



Beyond Cowboy Science

Simple Methods for Conducting
Credible and Valid Research

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Modified for Nursery Coop Use

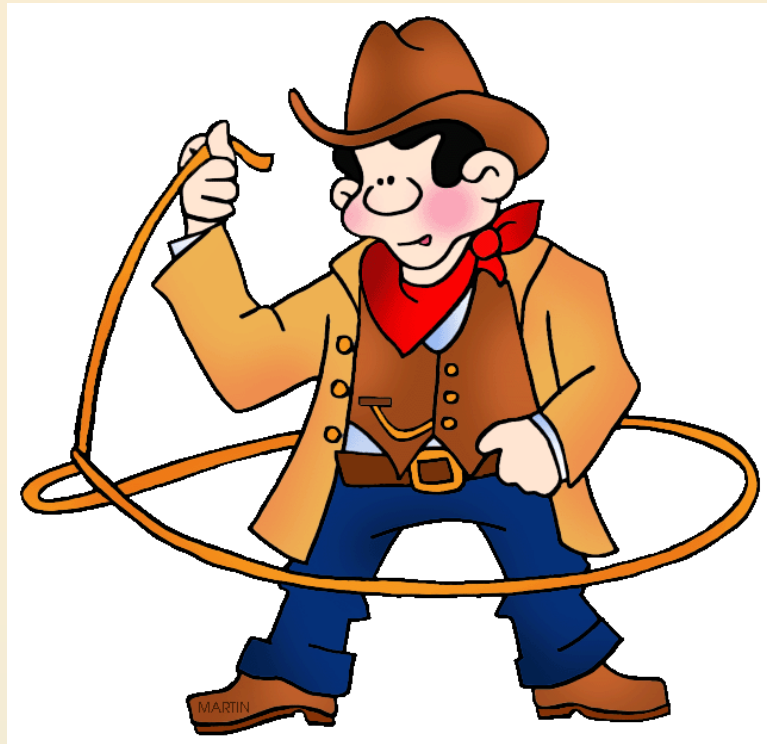


There will be
mention of...

StAtiStiCS



What is Cowboy Science?



Cowboy Science



- “quick and dirty” trials
- “demo plots”
- This approach can yield results that are statistically invalid and/or biologically untrue
- Using such data leads to erroneous conclusions

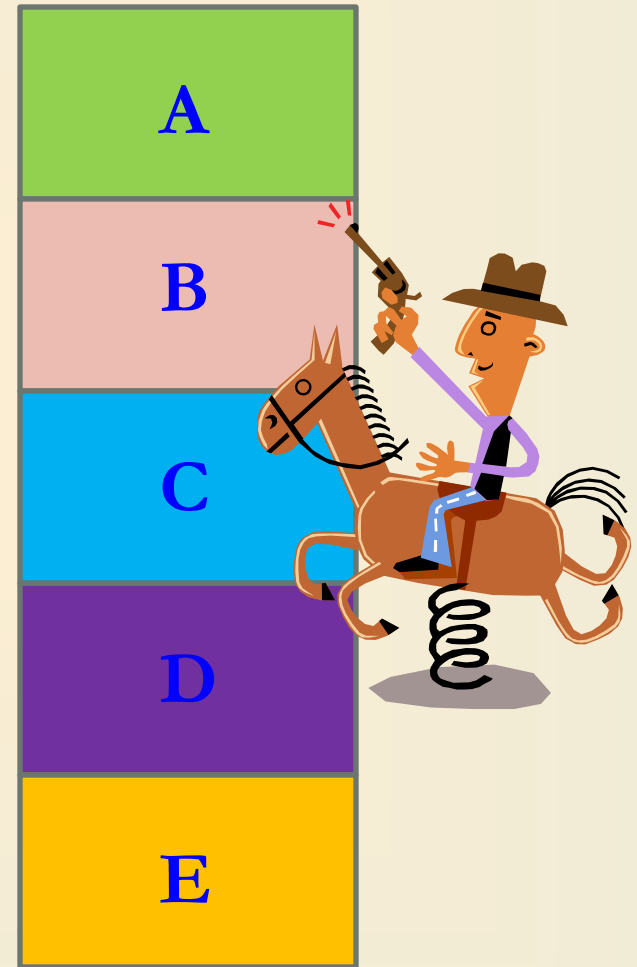
The Consequences

Using flawed results to make multi-million dollar management decisions can really get you in the end



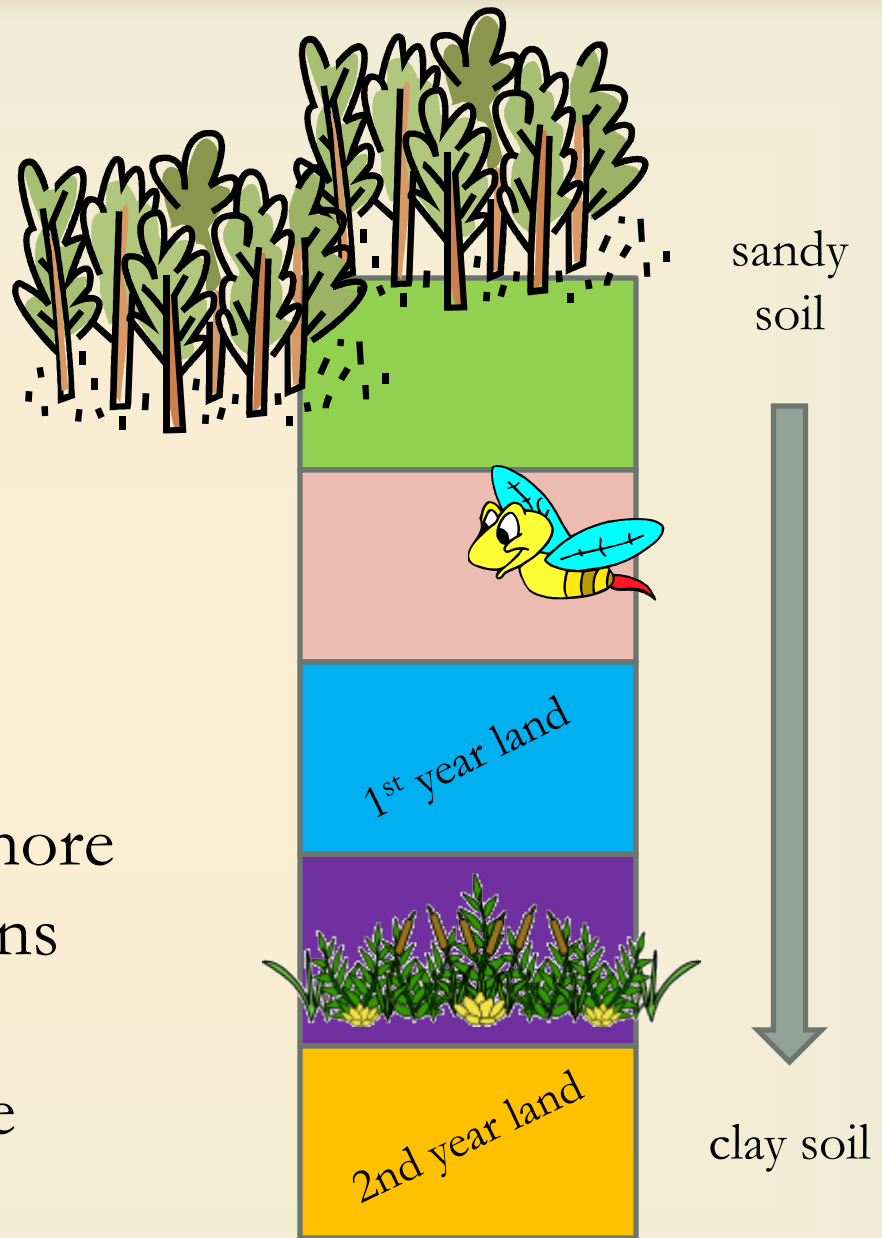
An Example

- Cowboy Joe has set up a trial to compare growth of seedlings from six different families.
- He established 5 plots (one in each production unit), each with 100 seedlings
- After 5 months, he finds that the Unit C grew the best seedlings and decides this is his best family.
- So....what's the problem?



