

**Descriptive science practice: Quantify variation in the Science Garden**  
Week 5 of BIOL 1615, fall 2021

**Objectives:** In this lab you will be able to: 1) make measurements consistently, 2) analyze data, 3) present the results in a lab report, and 4) collaborate with your group members. In previous weeks, you have observed and quantified variation across individuals (week 3) and among taxa (week 4). In this lab, you will collect and analyze data across plants from different populations of a single species that are a part of a real, long-term dataset to ask: **how are traits different across populations?** You will work with these plants during the upcoming biodiversity project.

- A population is all the organisms of the same species, which live in a particular geographic area, and are capable of interbreeding.
- A species is the largest group of organisms in which any two individuals of the appropriate mating types can produce fertile offspring. Many species are made up of multiple populations, but some species only have one.
- A taxon (plural is taxa) is a taxonomic group of any rank, such as species, family, or class.

**Background information:** Some people call alfalfa (*Medicago sativa*) the most important invention in 2000 years, because it provided year-long feed supply that enabled a reliable horse and animal culture. Alfalfa originated in the steppes of Asia, and now it is cultivated throughout Asia, Europe, Australia, North Africa, North and South America. There are at least 23 million acres of alfalfa in the US. Alfalfa, and other hay, is the third crop in value in the US, behind only corn and soybeans (Putnam et al., 2001). The primary use of alfalfa worldwide is to feed to dairy production, but it is used to feed horse, sheep, beef cows, and other animals as well. Thus, alfalfa is two steps removed from the dinner plate. However, mention the word “alfalfa”, and some people would associate the word with the sprouts used on their salad (a minor use) or with the Little Rascals. Few people might recognize the important role alfalfa plays in their lives in the form of milk, cheese pizza, ice cream, honey, leather, or wool sweaters. Fewer still would recognize the non-economic roles that alfalfa plays in maintaining a healthy environment (**Figure 1**).



**Figure 1.** Alfalfa from Victor, ID, housing several insects called treehoppers.

There is a wide range of alfalfa quality for livestock, from supreme to poor. “Purebred” alfalfa is usually higher quality than weedy (or roadside) alfalfa. It is best to “make hay while the sun shines,” since hay quality decreases when hay is rained on. Alfalfa harvested before bloom produces the highest quality hay which is low in fiber and high in protein. To produce the same amount of protein would require four times the acreage of corn. In addition, plant breeders are developing new alfalfa strains that are even higher yielding and more nutritious for livestock. They are also developing alfalfa that tolerate wheel traffic and grazing pressure, plants that tolerate high salinity so that it can be grown on salt-affected soils, and plants that tolerate ammonium-N so that they can endure wastewater or manure applications. Varieties with improved root systems for extracting water and absorbing nutrients or contaminants also are being developed (Putnam et al., 2001).

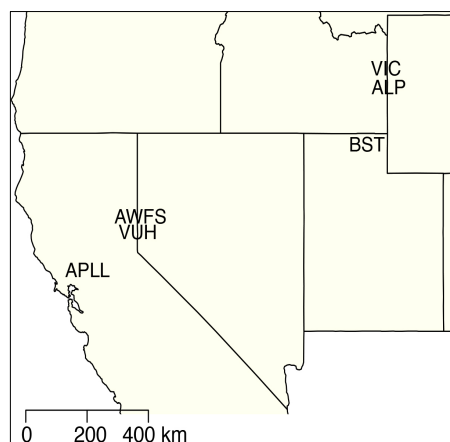
In terms of alfalfa's effect on the environment, it has deep roots (commonly 9-16 feet) and plentiful above-ground vegetation. Consequently, it protects the soil from becoming airborne and causing dusty conditions, or from being washed into rivers as sediment (millions of tons of soil are lost permanently each year; Putnam et al. 2001). Whereas it is true that alfalfa uses a lot of water per year, it uses water relatively efficiently due to its deep roots. In addition to growing long, alfalfa's roots house bacteria (*Sinorhizobium meliloti*) that fix nitrogen; therefore, no nitrogen fertilizers are needed for alfalfa growth. Moreover, the nitrogen alfalfa's bacteria

release into the soil aid the crops farmers grow following the alfalfa.

