UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

FAKULTI PERTANIAN
FACULTY OF AGRICULTURE
فاكولتي ڤرتانين

AGR5201
Advanced Statistical Methods

Principles of Experimental Design

Topic outline

1.0 Experimental design

Definition, types of experiment, steps in experimentation

2.0 Terminology in experiment

2.1 Experimental error

2.2 Control of experimental error

2.3 Conducting an experiment

1.0 Experimental design

Why conduct experiments?...

- To explore new technologies, new crops, and new areas of production
- To develop a basic understanding of the factors that control production
- To develop new technologies that are superior to existing technologies
- To study the effect of changes in the factors of production and to identify optimal levels
- To demonstrate new knowledge to growers and get feedback from end-users about the acceptability of new technologies

1.0 Experimental design

Definition

- The rules and procedures used in conducting research.

Includes the following topics:

I. Physical layout or arrangement of treatments.
II. How to plan, organize, and develop the experimental protocol.
III. Data collection - it’s organization.
IV. Data analysis - the statistical model and assumptions.
V. Inferences and Conclusions.

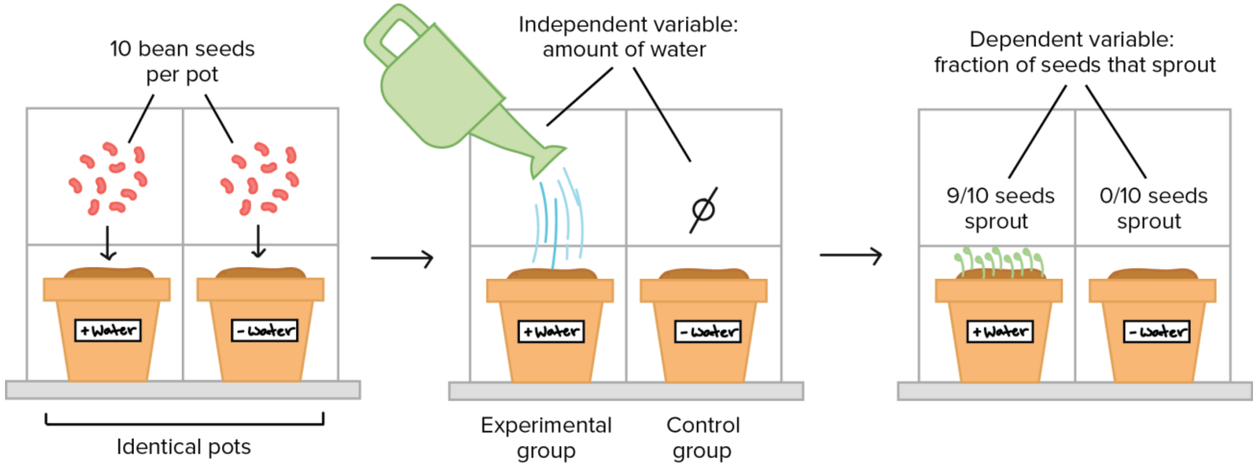
1.0 Experimental design

Experiment

A scientific procedure (method) undertaken:

- to make a discovery
- test a hypothesis
- demonstrate a known fact

- Synonyms: test, investigation, trial, examination, observation



1.0 Experimental design

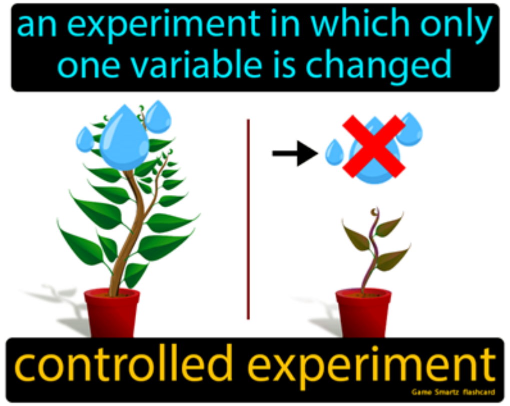
Types of experiment

DESIGNED EXPERIMENT

OBSERVATIONAL STUDY

1.0 Experimental design

What is a designed experiment?



- Treatments are imposed (manipulated) by investigator using standard protocols
  - The inference of response was due to the treatments
- Potential problems:
  - As we artificially manipulate nature, results may not generalize to real life situations
  - As we increase the spatial and temporal scale of experiments (to make them more realistic), it becomes more difficult to adhere to principles of good experimental design

1.0 Experimental design

What is an observational study?

- In this experiment, the treatments are defined on the basis of existing groups or circumstances.
- Uses
  - Early stages of study – developing hypotheses.
  - Scale of study is too large to artificially apply treatments (e.g. ecosystems).
  - Application of treatments of interest is not ethical.
    - Eg: To study the effect of wildfire to animal population in the forest ➔ not ethical to burn the forest purposely for this kind of research

1.0 Experimental design

What is an observational study?

- May determine associations between treatments and responses but cannot assume that there is a cause-and-effect relationship between them.
- Testing predictions in new settings may further support our model, but inference will never be as strong as for a designed (manipulative) experiment.