The Problem

- Cowboy Joe's site conditions are not uniform
 - (They rarely are!!)
 - Some plots have more favorable conditions than others
 - The treatments are confounded





Confounding

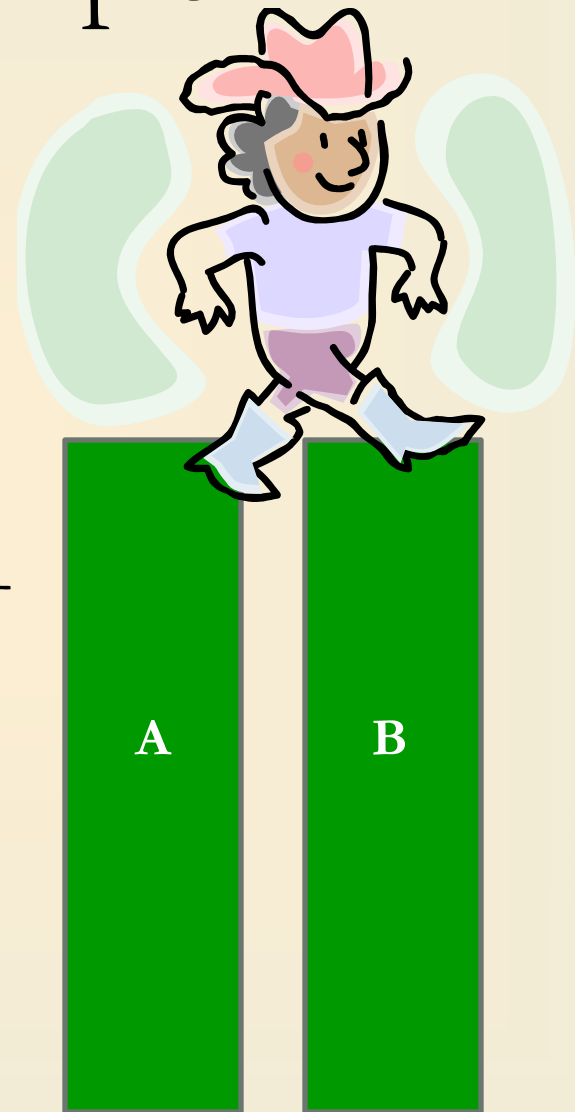


1. to throw into confusion or disorder.
2. to treat or regard erroneously as identical; mix or associate by mistake: truth confounded with error.
3. to mingle so that the elements cannot be distinguished or separated.

In Cowboy Science, confounding can result in differences among treatments which are not actually due to the treatment

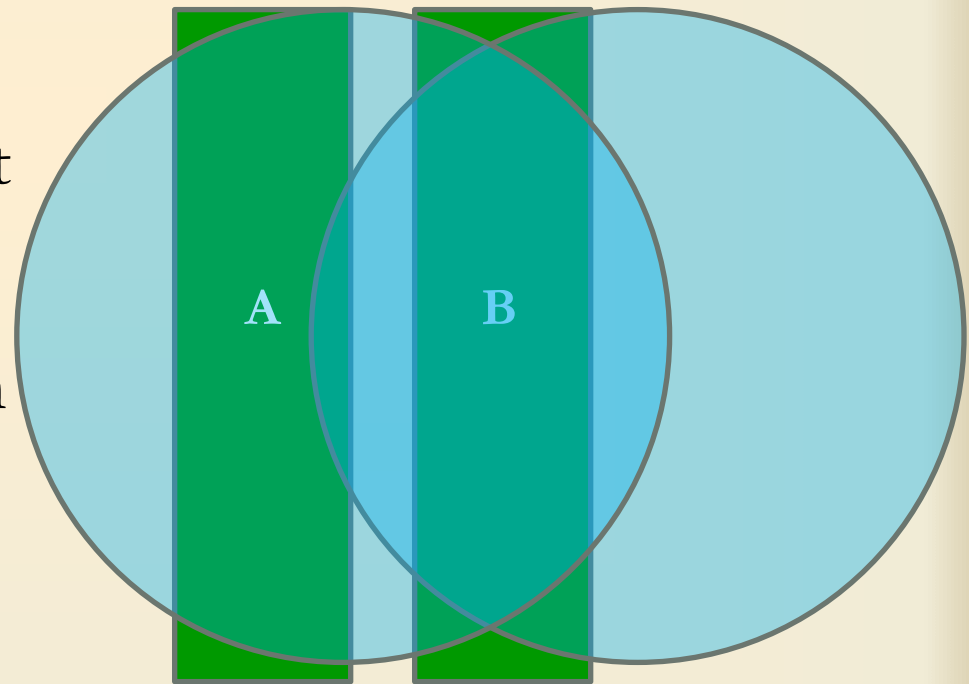
Another Example

- Cowgirl Jane sets up a nursery trial to test two products that the manufacturer claims will increase root growth
- She treats one nursery bed with just water - Treatment A and the other with the test product - Treatment B
- Seedlings treated with Treatment B grow much better so she decides to order that product for her entire crop



The Problem

- The irrigation patterns result in one bed receiving more water than the other
- The treatments were applied to seedlot Z which is a 2nd gen seedlot that grows differently than most other seedlots in the nursery
- Confounded with irrigation pattern and questionable applicability



Some Pitfalls of Cowboy Science

- Confounding
- No control treatment
- No study plan
- No labeling or mapping
- No follow through or maintenance
- Too many treatments
- Too few trees per treatment





About those
products that
seem too good
to be true...

they usually are!

Don't be taken in by glossy fliers
and smooth talking representatives!

Most products do not perform as
claimed and have been tested solely
by Cowboy Science

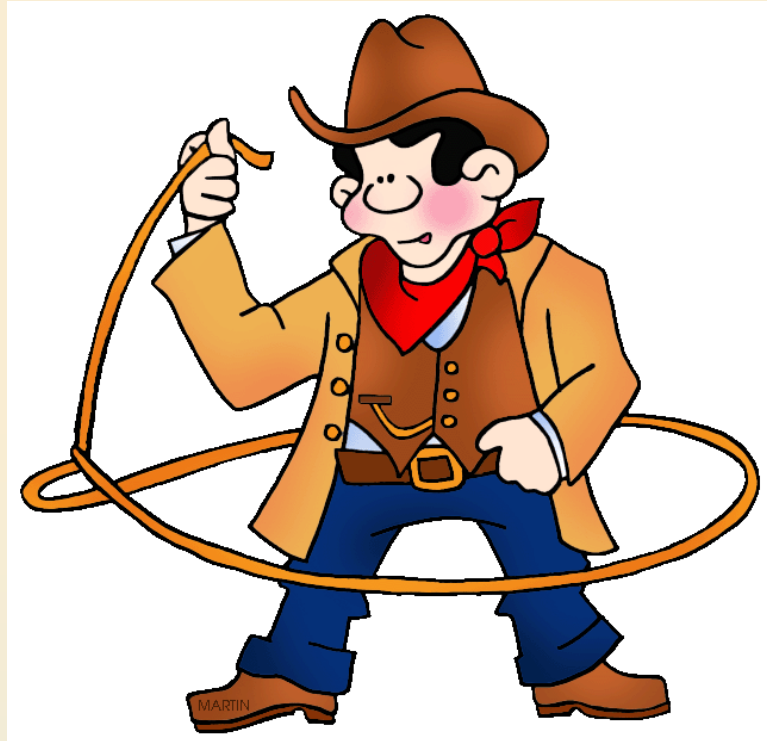


Also be wary of seeing what you want to see...

- Foresters and Nursery Managers/Growers gather around the biggest seedling like it's a campfire
- Don't fall for your own hopeful outcomes! Instead, rely on data generated from well-designed studies



The Three R's of Study Design





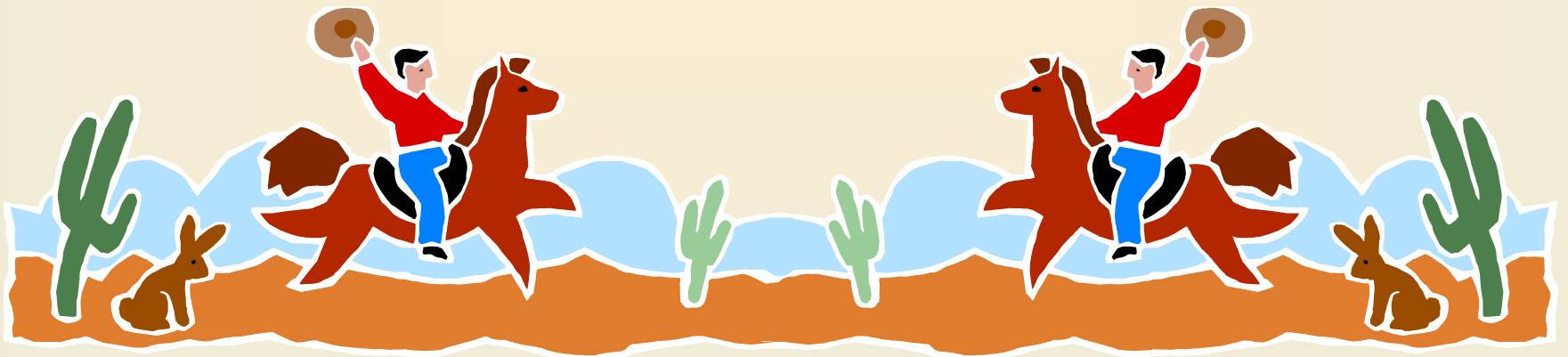
It's all About Variation

Controlling the
causes of data
variability is key

✓ Randomization

✓ Replication

✓ Representation



Randomization

- The circumstance in which each plot/tree could have been assigned to any of the treatments
- Prevents bias
 - Bias is any process which tends to produce results or conclusions that differ systematically from the truth



Implementing Randomization

- Assign treatments to trees or plots using a random, non-biased method



- roll the dice

- draw a playing card



- use a random number generator

- draw out of a hat

- To save time and avoid on-the-ground bias, do this in the office, prior to establishing the study



Replication

- Gives the ability to measure variation in a population whether it is due to the treatments, the individual trees, or the physical conditions on the site
- Failure to replicate makes it impossible to make valid comparisons between treatments
- Without replication, all you have is a one-time event which may or may not be repeatable
- At least 4 replications are recommended



If this cowboy rides the bull successfully just once, can we really predict that he will do so from now on?

