4. DESIGN OF EXPERIMENT AND TESTING A HYPOTHESIS

4.1. *Study / experimental design*

- SD is the *number* and spatio-temporal distribution of sampling *units* [1], *manipulations* (treatments) and/or *observations* to be made on each *unit* to answer a specific research question [2].

http://www.countrysideinfo.co.uk

- Manipulative exp. can help to establish *causality*.

- There are several techniques that are used to evaluate *cause-effect* relationships in Ecology.

[1] experimental & observational; the usual sampling unit is a ***quadrat*** (normally consist of a square frame), the most frequently used size being 1 m^2;

[2] designing an experiment ***properly*** will not only ***help*** you in analyzing data – it may determine ***whether*** you can ***analyze*** data at all!

4.2. Experimental requisites (requirements)

- Interpretation of *causality* depends on:

- controls;
- treatments;
- replication of independent units [1];
- randomization and interspersion /ɪntəˈspəːsɪon/ of treatments [2].

1. **Controls**:

- are *treatments* / *samples* against which manipulations are compared;
- remains the *same* or *equal* throughout the experiment.

[1] ***repetition*** of an experimental condition so that the ***variability*** associated with the phenomenon can be estimated;

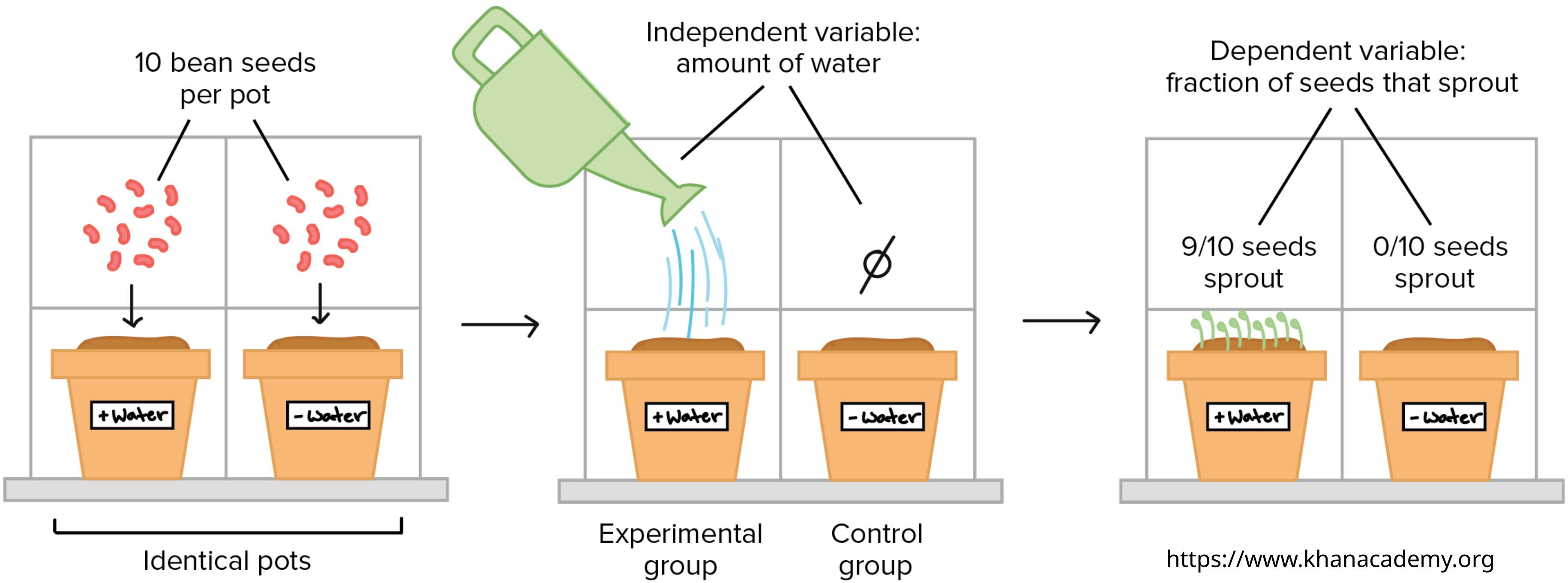
[2] пересічення (how to scatter here and there or place at intervals among other things.

- Biological systems *change* over time.

- So, any differences may be due to *treatments* or other *changes* occurring during time period.

- Therefore, the *effects* caused by *treatments* can (and should) be separated from those caused by other *changes*.