1.0 Experimental design

The scientific methods

- **Formulation** of a **hypothesis**
- **Planning an experiment** to objectively **test the hypothesis**
- **Careful observation** and **collection of data** from the experiment
- **Interpretation** of the experimental **results**

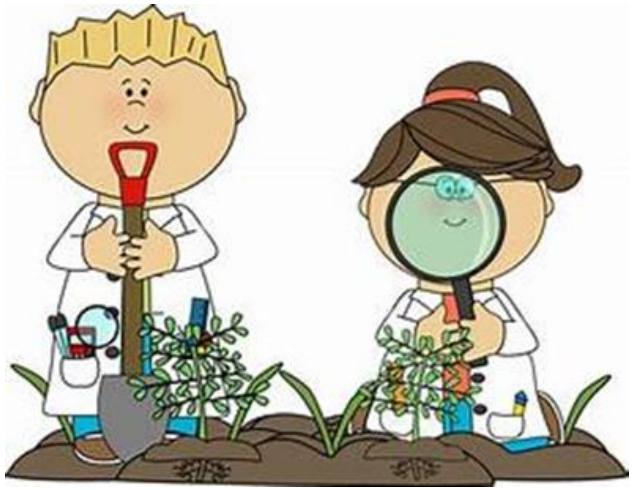
1.0 Experimental design

Steps in experimentation

Hypothesis	✓Definition of the problem (by observation) ✓Statement of objectives
Planning of experiment	✓Selection of treatments ✓Selection of experimental material ✓Selection of experimental design ✓Selection of the unit for observation and the number of replications ✓Control of the effects of the adjacent units on each other ✓Consideration of data to be collected ✓Outlining statistical analysis and summarization of results
Data	✓Conducting the experiment – to observe and obtain data!
Interpretation	✓Analyzing data and interpreting results ✓Preparation of a complete, readable, and correct report

1.0 Experimental design

The well-planned experiment

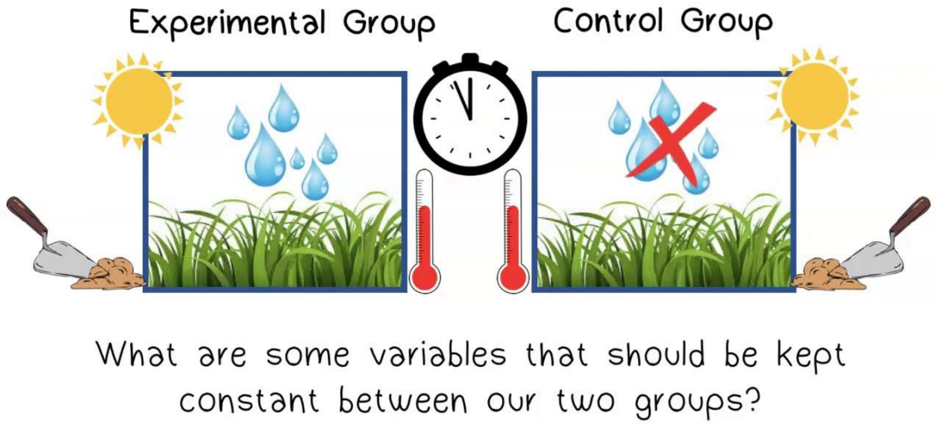


- Simplicity
  - don't attempt to do too much
  - write out the objectives, listed in order of priority
- Degree of precision
  - appropriate design
  - sufficient replication
- Absence of systematic error
- Range of validity of conclusions
  - well-defined reference population
  - repeat the experiment in time and space
  - a factorial set of treatments also increases the range
- Calculation of degree of uncertainty

2.0 Terminology in experiment

1. Comparative experiment:

- The process of applying, measuring, and analyzing two or more treatments.
- Example: Compare two fertilizer rate treatments on corn yield



2. Treatment:

- The combination of all biological and physical constraints used to define a system or practice of interest.
- The procedure whose effect will be measured

2.0 Terminology in experiment

3. Factor

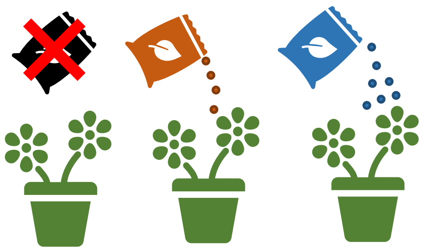
- class of related treatments

4. Levels

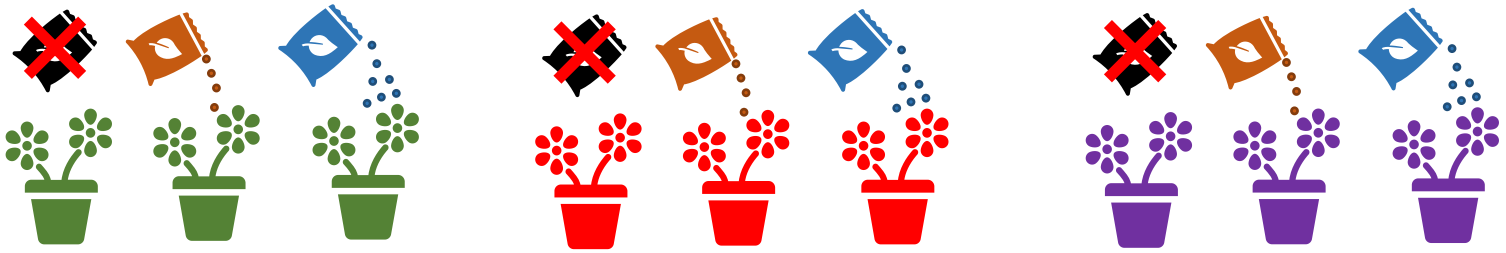
- states of a factor

Number of factor	Factor	Level
Single factor 1. Fertilizer rate	Fertilizer rate	0 kg/ha 50 kg/ha 100 kg/ha
Two Factors 1. Fertilizer rate 2. Variety	Fertilizer rate	0 kg/ha 50 kg/ha 100 kg/ha
	Variety	A B C

Single factor



Two factors (factorial)