Ahh.. A treatment effect....

Non - Sprayed Bed



Herbicide Sprayed Bed



Non - Sprayed Bed





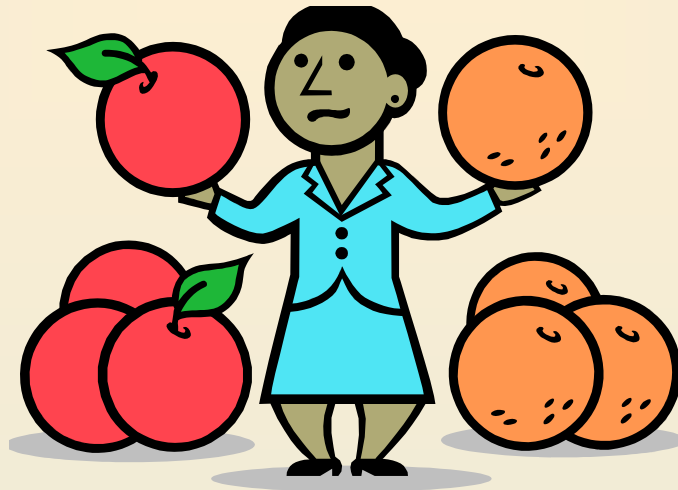
Implementing Replication

- Replicate by individual trees or plots
 - Plots are usually best for forest/nursery trials
 - Individual trees are good for short-term studies in small areas with uniform conditions
- Row plots vs. square plots
 - Square plots are best for longer-term
 - Recommend a minimum of 25 seedlings or 10 established trees per plot
 - Nursery – we use seedlings within counting frame
- Number of replications
 - Recommend a minimum of four replications
 - More is better than less!!

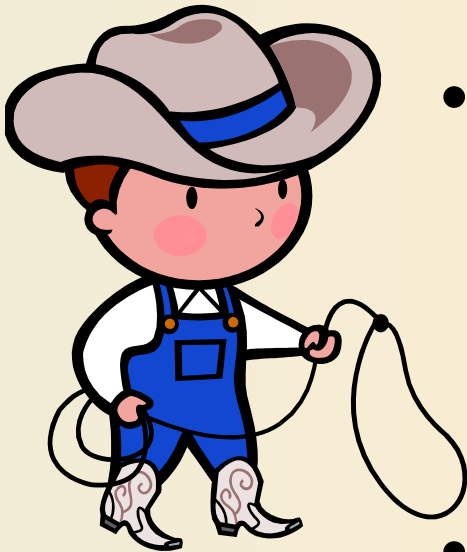


Representation

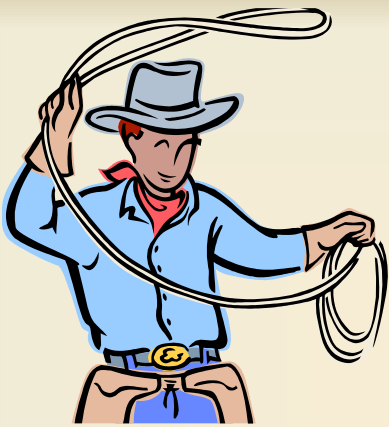
- Gives the ability to apply the results to the specific trees/situations of interest
- Provides the “Scope of Inference” for the data
 - the population and circumstances to which the results can be applied



Implementing Representation



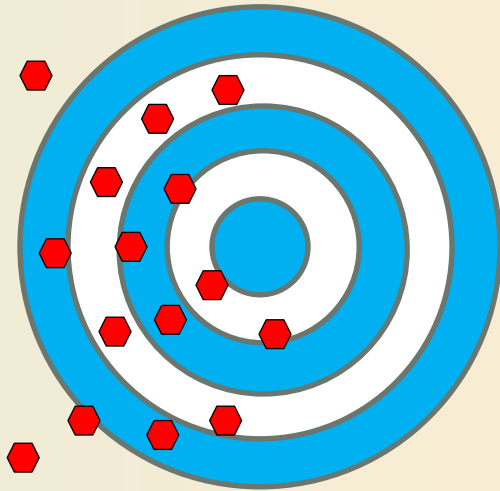
- Think through the desired outcome of the study
- What is the desired scope?
 - What population?
 - Under which circumstances?
 - Consider: what, when, where, why, who, and how
- With this in mind, choose your study population and circumstances accordingly
- Why test using 1.5 gen seedlings when you are more interested in 3.0+ gen seedlings



What about “operational” trials?

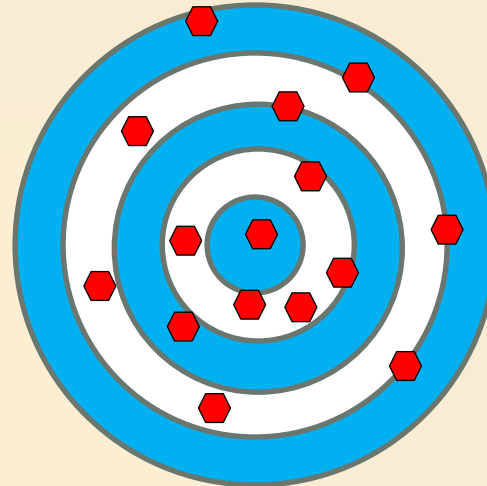
- The study objective is to generate results which can be applied to operational practices
- However, using operational practices during the study often results in excess “noise” (variation) and an inability to assess treatment effects with accuracy and precision
- Therefore, studies should be carried out with extra care and attention in order to minimize variation, focus on the objective, and determine the maximum treatment response potential

A quality study design maximizes Accuracy and Precision



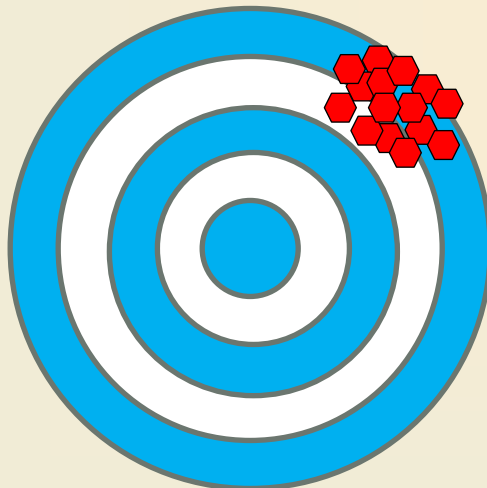
low accuracy
and
low precision

(biased and
imprecise)



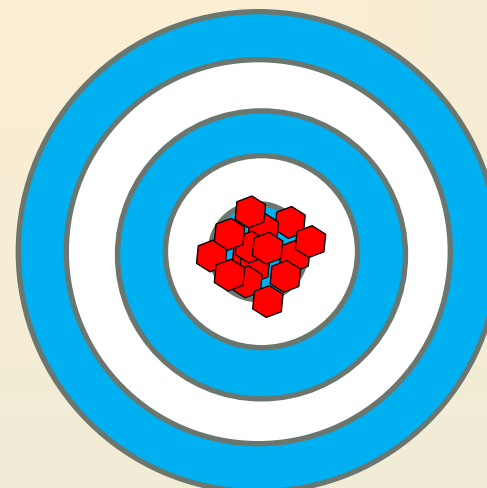
high accuracy
and
low precision

(unbiased and
imprecise)



