

PLANNING EXPERIMENTS

Experiments are conducted to obtain new facts or to confirm or deny results of previous experiments.

There are three types of experiments:

1. Preliminary - investigator evaluates a large number of treatments in order to obtain leads for future work.
2. Critical - investigator compares responses to different treatments using sufficient observations of the responses to obtain reasonable assurance of detecting significant differences.
3. Demonstration - performed by extension agents, experiment stations, or seed or chemical companies to compare new treatments with a standard.

PLANNING THE EXPERIMENT

1. Write down objectives in order of importance. This will help you choose the correct experimental design.
2. Write down the hypothesis to be tested.
3. Define the population you plan to sample.
4. Select the specific treatments for the population.

Example:

Determine the response of different barley cultivars to foliar applied fungicides that control wheat stem rust.

1. Objectives - to determine the effects of foliar applied fungicides that control stem rust on agronomic traits of barley.
 - a. yield
 - b. disease score
 - c. kernel plumpness
 - d. kernel weight
 - e. test weight
 - f. grain protein
 - g. kernel color
 - h. plant height
 - i. days to maturity
 - j. lodging
2. Hypothesis - An application of foliar fungicides to barley has no affect on agronomic characteristics of barley.

3. Population to sample - barley cultivars grown in North Dakota:
4. Fungicides labeled for use on barley.
5. Specific treatments (i.e. cultivars and fungicides be used).

Before treatments are considered, many items need to be considered:

- a. Where to conduct the experiments.
- b. How many and which cultivars to use.
- c. How many and which fungicides to use.
- d. Size of plots
- e. Seeding rate
- f. Spraying rate
- g. Method of spraying
- h. Amount of pressure to use for spraying.
- i. Amount of water to use to carry fungicide.
- j. What surfactant, if any, to use.
- k. Stage of growth in which fungicide is to be applied.
- l. Will plants be inoculated.
- m. What notes to take.
- n. How much time will the experiment take.
- o. Amount of labor needed to conduct the experiment.
- p. Amount of money needed to conduct the experiment.

EXPERIMENTAL UNIT - unit of material to which one application of a treatment is applied.

TREATMENT - the procedure whose effect is to be measured.

Given the following situations:

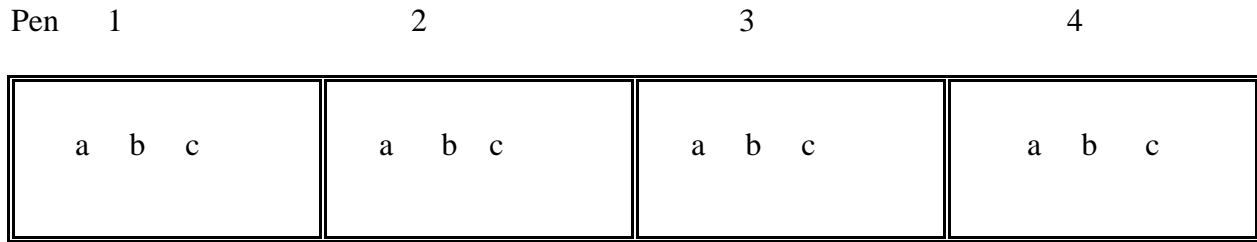
- a. what is the experimental unit?
- b. what is the treatment?

Example 1 - Yield trial experiment comparing 10 oat cultivars.

8	2	5	3	10	9	1	4	7	6
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- a. Experimental unit =
- b. Treatment =

Example 2 - Feed study using hogs. Four rations are to be compared. Each ration is fed to three hogs within a pen.



Ration 3 1 4 2

- a. Experimental unit =
- b. Treatment =

EXPERIMENTAL ERROR

Experimental error:

- 1. Measure of variation that exists among observations on experimental units treated like.
- 2. Failure of observations on experimental units treated alike to be the same.

Two sources of variation causing Experimental Error are:

- 1. Inherent variability that exists in experimental material treated alike (e.g. variability in the field, different starting weight of animals, etc).
- 2. Variation that results from lack in uniformity in the physical conduct of the experiment (e.g. poor experimental technique, poor emergence in the field, etc.).

REPLICATION

Replication - repeating a treatment more than once in an experiment.