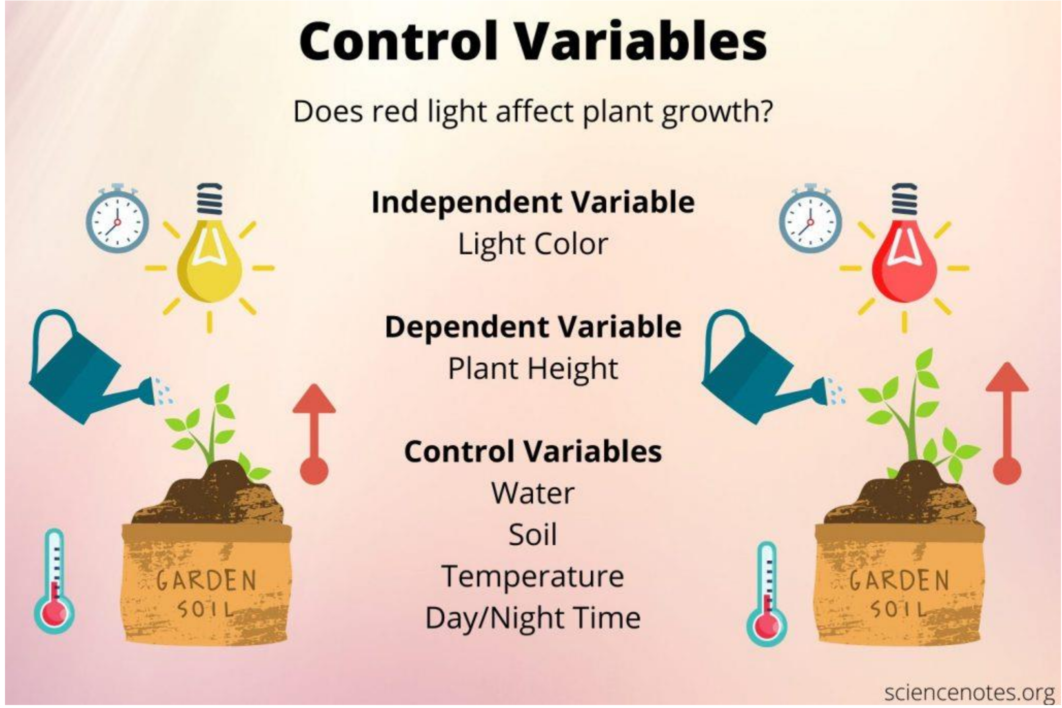
2.0 Terminology in experiment

5. Variables

i. Independent variable (Treatment)
   - The change made by the researcher
   - E.g.: Fertilizer rate, variety, etc.

ii. Dependent variable (Measured variables)
   - The change resulted from the treatment
   - Measurable characteristic of a plot / experimental unit
   - E.g.: plant height, dry weight, number of fruits

iii. Controlled variable
   - Other variables that should be kept constant/consistent
   - E.g.: soil, watering, temperature, etc.



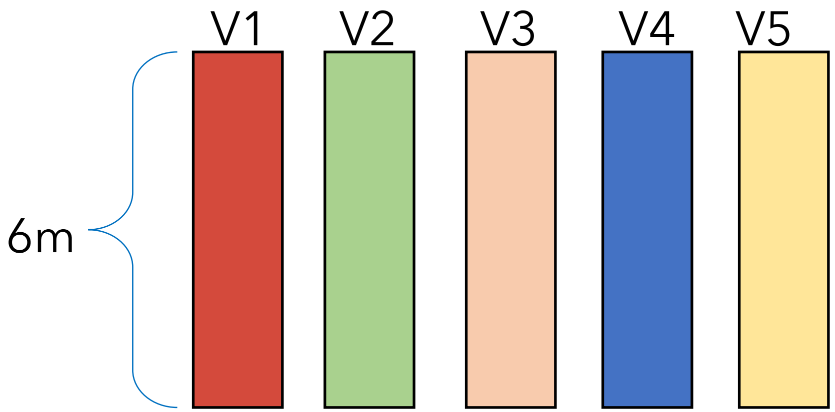
2.0 Terminology in experiment

6. Experimental unit (E.U.):

- The smallest unit to which a treatment is applied

- *Example 1:*

  Each hybrid is grown in a single row, 6 m long plot.
  What is the experimental unit for:
  - Case 1- Measure grain yield on the entire plot = __________
  - Case 2 - Measure grain yield on 10 ears within the plot = _________