low accuracy
and
high precision

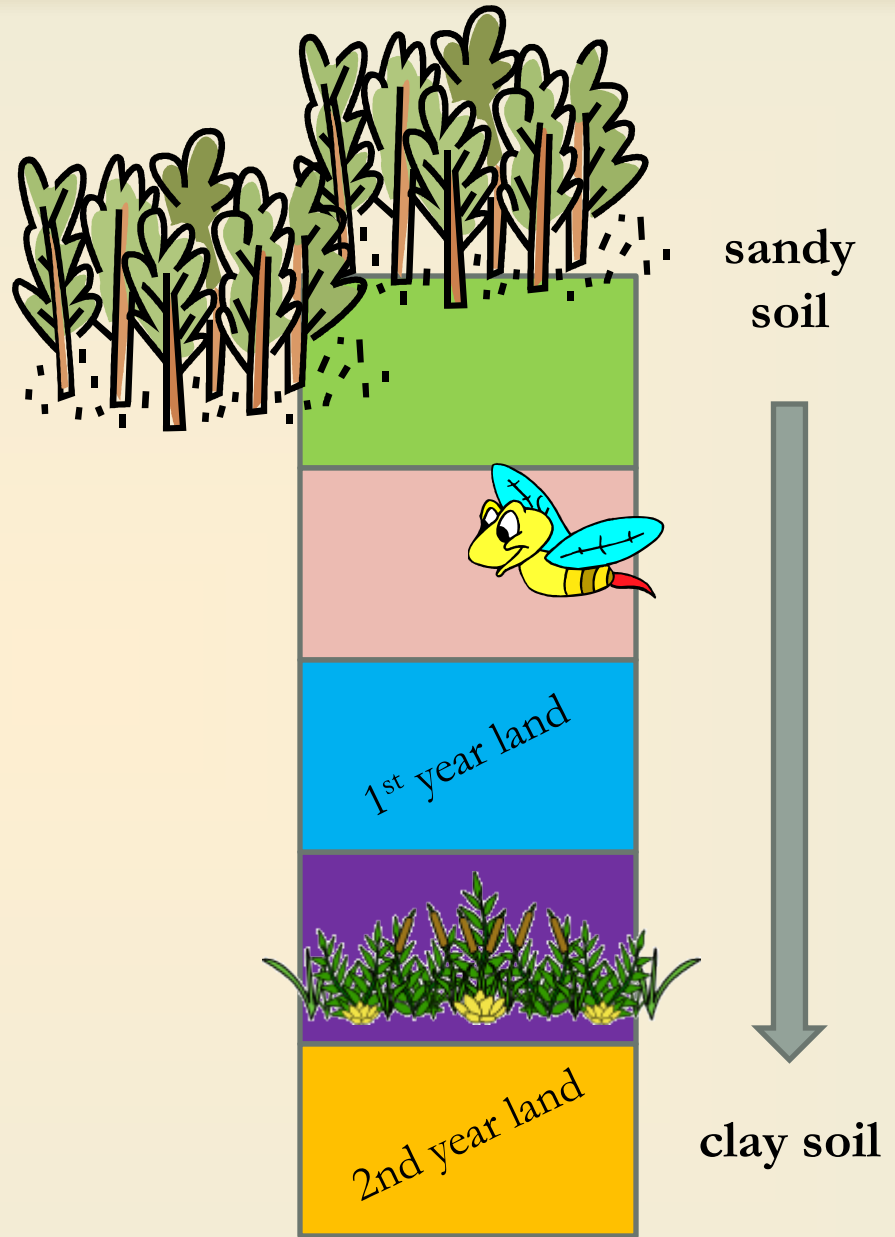
(biased and
precise)



high accuracy
and
high precision

(unbiased and
precise)

What could
Cowboy Joe
do differently?



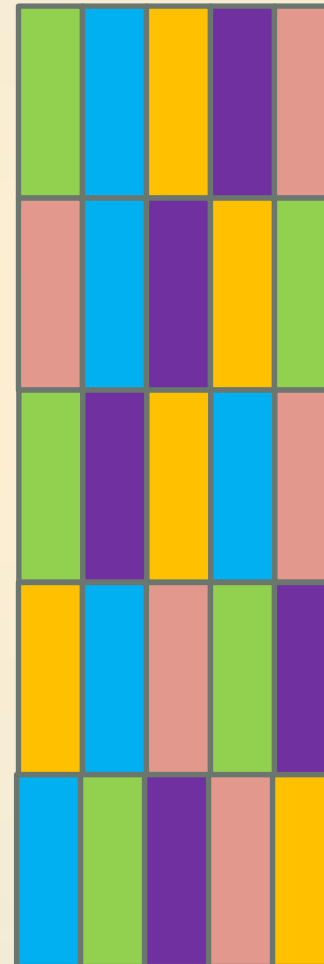


It's all
About
Variation

Control Variation



- Replicate and randomize to eliminate confounding
 - Chose units without obvious problems (insects, excessive weeds)
 - Choose units in same fumigation year
 - Don't mix seedlots or genetics
- Not “operational” but eliminates excess “noise” to allow for a true comparison among groups



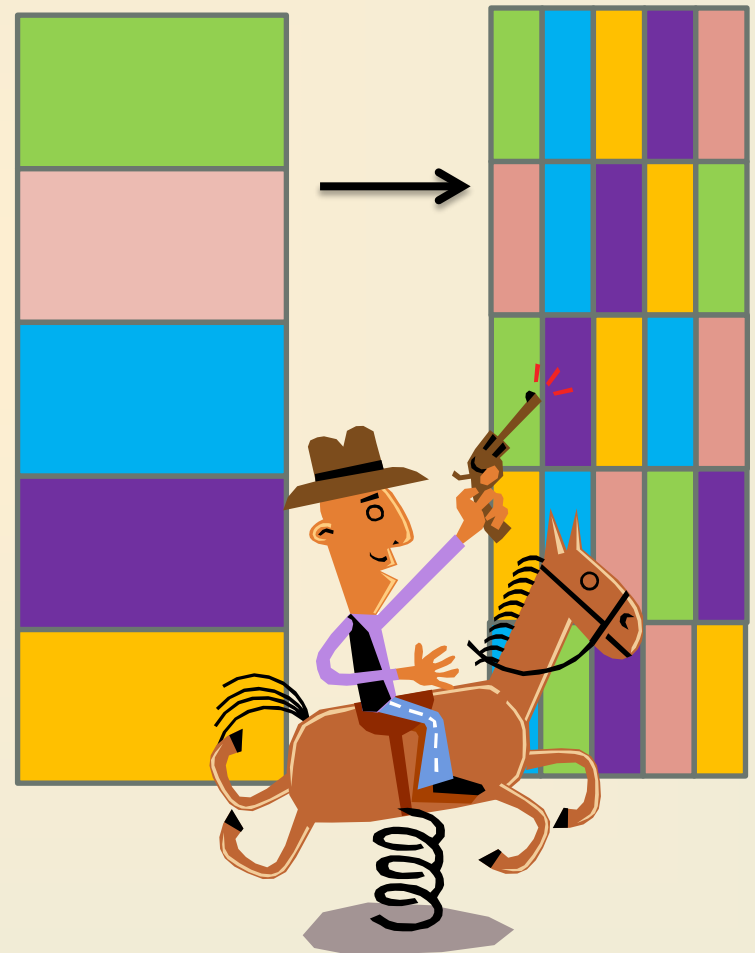
sandy
soil



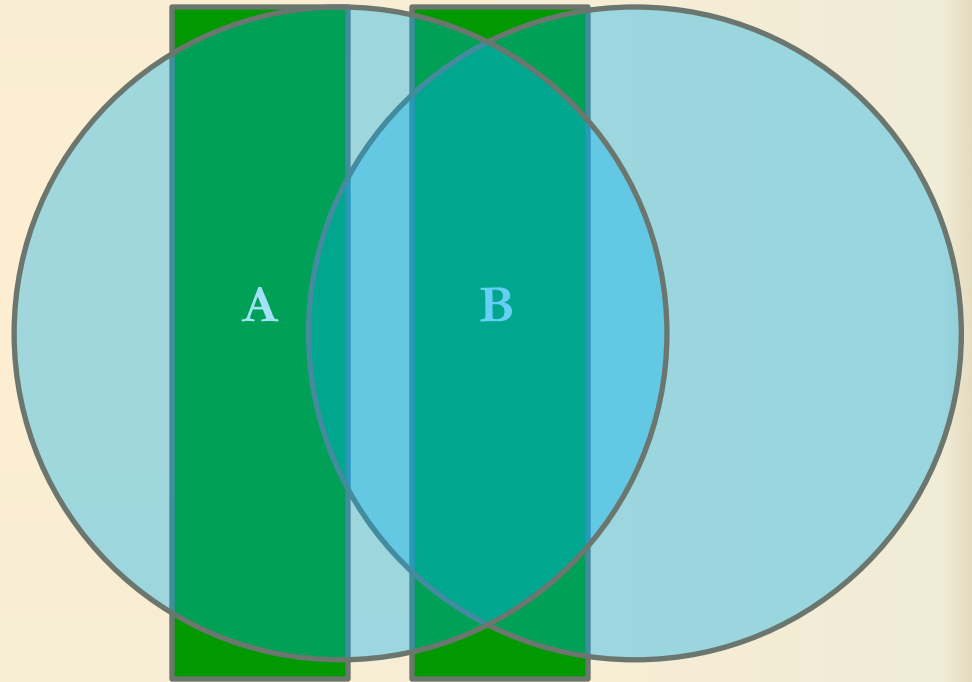
clay soil

Proper design does not have to cost more or take a lot more time....