Four functions of replication are:

- 1. Provide an estimate of experimental error.
 - Needed for tests of significance.
 - Without replication, an estimate of experimental error cannot be calculated; therefore, tests of significance cannot be conducted.

- To improve the precision of an experiment by reducing standard deviation of the mean (i.e. standard error).

$$s_{\bar{Y}} = \sqrt{s^2/n} = s/\sqrt{n}$$

$$\text{where: } s^2 = \frac{\sum Y_i^2 - \left(\frac{(\sum Y_i)^2}{n}\right)}{n-1}$$

- Note that $s_{\bar{Y}}^2$ is always less than s^2 .
- As stated earlier, replication reduces the standard deviation of the mean.

Example

- Conduct an experiment with four replicates

$$s_{\bar{Y}} = s/\sqrt{4} = s/2$$

- Conduct an experiment with 16 replicates

$$s_{\bar{Y}} = s/\sqrt{16} = s/4$$

- This shows that by increasing the number of replicates from 4 to 16, the standard error was reduced by a factor of two.
 - Later, it will be shown that as the number of observations in an experiment increases, the chance of detecting smaller significant differences between treatment means increases.
- Increase the scope of the experiment.
 - The scope of the experiment is increased by conducting the experiment at different times because conditions (e.g. environment, experimental units) will vary each time the experiment is conducted.

Precision - related to the repeatability of the experiment (i.e. the ability to obtain the same results each time the experiment is conducted).

Accuracy - related to obtaining the correct answer.

4. To control error variance by grouping similar experimental units together into replicates. The goal is to **minimize** variation **between** experimental units and **maximize** variation **between** replicates.

FACTORS THAT INFLUENCE THE NUMBER OF REPLICATES

The number of replicates in an experiment depends on:

1. Amount of precision required.
 - The smaller the differences you wish to detect between treatment means, the more replicates you will need.
 - It is important to have the correct number of replicates needed to detect the differences you desire.
 - Too many replicates are expensive.
 - Too few replicates may not allow you to detect the desired differences.
2. Type of experimental material.
 - Heterogeneous material requires more replicates.
3. Type of experimental units.
 - Homogeneous experimental units require fewer replicates.
4. Number of treatments.
 - The number of treatments affects the precision of an experiment.
 - Precision or amount of information of an experiment (I) is measured as the reciprocal of the variance of the mean.

$$\text{Where } I = \frac{1}{\sigma_{\bar{y}}^2} = \frac{1}{\sigma^2/n} = \frac{n}{\sigma^2}$$

- Remember, decreasing the standard error increases precision.
- If we increase the number of treatments while holding the number of replicates constant, the size of the experiment increases along with the precision.

- If the size of the experiment is held constant and number of treatments is increased, the number of replicates must be decreased. This results in a reduction in precision.
- Size of the experiment = n
- n = number of replicates X number of treatments

Example:

	Exp 1	Exp 2	Exp 3	SOV	df	Exp 1	Exp 2	Exp 3
Trt	3	9	18	Trt	t-1	2	8	17
Rep	4	4	2	Expt. error	t(r-1)	9	27	18
n	12	36	36	Total	rt-1	11	35	35

- Experiment 3 has the same number of observations as experiment 2, but has fewer degrees of freedom (df) for the experimental error than that in experiment 2.
- This indicates that experiment 3 may be measured with less precision than experiment 2.
- Thus, you may be able to detect smaller significant differences between treatment means with experiment 2.

Take home story: the number of replicates and treatments must be such that precision is at the desired level. To do so, the experimental error degrees of freedom should be at least 20 or greater.

RELATIVE EFFICIENCY AND PRECISION

- The precision or amount of information in an experiment is measured by $I = n/\sigma^2$.
- Thus, I is affected by n and σ^2
 - As n increases, I increases.
 - As σ^2 decreases, I increases.

The variance, σ^2 , can be decreased by increasing the degrees of freedom for the experimental error.

This can be accomplished by:

1. Increasing the number of treatments.
2. Increasing the number of replicates.
3. Choice of experimental design.

Example: The effect of choice of experimental design on the experimental error degrees of freedom.

Given 5 treatments and 5 replicates.