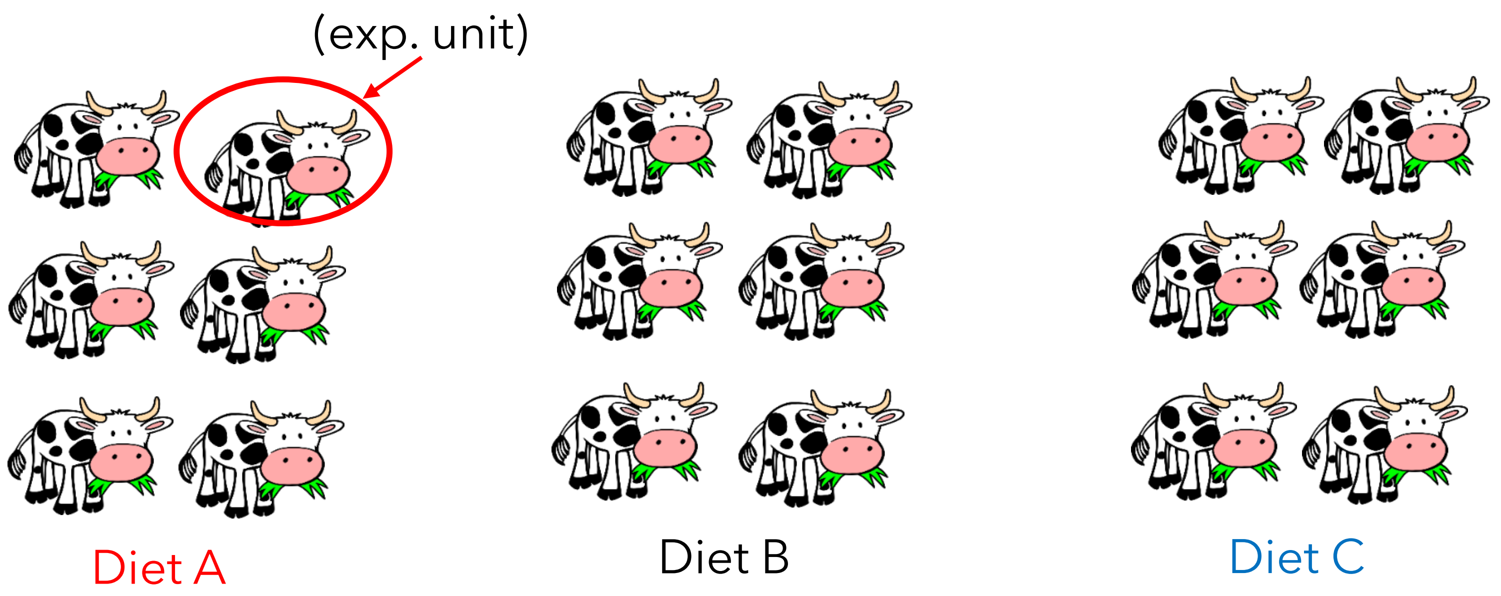


2.0 Terminology in experiment

- *Example 2:*

Six cows are each fed one of three diets. What is the experimental unit for:

- Case 1- Measure milk production for 9 weeks:_______
- Case 2 - Sacrifice the cows and measure levels of enzyme activity in the liver = ______
- Case 3 - Take a blood sample on treatment & control = _____

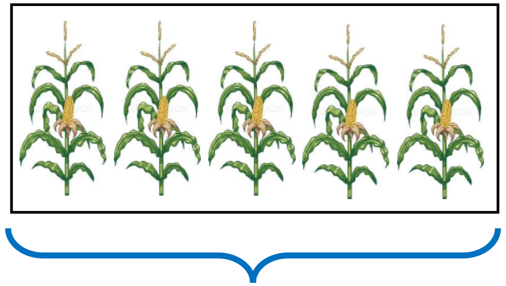


2.0 Terminology in experiment

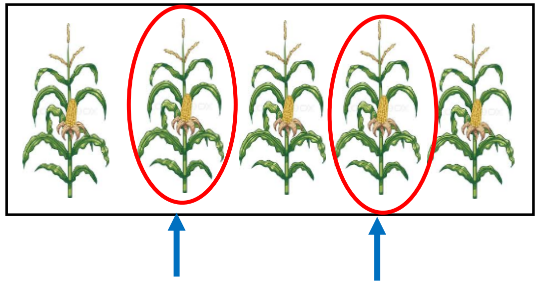
7. Sampling unit

Some part of an experimental unit that is measured:

- *Example 1*: corn treated with different rate of fertilizer



Experimental unit = a row of corn



Sampling unit – a few corn plants

- *Example 2*: cows fed with three different diets

Sampling unit = blood samples from a cow

Experimental unit = a cow

2.0 Terminology in experiment

8. Replication

- Repetition of experimental units that receive the same treatment.
- To increase precision – se
- Important for the measurement of experimental error!
- Each treatment is applied independently to two or more experimental units
- Variation among plots treated alike can be measured
- Increases precision - as n increases, error decreases

Standard error of a mean

$$s_{\bar{y}} = \frac{s}{\sqrt{n}}$$

Sample standard deviation

No. of replications

T2-R3	T1-R2	T2-R4	T1-R3	T3-R4	T2-R2
T1-R1	T2-R1	T3-R2	T3-R1	T1-R4	T3-R3

2.0 Terminology in experiment

9. Randomization

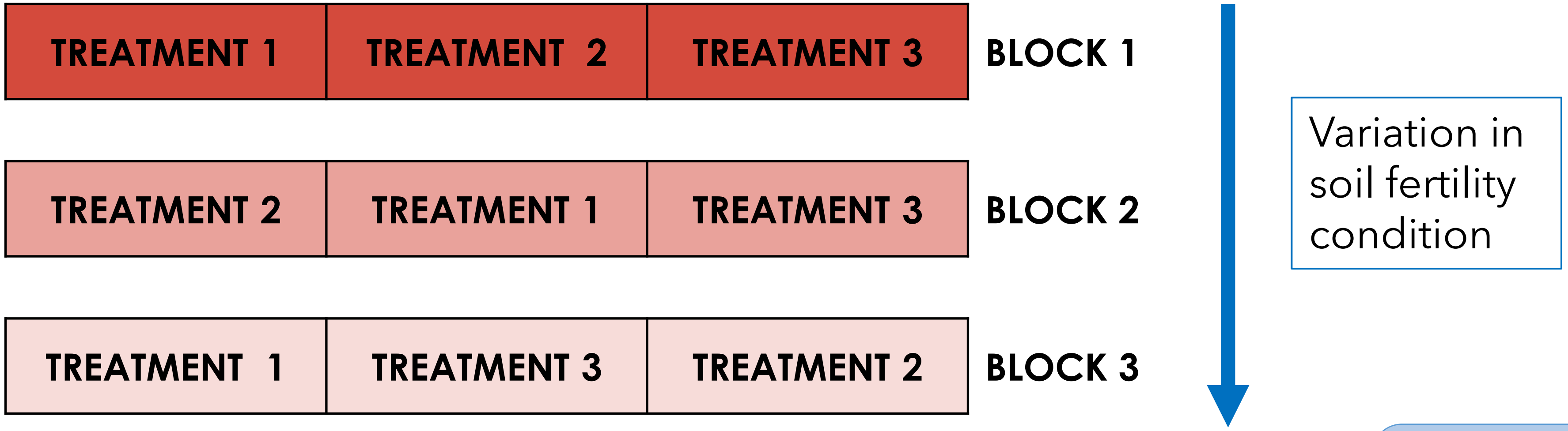
- To eliminate bias (to increase accuracy)
- To ensure independence among observations
- Required for valid significance tests and interval estimates

In each pair of plots, although replicated, the new variety is consistently assigned to the plot with the higher fertility level.