- For, Cowboy Joe, the revised design uses the same amount of space and seedlings and just a little more time to randomize, lay out, and maintain the plots
- Furthermore, the original design produced confounded data and was a 100% waste of time and resources

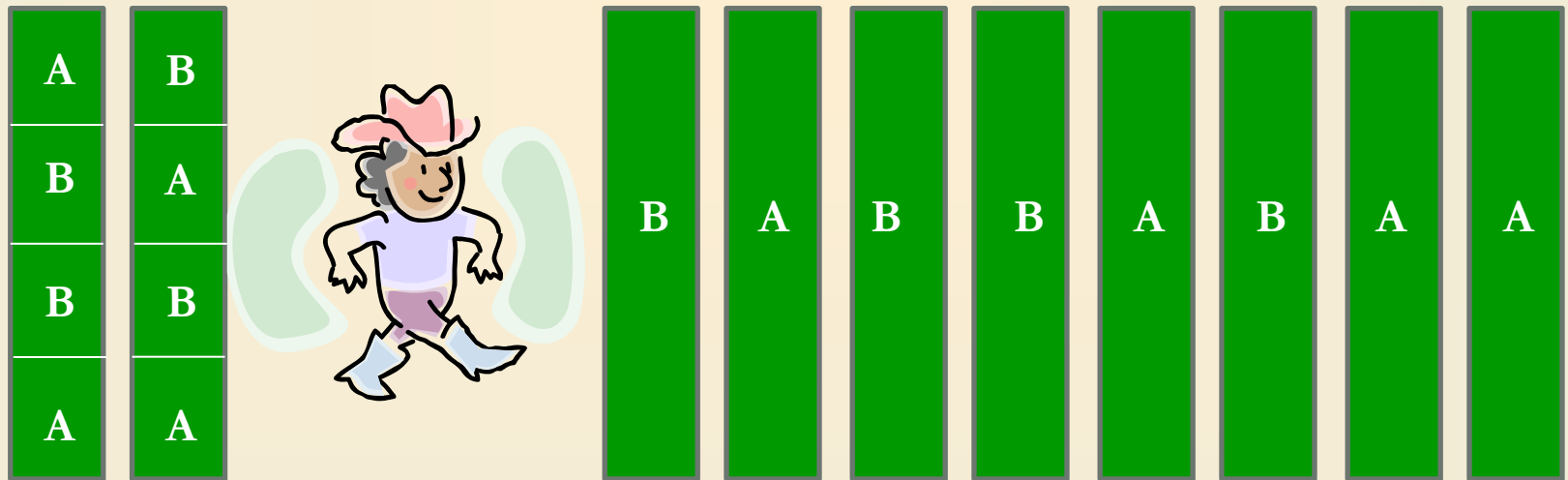


What could
Cowgirl Jane
do differently?



Control Variation and Ensure Representation

- Replicate and randomize to avoid confounding
- Choose a representative lot or lots so that the results will be applicable for a majority of the crop



She can randomly assign treatments to replications within nursery beds

Or, she can randomly assign treatments to several nursery beds or units

Completely Randomized Design

- Plots of trees (or individual trees) are identified and each are randomly assigned to a treatment
- Must be installed in an area that is relatively uniform

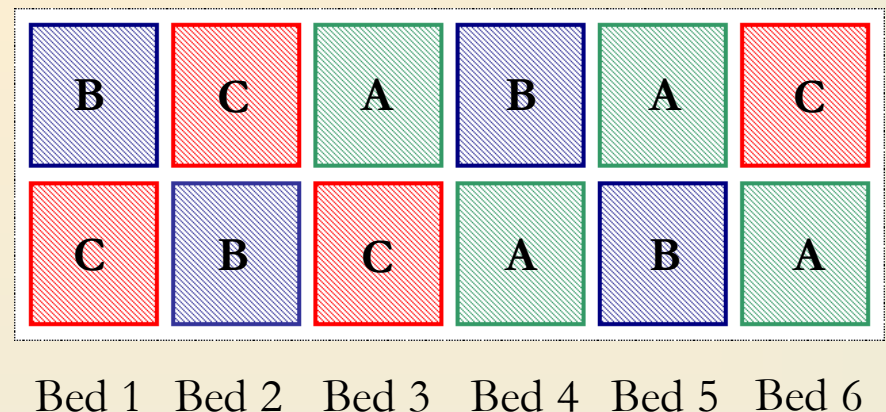
3 Treatments:

A

B

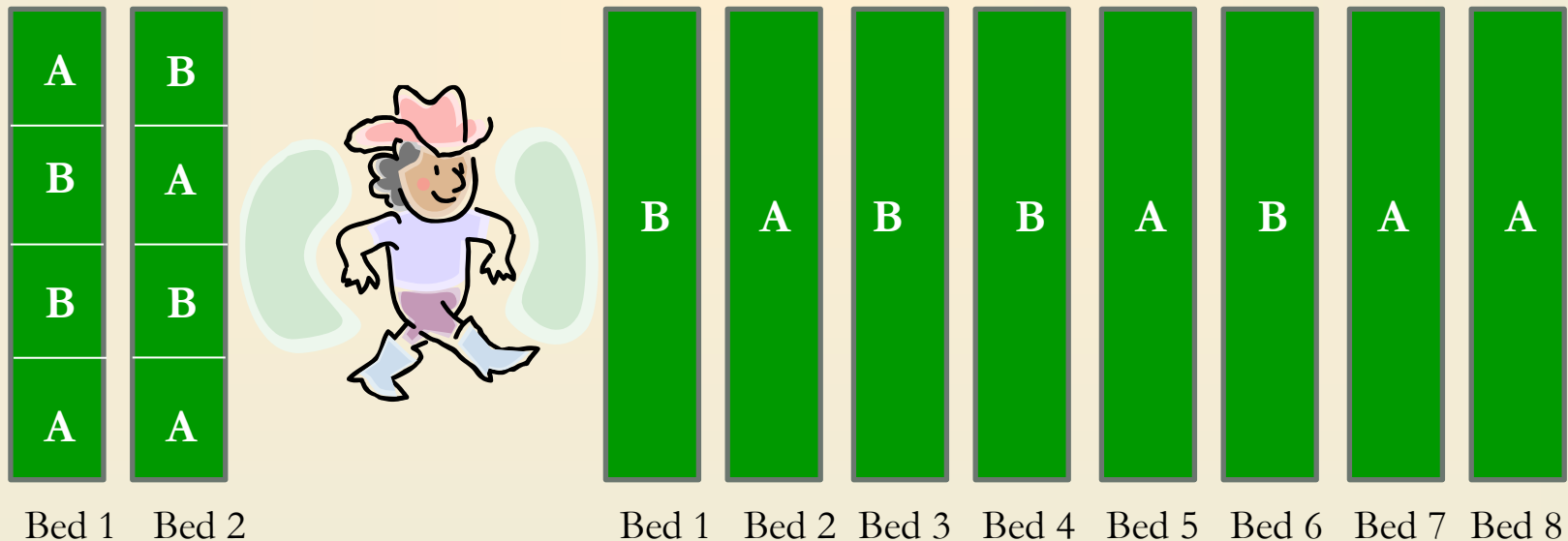
C

4 Replications



Cowgirl Jane's study design is a completely randomized design

- Four replications
- Two treatments
- Randomly assigned within the study area





Randomized Complete Block

- Treatment plots are grouped into blocks
 - Conditions among blocks can vary significantly
 - Conditions within each block should be relatively uniform
 - Every treatment is randomly assigned within each block
- Blocking is based on any condition or gradient that could affect treatment responses
 - slope, drainage, aspect, soil, vegetation, site



Cowboy Joe's study design is a randomized complete block

- Five blocks (replications)
- Five treatments randomly assigned within each replication

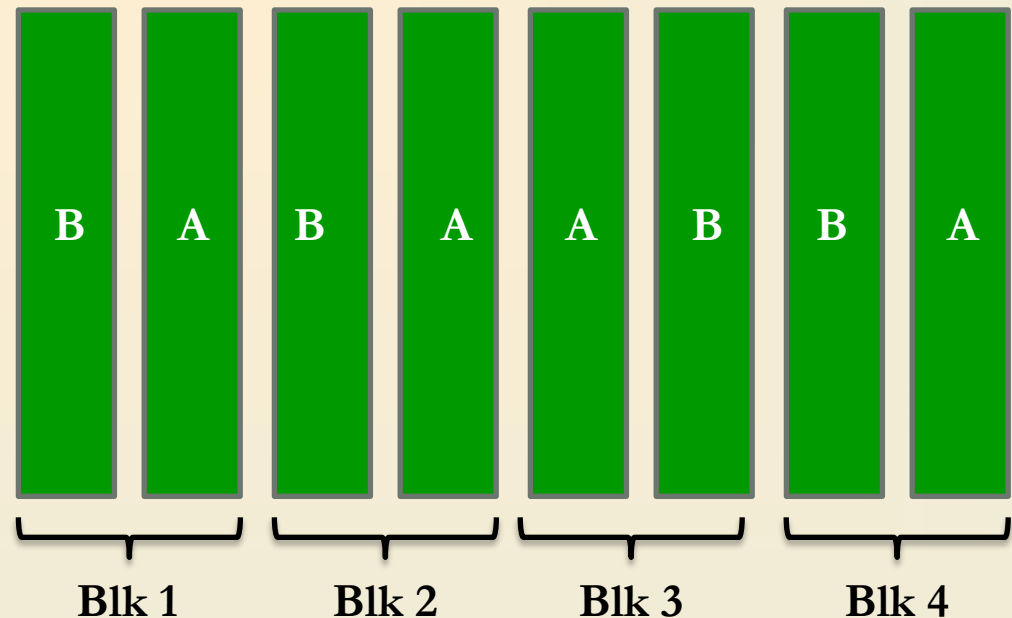
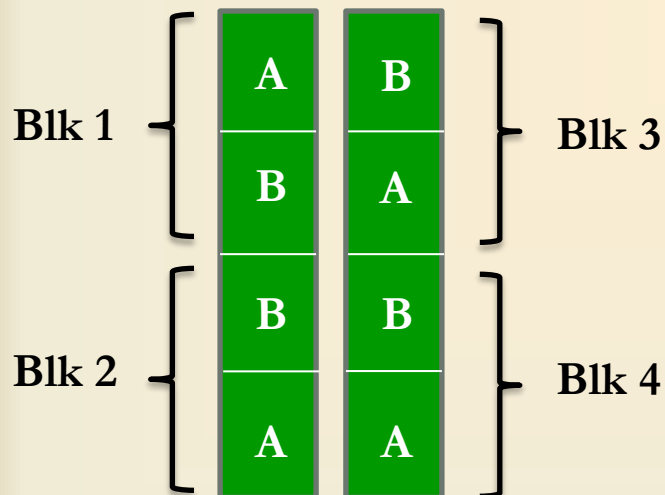