Experimental Design					
CRD ¹		RCBD ²		Latin Square	
SOV	df	SOV	df	SOV	df
Treatment	4	Replicate	4	Row	4
Error	20	Treatment	4	Column	4
Total	24	Error	16	Treatment	4
		Total	24	Error	12
				Total	24

¹ CRD = Completely random design.

² RCBD = Randomized complete block design.

- More complex designs have fewer df in their experimental error relative to simpler designs.
- It is more difficult to detect differences among treatment means when the error degrees of freedom are less than 20.
- To compare the amount of information provided by two experimental designs, one can look at the relative efficiency (RE).

$$RE = \frac{(n_1 + 1)(n_2 + 3)s_2^2}{(n_2 + 1)(n_1 + 3)s_1^2}$$

where: n_1 = error df for the least complex design
 n_2 = error df for the more complex design

$$s_1^2 = \text{error MS for the least complex design}$$
$$s_2^2 = \text{error MS for the more complex design}$$

- The RCBD is considered more complex than a CRD.
- The Latin square design is considered more complex than an RCBD.
- The Lattice design is considered more complex than an RCBD.

- The more complex design is considered more efficient than the least complex design if RE is greater than 1 or 100%.

- Often see RE given on outputs for analyses of lattice designs.

ERROR CONTROL

Error control can be accomplished by:

1. Choice of experimental design.
2. Use of concomitant observations (analysis of covariance).
3. Choice of size and shape of experimental units.

Choice of Experimental Design

- Choice of design is made so the natural variation among the experimental units contributes a minimal amount to difference among treatments.
- Want differences to be due to differences between treatments, not due to variation between experimental units (inherent variability).
- For example, in an RCBD, experimental units are blocked such that each block contains each treatment at least once. The precision is increased because variation among experimental units within the same block (replicate) is minimized.
- When the number of treatments is large (e.g. 121), it becomes difficult to minimize the variation with a block; thus, the experimental error increases. There are designs where the block is subdivided into incomplete blocks (sub-blocks). Each incomplete block contains only a portion of the treatments. Precision is increased because variation among experimental units within a sub-block is minimized. These designs are called incomplete block designs. A lattice is an incomplete block design.

The best design is the one that is simplest to use and still provides the information you desire.

Use of Concomitant Observations

- In some experiments, the precision of the experiment may be reduced due to variation in some character that cannot be controlled by the choice of experimental design.
- The starting weight of an animal in a feeding trial may affect the rate of weight gain.
- The number of plants per row may influence yield of that row.
- These characters that influence the outcome of the experiment are called concomitant observations.
- A technique called analysis of covariance can reduce the effects of these concomitant observations.
- This technique is taught in CWS 734 - Field Designs II.