2.0 Terminology in experiment

10. Block

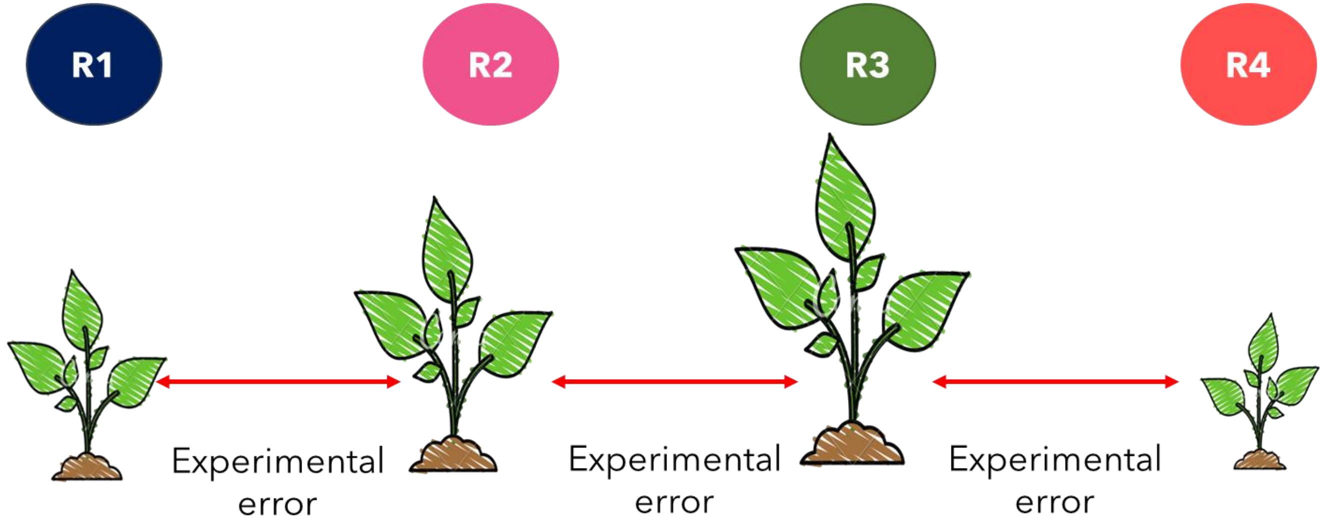
- Group of homogeneous experimental units:
- Example:



2.0 Terminology in experiment

11. Experimental error

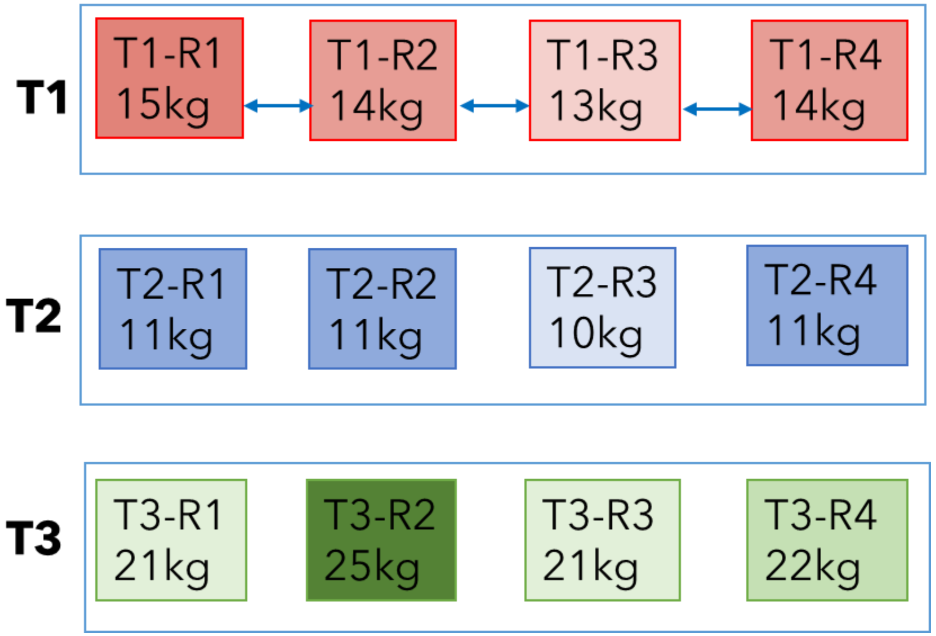
- **Definition:** The variation among experimental units that are treated alike
- *This variation* is always present (although treatments are the same)
- The experimental error *can be reduced* by using appropriate experimental design when conducting experiment



2.0 Terminology in experiment

11. Experimental error

Rearrange the result from a randomized experiment



- A modern experimental design should:
  - provide a measure of experimental error variance
  - reduce experimental error as much as possible

2.0 Terminology in experiment

11. Experimental error – a hypothetical example:

- Below are hypothetical data from an experiment on the effect of nitrogen (N) rate on the dry weight of corn seedling.
- What do we expect when we apply the same rate of treatment on a few experimental units (plants)?