A	C	E	D	B	Block 1
B	C	D	E	A	Block 2
A	D	E	C	B	Block 3
E	C	B	A	D	Block 4
C	A	D	B	E	Block 5

Cowgirl Jane's study could also be set up as a randomized complete block design



- This will account for gradients and other variations among nursery beds
- Four replications (blocks)
- Two treatments randomly assigned within each block



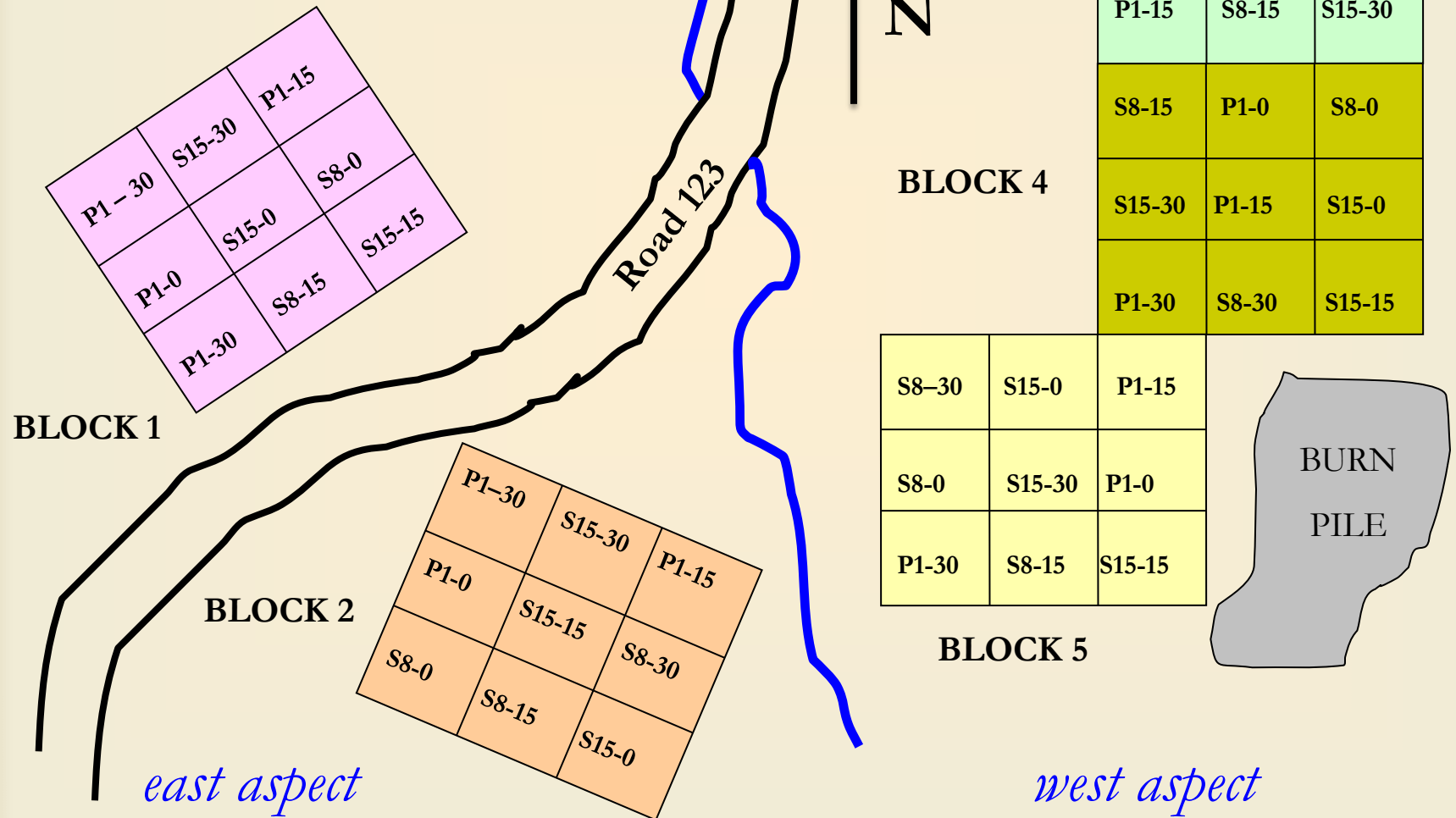


Factorial Treatments

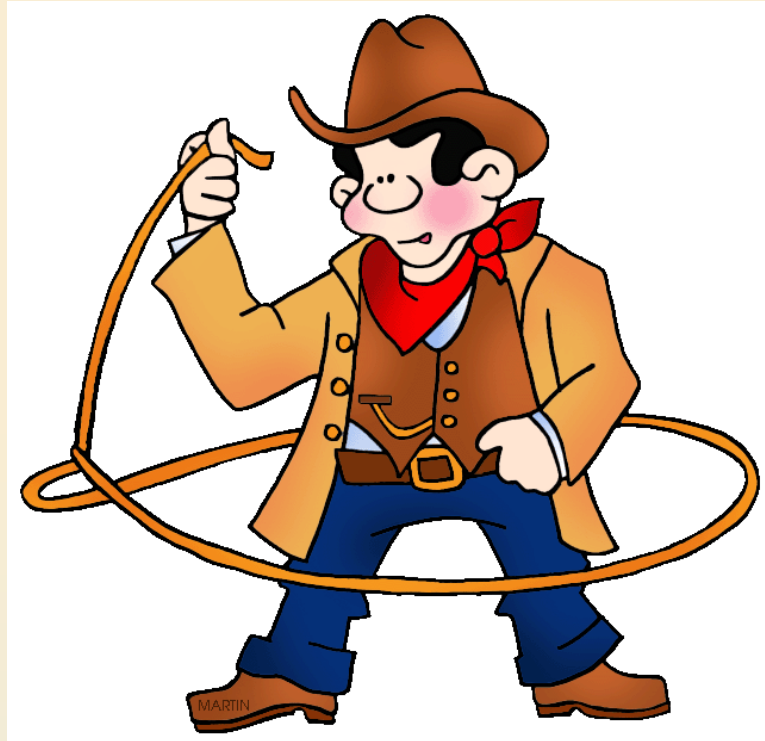


- Allows you to evaluate two treatments in the same study
 - For example, fertilizer type (Factor A) and families (Factor B)
 - You must include all combinations for a true factorial
 - Avoid too many treatments; they add up quickly!
- Allows you determine if there are interactions between the two factors
 - For example, is the response to fertilizer the same for every stocktype?
- Keep it simple; do not go beyond two factors!
 - Increasing the number of treatment comparisons increases the odds of finding a difference when one does not exist
 - Three-way (or more) interactions are very challenging to quantify and interpret

Factorial trial to evaluate
3 families (P1, S8, S15)
and 3 fertilizer rates (0, 15, 30)
in a randomized complete block



Elements of a Study Plan



A study plan
should read like
a recipe that
anyone can
follow from
start to finish





Study Plan Elements:

1) Define the problem and state the objectives

- Be clear, concise, and specific.
- If the problem cannot be defined, it will be difficult to solve
- Do not try to answer too many questions in a single study
- Examples:
 - Loblolly Pine seedling survival is poor during the first two years after outplanting. We wish to determine if application of Fipronil increases seedling survival.
 - The effect of a new site prep technology on seedling performance is unknown. The objective is to evaluate effects of four site preparation methods on subsequent seedling growth and survival





Study Plan Elements:

2) Describe the experimental material

- The material selected should be REPRESENTATIVE of the population in question.
- Examples:
 - Loblolly Pine seedlings (seed lot 123-456, Central Coastal Plain) will be used. Seedlings were sown in 2013 at the WeGrow Nursery (Georgia)
 - A 3-year old plantation of Slash Pine planted in 2007 with seedlings from WeGrow Nursery, etc....





Study Plan Elements:

3) Describe the study site

- The site should be REPRESENTATIVE of environment associated with the problem and objectives.
- Examples:
 - Seedlings will be outplanted to a site 5 miles NW of Shubuta, MS. The site was harvested in 2012. Site preparation consisted of....Etc....





Study Plan Elements:

4) Describe the treatments

- Select treatments specific to the problem and objectives
- Be specific
- Examples:
 - Four Loblolly Pine families from WeGrow, Inc. (Georgia) will be evaluated: Fam 123, Fam 528, Fam2234, Fam 4435)
 - Three fertilizer treatments will be included in the study: a) unfertilized control, b) 10-25-4, c) 17-17-17, and d) 15-9-12. Fertilizers are controlled-release and manufactured by Apex.
 - Seedlings treated with mycorrhizal fungi obtained from Fungi, Inc. (Ecto, WA) will be compared to untreated seedlings.