Alfalfa is the beginning of a food chain for many animals, including arthropods like insects. And alfalfa is a home to many beneficial insects; less than 1% of the alfalfa arthropods inflict damage to alfalfa and cause concern to alfalfa growers. These beneficial insects help control pests on alfalfa and other crops. In general, alfalfa is genetically resistant to many pests, a result of traditional plant breeding. Consequently, alfalfa does not need a large amount of pesticide each year compared with other crops.

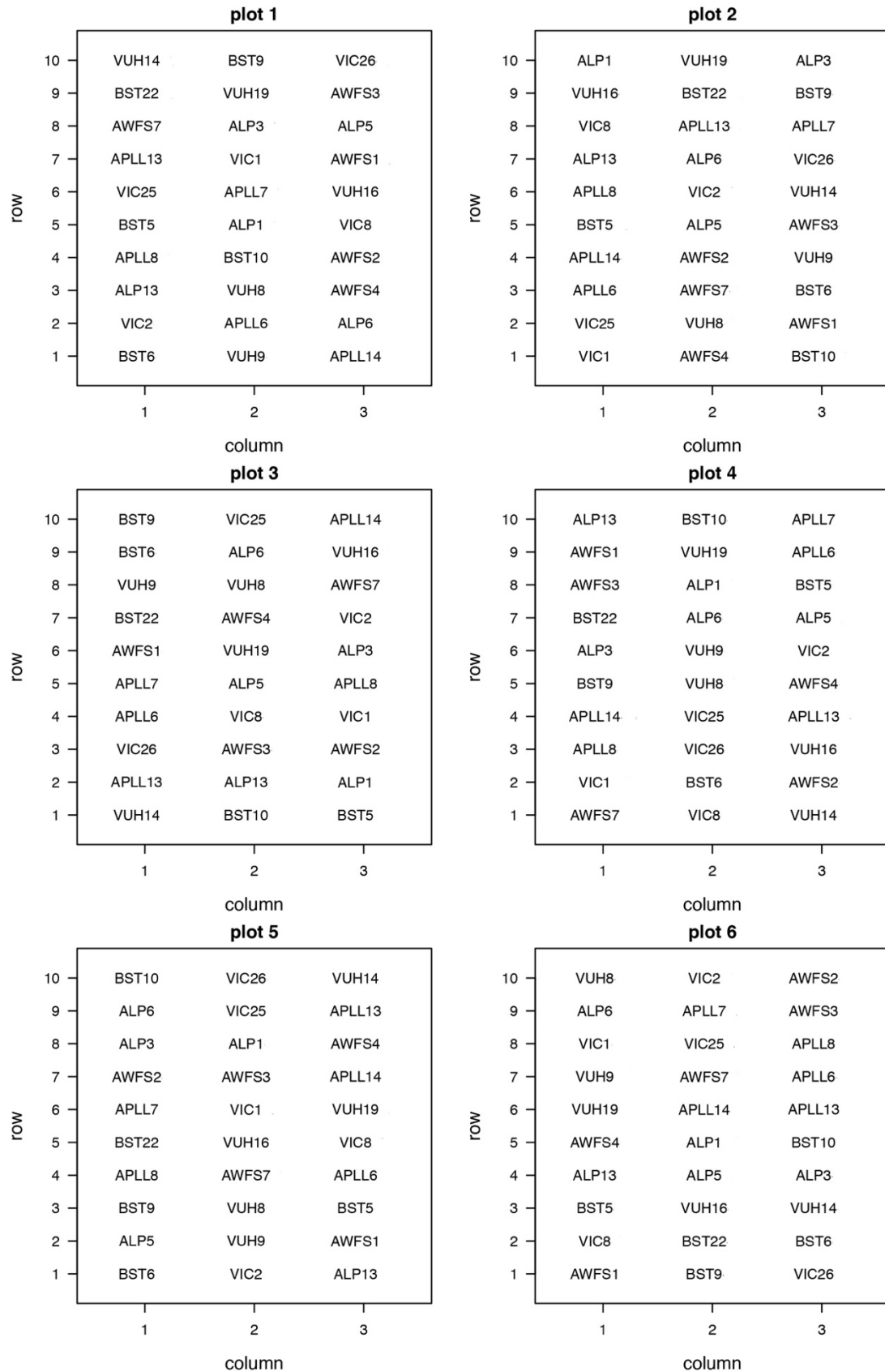
The alfalfa we will study in this lab project specifically are in the relatively new Dr. Gene Miller Life Science Garden Laboratory. You will contribute to the scientific history of these plants. Here is a brief history of the Science Garden. Also see the first video in the Week 5 Canvas page for an introduction to the garden and a tour through the plant traits.