Result A

	Weight (g)		
Treatment	Plant 1	Plant 2	Plant 3
T1 (no N)	10	10	10
T2 (50 kg/ha)	20	20	20
T3 (100 kg/ha)	35	35	35

Result B

	Weight (g)		
Treatment	Plant 1	Plant 2	Plant 3
T1 (no N)	9	10	11.4
T2 (50 kg/ha)	20	22	21
T3 (100 kg/ha)	38	36	34

2.0 Terminology in experiment

Examples of experimental design

- An '**experimental design**' is a plan for the assignment of the treatments to the plots in the experiment
- Designs differ primarily in the way the plots are grouped before the treatments are applied
  - The difference → How much restriction is imposed on the random assignment of treatments to the plots.
- Example:

A (R1)	B (R2)	D (R1)
D (R3)	C (R1)	B (R3)
C (R3)	A (R2)	D (R2)
B (R1)	C (R2)	A (R3)

Completely randomized design (CRD)

A	B	C
D	C	B
C	A	D
B	D	A
Block 1	Block 2	Block 3

Randomized complete block design (RCBD)

2.1 Experimental error

Natural sources of error in field experiments

- **Plant variability**
  - type of plant → larger variation among larger plants
  - competition → variation among closely spaced plants is smaller
  - plot to plot variation because of plot location (border effects)

- **Seasonal variability**
  - climatic differences from year to year
  - rodent, insect, and disease damage varies
  - conduct tests for several years before drawing firm conclusions

- **Soil variability**
  - differences in texture, depth, moisture-holding capacity, drainage, available nutrients
  - since these differences persist from year to year, the pattern of variability can be mapped with a uniformity trial

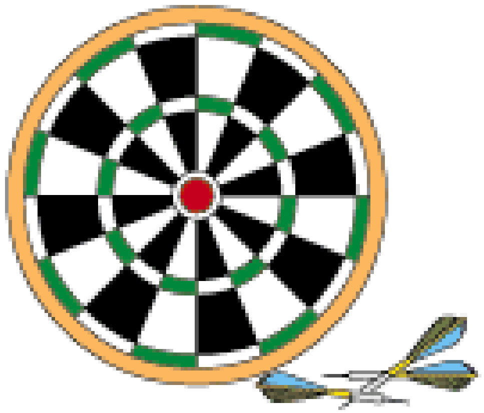
2.1 Experimental error

Strategies to control experimental error

1. Select appropriate experimental units ~homogeneous
2. Increase the size of the experiment to gain more degrees of freedom
   i. more replicates or more treatments
   ii. caution – error variance will increase as more heterogeneous material is used
3. Select appropriate treatments
   i. factorial combinations result in hidden replications and therefore will increase n
4. Blocking
5. Refine the experimental technique
6. Measure a concomitant variable (accompanying variable)
   i. covariance analysis can sometimes reduce error variance

2.2 Control of experimental error

Accuracy vs. precision



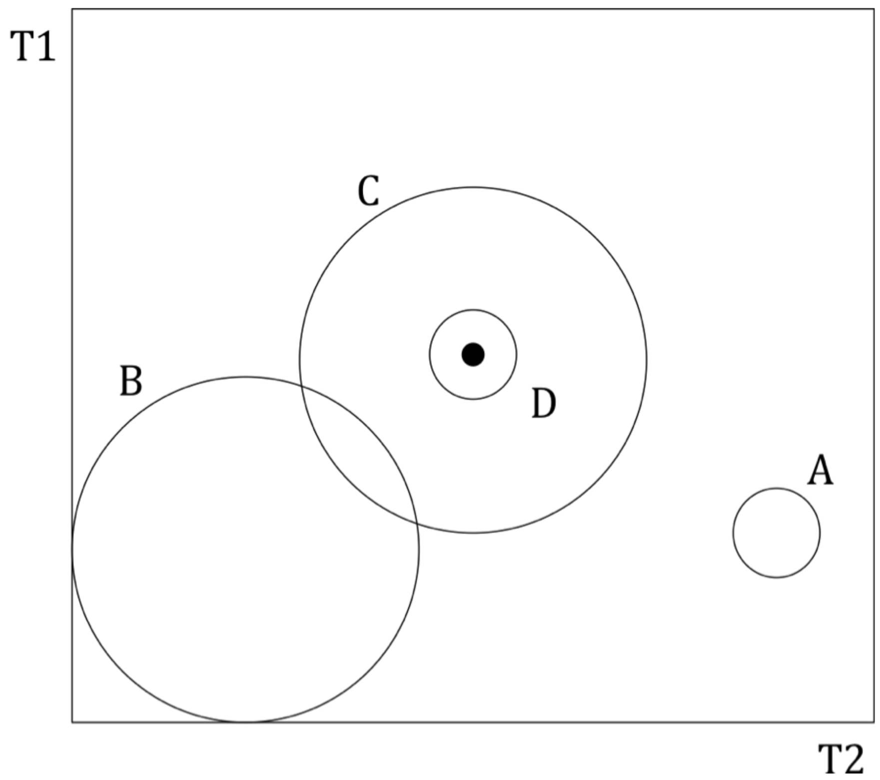
- In experiment, **both accuracy and precision are needed** to control for experimental error
- The analogy → Darts game.
- Bull's eye represents the true value of the parameter you wish to estimate

Precision = repeatability
- ➢ measurements are close together
- ➢ achieved through replication

Accuracy = without bias
- ➢ average is on the bull's-eye
- ➢ achieved through randomization

2.2 Control of experimental error

Accuracy vs. precision



Experiment	Accurate?	Precise?
A	no	yes
B	no	no
C	yes	no
D	yes	yes

2.2 Control of experimental error

Requirements in experiment

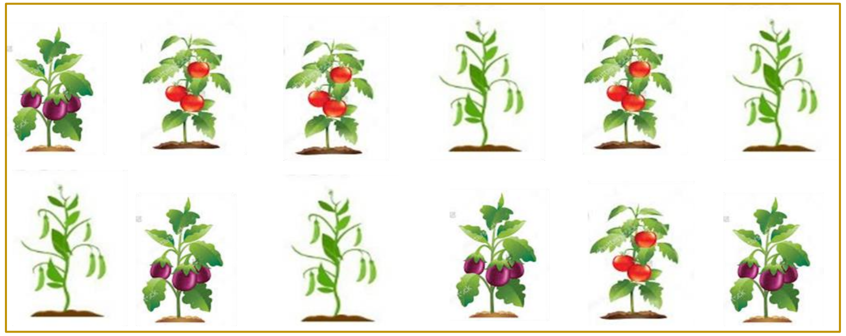
Randomization

- To eliminate bias (to increase accuracy)
- To ensure independence among observations
- Required for valid significance tests and interval estimates

In each pair of plots, although replicated, the new variety is consistently assigned to the plot with the higher fertility level.