Study Plan Elements:

5) Define the experimental design

- Choose the simplest design that will meet the objectives
- Include details about RANDOMIZATION and REPLICATION
- Examples:
 - Seedlings will be outplanted in a completely randomized block design with 5 blocks each consisting of 25 seedlings per treatment.





Study Plan Elements:

6) *Describe the installation*

- Include dates, labor, equipment, supplies, etc.
- Example:
 - The study will be installed in February 2013. Pin flags will mark each planting spot and each seedling will be tagged with block and treatment. Seedlings will be hand planted using four planters and will be monitored for quality.





Study Plan Elements:

7) List the desired data and how it will be collected

- Include timeline, tools, dates, etc.
- Be specific.
- Examples:
 - Each year from 2013-2017, seedlings will be measured in early October for height (groundline to base of terminal bud), stem diameter (1 cm above groundline), and survival.
 - Foliar samples will be collected in the July after planting from a branch in the upper half of the seedling and analyzed for concentrations of N, P, K, Mg, and B. Nutrient analyses will be conducted at Ion Lab, Ltd. (Bunson, ID).





Study Plan Elements:

8) *Describe how the data will be analyzed*

- The sources of variation and method of analysis should be determined ahead of time to ensure that the experimental procedures will generate the answers sought
- Examples:
 - Data will be analyzed using Analysis of Variance for a completely randomized design. Means will be compared using Duncan's Multiple Range Test





Study Plan Elements:

9) *Describe study maintenance and duration*

- Include necessary annual activities other than data collection
- Examples:
 - Competing vegetation will be controlled with herbicide (Killfast[®] @ 22 fl oz/acre) for the first three seasons after planting
 - Pin flags and seedling tags will be checked on each measurement date and moved as needed to avoid damage and growth restriction





Study Plan Elements:

10) *List the expected outcomes*

- Regardless of the results, the study can be used to address the objective, make management decisions, and determine future research needs.
- Examples:
 - Results of this study will be used to determine which loblolly pine family has the greatest growth potential on specific sites in Georgia
 - A report of this study will be presented at the 2015 Nursery Cooperative Advisory Meeting
 - An article will be prepared and submitted to *Tree Planters' Notes* for publication.





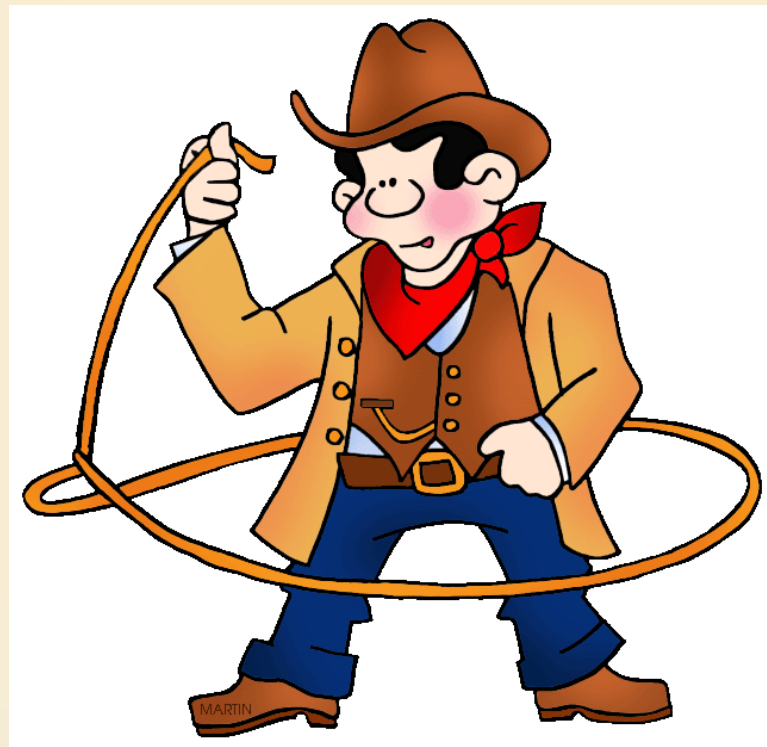
THINK AHEAD!

Plan for EVERYTHING

Be Specific

Be Detailed

Setting up the Study





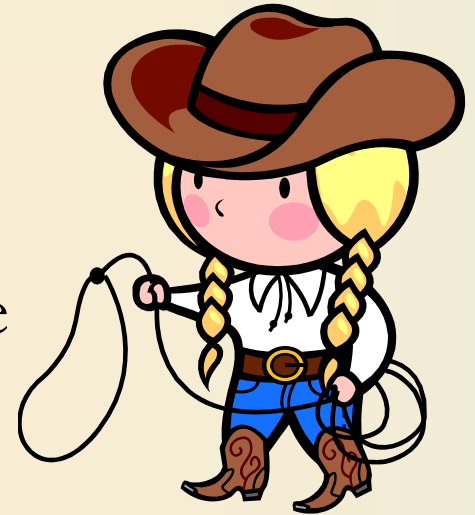
Avoid adding more variation



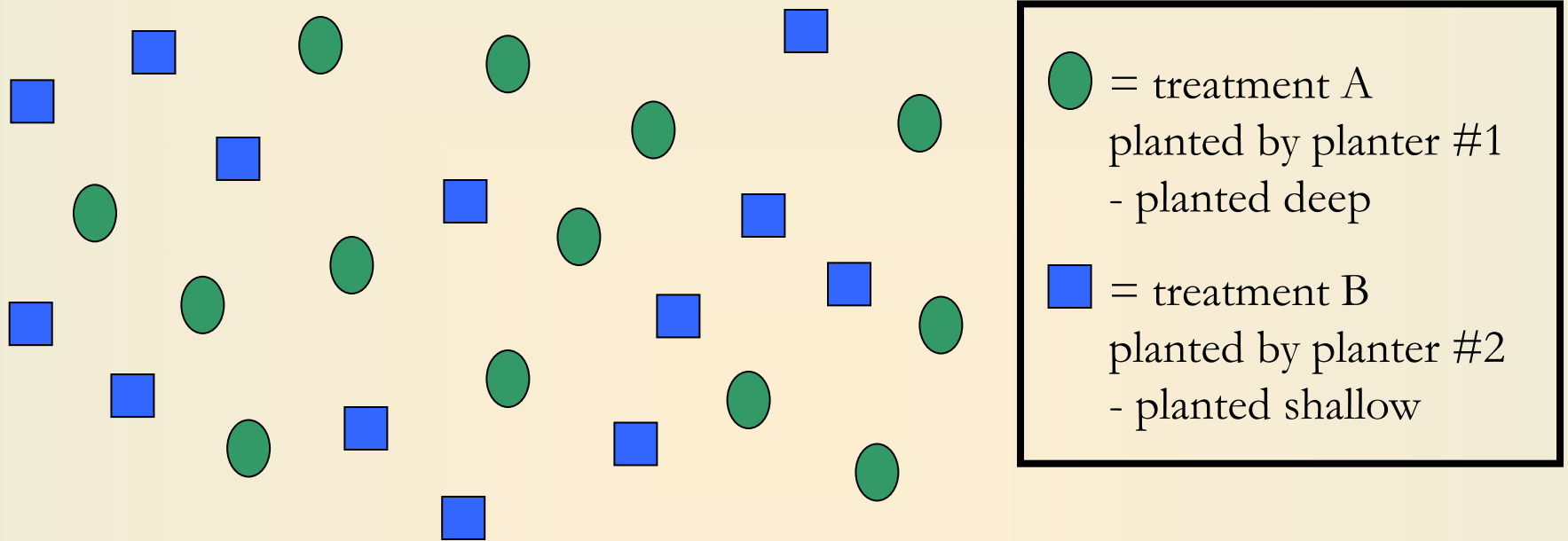
- Make sure conditions within a replication are as uniform as possible
- Plant carefully! Use your best planters.
- Protect from browse and other factors which create more variation and can mask potential treatment responses
- Stick to the study plan
 - If anything must be changed; record it in detail

Avoid confounding

- Use seedlings from the same seedlot and nursery
 - unless the objective is to determine differences among seedlots or nurseries
- Plant/Apply treatments at the same time
 - unless planting date is a treatment
- Et cetera

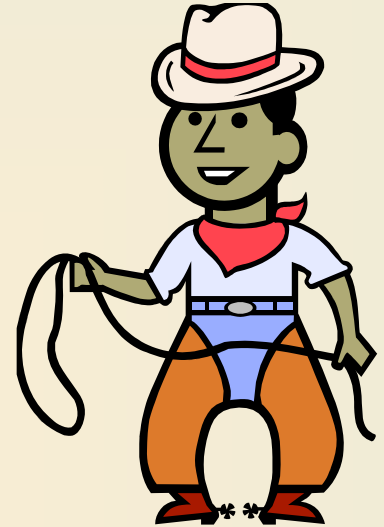


Example of Confounding



- Seedlings in treatment A are shorter than those in treatment B but not because of the treatment
- An otherwise perfectly designed study has now been compromised