Size and Shape of Experimental Units

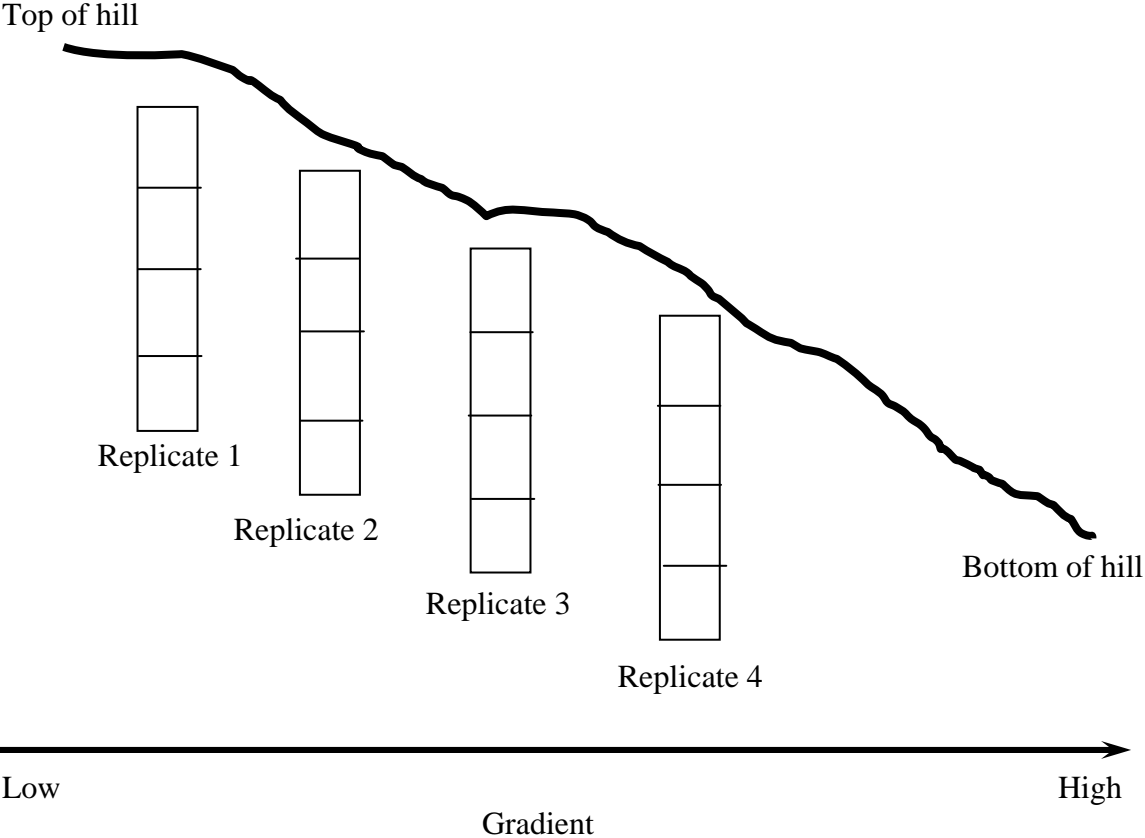
- Larger experimental units generally are better than small ones because large ones have less variation.
- However, increasing the size of the experimental unit often results in a decrease in the number of replicated because of size constraints.
- Adequate replication of small plots is easier to obtain than is adequate replication of large plots.
- In field plot experiments:
 - 0. Long narrow rows are better than square plots.
 - 0. Replicates or incomplete blocks should be square.

This arrangement of experimental units and blocks has been found to minimize variation within the block while maximizing variation among blocks.

Plots should be parallel to any gradient in the field

Blocks should be perpendicular to the gradient.

Diagram showing layout of plots perpendicular to field gradients.



REFINEMENT OF TECHNIQUE

It is the responsibility of the experimenter to ensure that everything possible is done to guarantee the use of careful technique.

No analysis, statistical or otherwise can improve data obtained from poorly conducted experiments.

Variation resulting from careless technique is not random variation and thus, is not subject to the laws of chance of which statistical inference is based.

Points of Technique to Consider:

1. It is important to have uniformity in application of treatments.
 - spraying chemicals
 - seeding rates
 - pipeting
2. Influences from external sources should be minimized.
 - Plot should be uniform before treatments are applied.
 - Harvest of the complete experiment should be done in one day or harvest at least one complete replicate or incomplete block.
3. Treatment effects should be measured in an unbiased manner.

Faulty Technique May Increase Experimental Error in Two Ways

1. May introduce fluctuations of a more or less random nature that may be subject to the laws of chance.
 - These may be revealed in the Coefficient of Variation (CV).

$$CV = (s/y) \times 100$$

- CV is a unitless measure of relative variation.

Considerations of the CV

- Knowing what is an unacceptable or acceptable CV takes experience with working in your area.
- In general, physical measurements (e.g. plant height, seed weight, enzyme assays) have lower CV's.

- Visual scoring measurements (e.g. herbicide injury, lodging score, disease score, etc.) tend to have greater CV's as compared to physical measurements.
2. May introduce non-random mistakes.
- The mistakes are not subject to the laws of chance.
 - The may not always be detected by observation of individual measurements.

RANDOMIZATION

- Randomization is done to ensure that there is a valid or unbiased estimate of experimental error, and of treatment means and differences among them.
- Randomization is done to ensure that a particular treatment is not always located next to the same treatment in all replicates.
- Numerous studies have show that adjacent experimental units (e.g. plots) tend to be more alike in their results than those experimental units far apart.
- Always having the same treatments side by side may result in correlation among experimental errors. This violates the assumption that the errors for each observation are normally and independently distributed.
- Correlated responses are more likely to occur in systematic arrangements.