Replication

- Each treatment is applied independently to two or more experimental units
- Variation among plots treated alike can be measured
- Increases precision - as n increases, error decreases
- Important for the measurement of experimental error!



$$S_{\bar{y}} = \sqrt{\frac{S^2}{n}}$$

Sample variance

Standard error of mean

Number of replications

2.2 Control of experimental error

What determines the number of replications?

- Pattern and magnitude of variability in the soils
- Number of treatments
- Size of the difference to be detected
- Required significance level
- The amount of resources that can be devoted to the experiment
- Limitations in cost, labor, time, and so on

2.3 Conducting an experiment

Choice of experimental site

- Site should be representative
- Grower fields may be better suited to applied research
- Suit the experiment to the characteristics of the site
  - make a sketch map of the site including differences in topography
  - minimize the effect of the site sources of variability
  - consider previous crop history
  - if the site will be used for several years and if resources are available, a uniformity test may be useful

2.3 Conducting an experiment

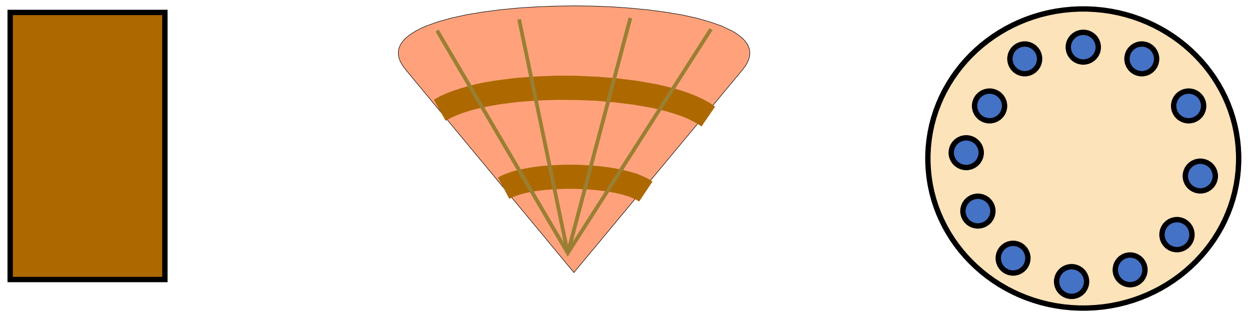
Greenhouse effects

- Greenhouse and growth chambers are highly controlled, but in practice may be quite variable
- Not representative of field conditions
  - light
  - growth media
  - unique insect pests and diseases
- Experiments can be conducted in the off-season

2.3 Conducting an experiment

The Field Plot

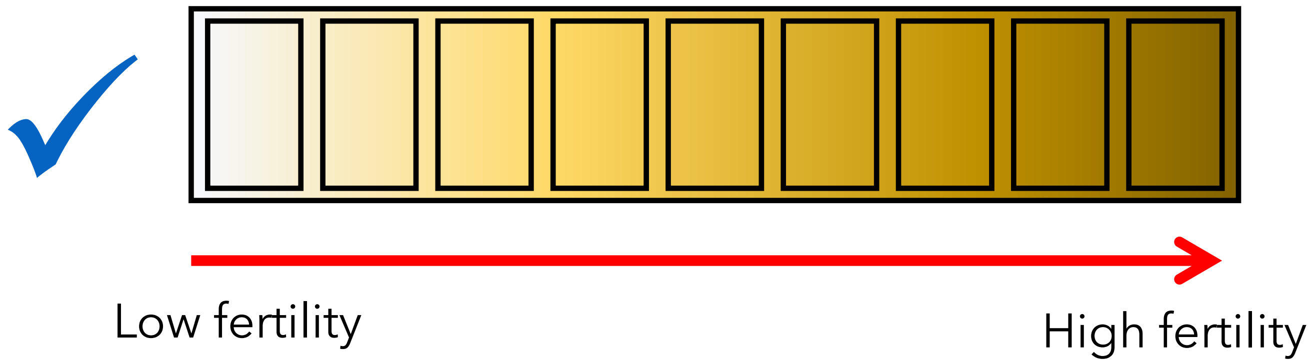
- The experimental unit: the vehicle for evaluating the response of the material to the treatment
- Shapes
  - Rectangular is most common - run the long dimension parallel to any gradient
  - Fan-shaped may be useful when studying densities
  - Shape may be determined by the machinery or irrigation