Detailed field survey
https://www.intechopen.com/chapters/46032



- For example, there are two groups of *<u>identical</u>* pots in the experiment (fig):
- one group (**<u>experimental</u>**) receives a *<u>treatment</u>* (water) while the other ("no treatment", ***<u>control</u>***) does not.
- The control group provides a baseline that lets us see if the *<u>treatment</u>* has an effect.

- The *control* in some exp. is not *necessary* “no treatment”.

- E.g., we want to compare plant’s *growth* at “normal, current ambient condition” and elevated level of CO_2 in the air.

- One *way*: current ambient condition (control) and elevated level (e.g. 25% higher level, treatments)*.

- Another *way*: current ambient condition (treatments) and lower level (e.g. 25% lower, control)**.

```
* e.g. in year 2050;

** e.g. before the Industrial Revolution.
```

4.3. *Rules for experimental design in Ecology*

1. Begin by *identifying* a *hypothesis* [1].

2. Define *parameters* for the exp. *clearly* [2].

3. Decide if you would like to perform a *mensurative* or *manipulative* exp. [3].

[1] i.e. an explanation of ***how*** you ***think*** a system works based on ***observation***. The hypothesis will be either ***accepted*** or ***rejected*** based on the data collected.

[2] i.e. clearly define terms, you can focus in on exp. ***methods*** and avoid ***ambiguity*** /ambɪˈgjuːɪti/ - the ***quality*** of being open to more than one ***interpretation***; e.g. Some researchers define **disturbance** as any event that changes ecosystem structure or function (such as fires, storms, or logging) while others use a narrower definition (considering only natural events like wildfires or floods as disturbances, excluding human-caused changes). it can lead to: misinterpretation of results; challenges in comparing studies; inconsistent management recommendations-.

[3] a ***mensurative*** exp. involves making ***measurements*** at different times or in different areas (a ***manipulative*** exp. involves physically ***altering*** a treatment group, thus there always are two or more treatments).

4. Choose an appropriate *sample size* that is fitting for the results you wish to obtain [1].

5. Introduce a *control* group [2].

6. *Randomize* assignment [3].

7. *Replicate* [4].

Precipitation manipulation experiment, Sevellita LTER, New Mexico. The troughs exist to limit precipitation on plants, simulating drought conditions. William T. Pockman, University of New Mexico https://www.fs.fed.us/research

[1] often a ***smaller*** sample size produces ***inaccurate results*** for generalization (1) and thus should be avoided;

[2] biol. systems tend to exhibit ***temporal change***, so a ***control*** is ***necessary***.

[3] randomization allows avoid ***bias***.

[4] the number of replicates necessary will ***vary*** with design, however it ensures ***precision*** in experiments.

8. Ensure samples are *dispersed* in *space* or *time* to avoid *pseudoreplication* [1].

9. Use *linear* regression based analysis *first*, before introducing ANOVA [2].

10. *Refrain* /rɪˈfreɪn/ from *deducing* results based on P value alone [3].

[1] interspersion /ɪntəˈspəːsɪon/, which ensures replicates are statistically ***independent***. If data collected on the samples from the same unit, it is not a genuine ***replication***.

[2] Linear ***regression*** is more ***powerful*** to analyze obtained data, and indicates how ***dependent*** variables change with the ***independent*** variable.

[3] The ***p value*** is ***indicative*** of confidence interval, however it fails to indicate how a system actually changes. Effect ***size*** measures are more ***meaningful*** in ecology.

4.4. *Sampling in the field: subjective locations of plots*

1. *Subjective* locations of plots (individual samples).

- E.g. we are interested in observing species *response* to *gradients*.
- It would be valuable to have the gradient *extremes* [1].
- We may also want to have good representation of the *intermediates*.
- Subjective locations are easier to implement than objectively-located samples.

[1] e.g. very wet and very dry, or very steep and very level, or very acidic to very basic.