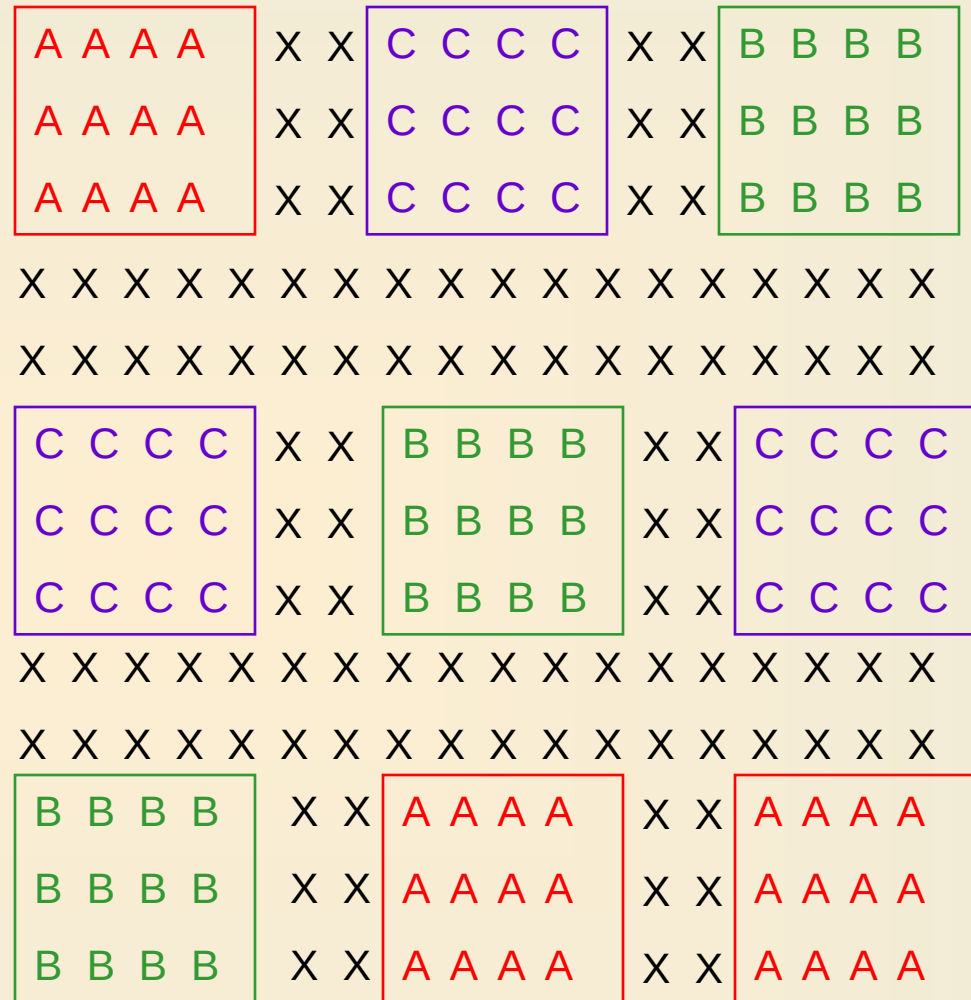
Avoid Bias



- During Plot Establishment
 - “This plot looks weedy; let’s put the veg. control treatment here.”
 - “This area is close to the road, let’s install the fertilizer treatments here so we don’t have to carry it up that hill.”
- During Treatment Application
 - “This seedling is pretty small. It looks like it could use a little extra fertilizer.”



If necessary,
control effects
of adjacent
treatments on
each other with
border rows or
buffer strips



A=treatment 1, B=treatment 2,

C=treatment 3, X=buffer rows

Identification

- Pin flags
 - Blue lasts the longest;
red lasts shortest
 - Permanent ink markers fade very quickly
- Tags
 - If on the main stem, will need to be moved after 1-2 years to avoid girdling
- Maps
- Stakes and posts
- Color coding



Block 5

Block 3

Block 2

Block 1

Block 6

Block 4

Rd. 131

Rd. 130

Rd. 110

Rd. 100

GATE

GETTHERE ROAD



Map It!

not to scale

Jesup Nursery

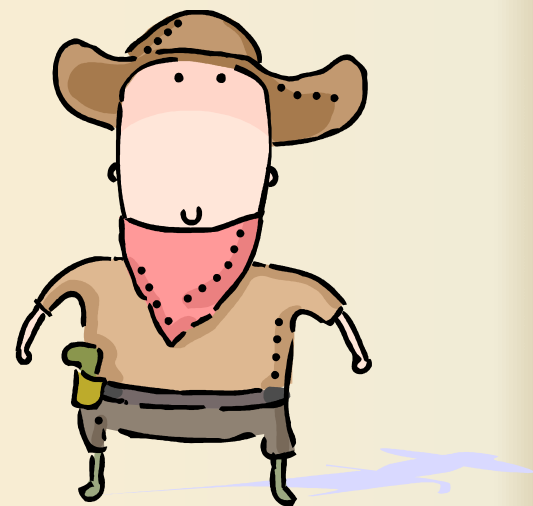
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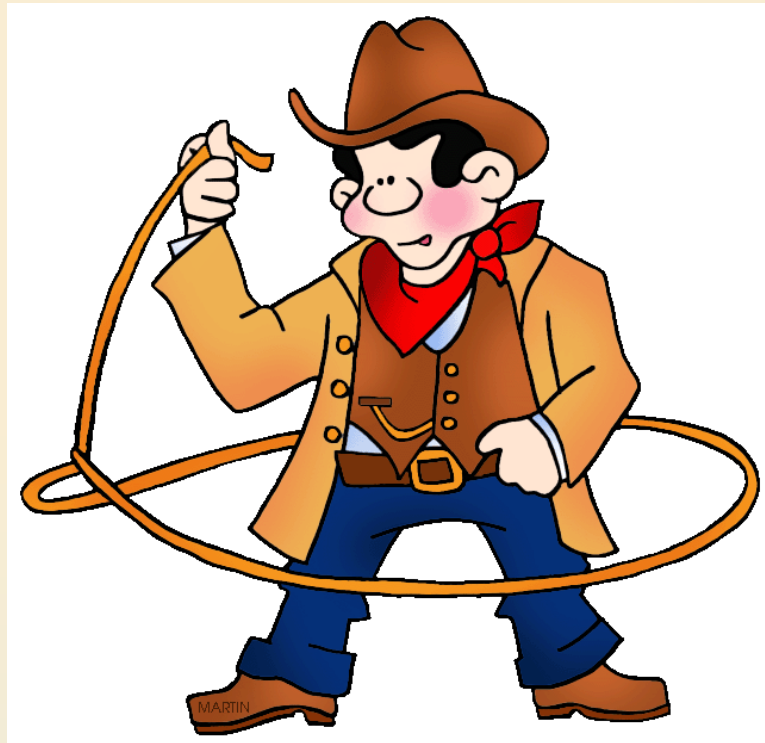
Google earth

Longevity

- Accessibility
 - Will the road be open for the duration of the study?
- Are there sufficient resources to continue the study to completion?
- Protect the study from damage due to factors such as browse, competition, and handling which are not questions of interest

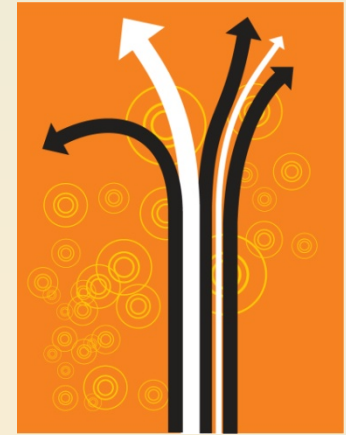


Data Collection





Avoid adding more variation



- Be consistent!
- Measure under ideal conditions if possible
 - Avoid fatigue or severe weather conditions
- Do not confound data during measurement
 - One person measures all of one treatment
 - Some treatments are measured earlier than others
- Do not damage seedlings during measurement
 - Calipers can girdle seedlings



Avoid Bias

- Systematic over/under estimation by treatment
 - “This tree has nice foliage. Let’s sample it for foliar nutrient concentration.”
 - Short person measures DBH below 4.5’
 - Warped meter stick underestimates height
 - Caliper is measured higher up on seedlings growing in prickly weeds



Put data in Excel

Blk	Trmt	Tree	Ini hgt (cm)	Ini dia (mm)	Yr1 hgt (cm)	Yr1 dia (mm)	Yr1 surv	Yr1 comm
1	4	1					1	brws
1	4	2					0	dead
1	4	3					0	dead
1	4	4					1	
1	4	5					1	mt
1	4	6					1	
1	4	7					1	
1	4	8					0	dead
1	4	9					1	
1	4	10					1	brn
1	4	11					1	
1	4	12					0	dead

Record everything!

- Deviations from the study plan
- Unusual observations
- Extreme weather events
- Site history and environment
- Dates: planting, measuring, sampling, etc.
- Take LOTS of photos



Questions?





Thank you!

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