2.3 Conducting an experiment

Plot shape and orientation

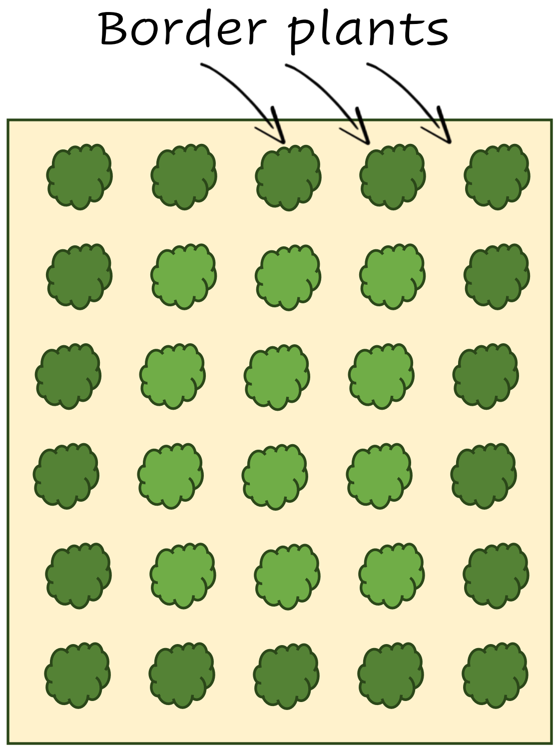
- Long narrow plots are preferred
  - usually more economical for field operations
  - all plots are exposed to the same conditions
- If there is a gradient - the longest plot dimension should be in the direction of the greatest variability



2.3 Conducting an experiment

Border effects

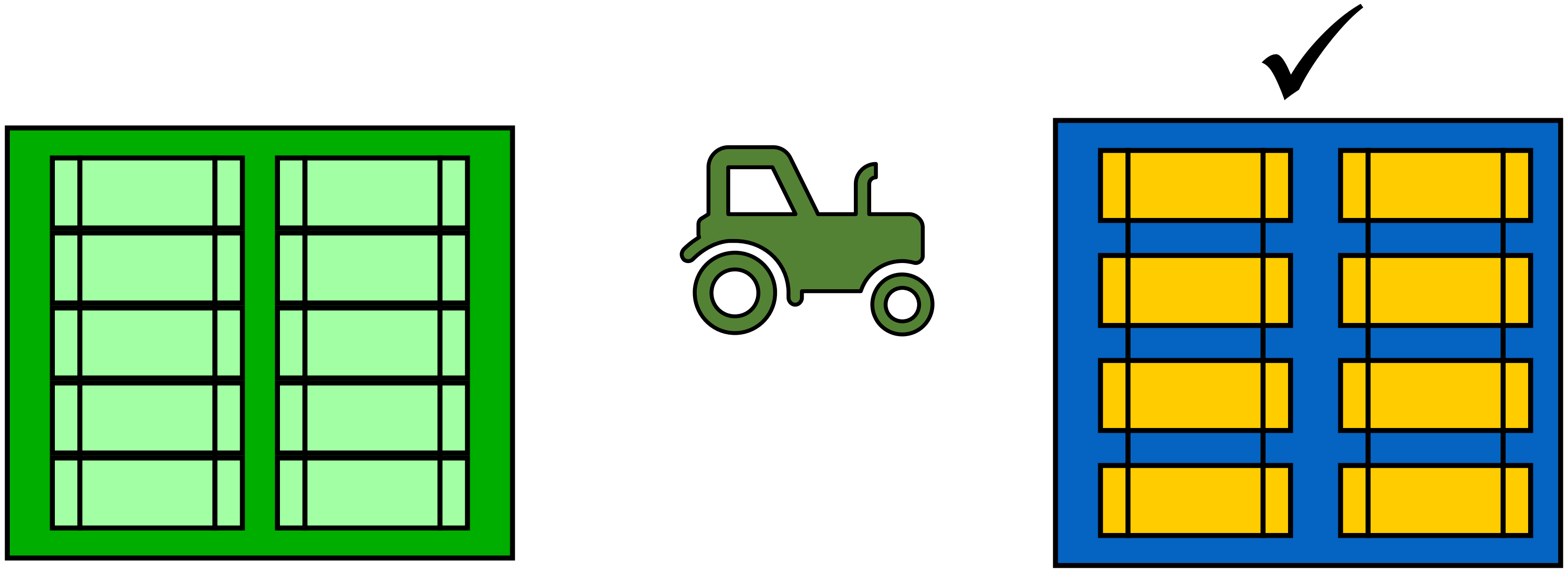
- Plants along the edges of plots often perform differently than those in the center of the plot.
- Border rows on the edge of a field or end of a plot have an advantage – less competition for resources.
- Plants on the perimeter of the plot can be influenced by plant height or competition from adjacent plots.
- Machinery can drag the effects of one treatment into the next plot.
- Fertilizer or irrigation can move from one plot to the next.
- Impact of border effect is greater with very small plots.



2.3 Conducting an experiment

Minimizing border effects

- Leave alleys between plots to minimize drag
- Remove plot edges and measure yield only on center portion
- Plant border plots surrounding the experiment



2.3 Conducting an experiment

Rounding and Reporting Numbers

To reduce measurement error:

- Standardize the way that you collect data and try to be as consistent as possible
- Actual measurements are better than subjective readings
- Minimize the necessity to recopy original data
- Avoid “rekeying” data for electronic data processing
  - Most software has ways of “importing” data files so that you don’t have to manually enter the data again
- When collecting data - examine out-of-line figures immediately and recheck

References (Books)

- Casella, G (2008). Statistical Design. New York: Springer.
- Clewer, A.G. & Scarisbrick. D.H. (2001). Practical Statistics and Experimental Design for Plant and Crop Science. New York: John Wiley & Sons.
- Gomez, K.A. & Gomez, A.G. (2005). Statistical Procedures for Agricultural Research (4th Edition). New York: John Wiley & Sons.
- Hinkelman, K. & Kempthorne, O. (2007). Design and Analysis of Experiments, Introduction to Experimental Design (2nd Edition). New York. Wiley-Interscience.
- Peterson, R. G. (1994). Agricultural Field Experiments: Design and Analysis. New York: Marcel Dekker.