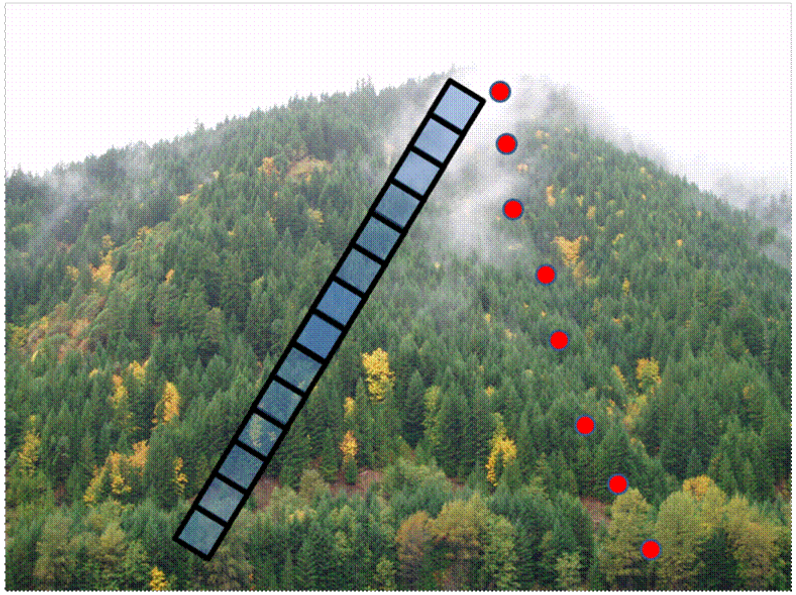
2. Gradsect.

- E.g. we are interested in species ***responses*** to an environmental ***gradient*** that varies directionally in space [1].

- We subjectively choose the ***gradient*** we are interested in.

- We can locate our samples in a ***transect*** [2], ***systematically*** in a direction of ***max***. change in the gradient (gradsects).

- E. g. the blue squares - ***quadrats*** for plant communities; the red ***dots*** - point samples (pitfall traps).



http://ordination.okstate.edu/sampling.htm

[1] E.g. in bird species distributions as a function of elevation on a mountain;

[2] a ***path*** along which one counts and records occurrences of the species of study (e.g. plants).

4.5. *Sampling in the field: completely random and systematic placement*

1. ***Random*** plot locations: ***every point*** in the sampling area has an ***equal chance*** of being ***chosen*** [1].

- Another way to use a random number generator on a computer [2].

- Just due to chance, some areas will be ***oversampled*** and some areas will be ***undersampled***.

[1] e.g. by walking a certain number of steps with their eyes closed.

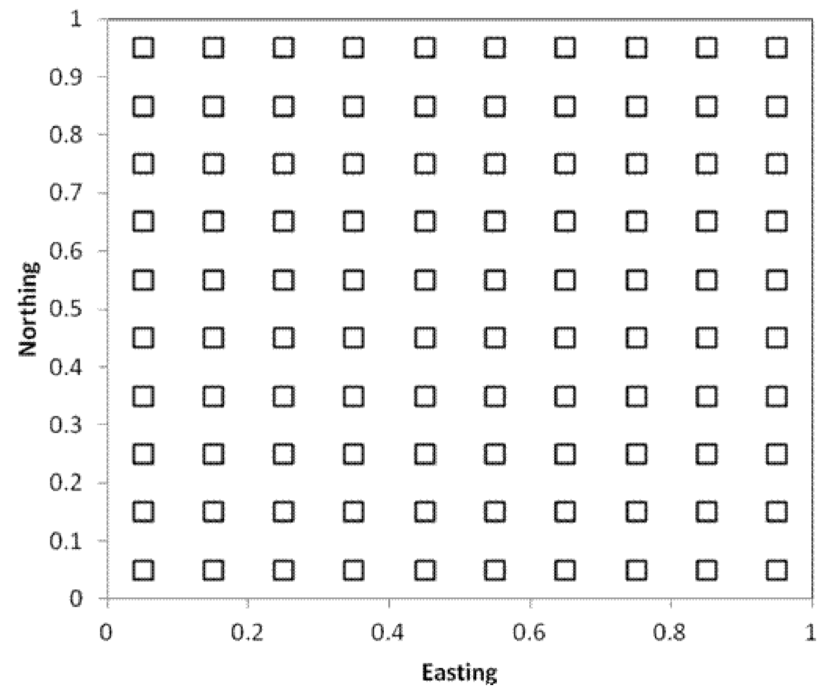
[2] In Excel using the formula “=RAND()”; e.g. we want to find a random point in a rectangle 100m by 50m, the formula is “=100*RAND()” and “=50*RAND()” to find **x** and **y** coordinates (GIS).

2. Systematic placement: plots regularly spaced along a transect or rectangular grid (occasionally hexagonal arrays [1]).

- E.g. can be used if we are interested in analyzing or quantifying *spatial* pattern.

- The plot locations are *objective*.

- A disadvantage: it is possible that *periodicity* exists in nature, i.e. spacing of plots may *coincides* with this periodicity [2].



http://ordination.okstate.edu/sampling.htm

[1] is exceptionally rare in nature;

[2] “шестикутні масиви”