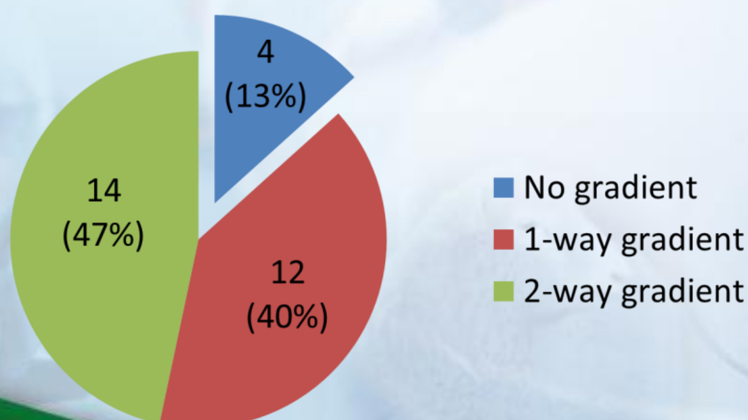
- **Multiple gradients are prevalent in field trials.**
- **Blocking in a single direction will only increase precision if the variation is successfully captured within those blocks.**
- **The Latin Square increased precision in over 2/3 of trials evaluated.**
  - **Efficiency is not sacrificed in single gradient systems**
  - **The number of treatments is restricted to equal the number of replicates: 4 x 4, 5 x 5, 6 x 6**

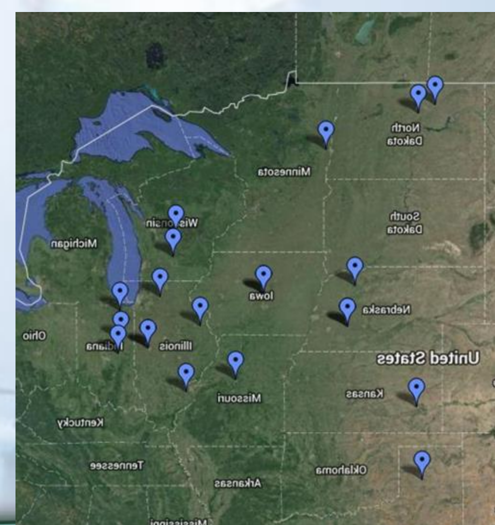
Recall

- Research strategies and principles of design
- The Randomized Complete Block Design (RCBD)
- Sources of variation in the field
- Features of Latin Square Design
- Relative Efficiency RCBD vs LS
- Case study of 30 Midwest trials designed as LS but ANOVA as RCBD and as LS









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C	D	A	B
D	A	B	C

References and Citations

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