- **In the summer of 2018:** Dr. Zach Gompert at USU, as well as researchers from four other institutions, received a five-year federally-funded grant to identify the key determinants of plant-insect-microbe interactions. Filling this gap in our understanding of the diversity of life might help us better predict how global change will affect eco-evolutionary dynamics, patterns of biological diversity, and ecosystem function. They are focused on insects and microbes associated with the human-introduced plant, alfalfa -- where it has escaped cultivation and grows in both disturbed and relatively intact habitat, in particular. This “wild” alfalfa hosts a high diversity of insects and microbes. They are using a combination of observational science, experiments, DNA sequencing, and models to analyze the evolutionary and ecological significance of genetic diversity, phylogenetic diversity of microbiome assemblages, and functional variation. As part of this large project, in summer 2018, Dr. Zach Gompert, Lauren Lucas and collaborators studied genetic differences among wild alfalfa populations and the effects these genetic differences have on plant traits and the herbivores that feed on the plants. The seeds used originally came from the following six alfalfa populations: Alpine, WY (“ALP”), north of Davis, CA (“APLL”), west Reno, NV (“AWFS”), Bonneville Shoreline Trail in Logan, UT (“BST”), Victor, ID (“VIC”), and Verdi, NV (“VUH”) (**Figure 2**). The plants were grown in a greenhouse. The plants were measured within the first few months of growth, and then were used to feed to caterpillars of three butterfly species, to answer the question: how does genetic variation of alfalfa affect the caterpillars that feed on them? Each caterpillar was fed plants from only one alfalfa population. Caterpillar weight was measured, and survival was recorded. One thing they learned was that caterpillars of all three species performed better when reared on alfalfa from two populations in particular, APLL and VIC.



**Figure 2.** A map of the six populations from which seeds were collected for this project.

- **In the fall of 2018:** Gompert and Lucas choose six offspring from each of 30 of the original wild alfalfa plants spanning the six alfalfa populations from the summer 2018 experiment to transplant into each of six plots (i.e., replicates) in the Science Garden. Then, a small team at USU transplanted these 180 plants into the garden. See **Figure 3** for a map of the alfalfa in the Science Garden.



**Figure 3.** Science Garden map. The garden is divided into six plots. Plants are randomized within a plot. The letters in the plant ID refer to population and numbers refer to family. Plots 1 and 2 are against the LSB building.

- In the summer of 2019: Gompert, Lucas, and collaborators continued to study these plants, similar to the summer 2018 research project, as well as many other alfalfa in a larger research field plot and along the Bonneville Shoreline Trail in Cache Valley to see if they can predict how herbivores perform across alfalfa genotypes.
- Since fall 2019: BIOL 1615 students measure several traits of the plants in the garden to ask whether there are significant differences across the six populations. These students also conduct a biodiversity project in the garden (details to come).

**Lab safety:** No PPE is required. Move through the garden with care. Pants and covered shoes will protect your legs and feet from injury in the garden. Insect stings and bites are rare but possible; tell your GTA and group members if you are allergic and you can be a data recorder. Your GTA has access to a first aid kit.

### **Equipment:**

For data recording: lab manual, clipboard, writing utensil

For plant height: meter tape

For leaf toughness: penetrometer, rig, labeled envelopes

For leaf area: digital calipers

For herbivory: N/A

For seed pods: N/A

For seeds within pods: labeled collection tubes, blank white paper

For insect counts: InsectaZookas, charged batteries, collection cups

For data analysis: RStudio Cloud Week 5 project (with pre-uploaded R script and two data sheets)

For scientific write-up: Google doc template (link on Week 5 page)

### **Methods: Part 1. Contributing to a long-term data set**

1) Before class, watch the first Week 5 video for how to measure each trait consistently across plants. We need to minimize measurement error and noise in the long-term data set.

2) You will meet in the classroom at the start of class. Your lab section is assigned a specific trait to measure (**Table 1**), and your group will be assigned specific plants to measure in a specific plot. Before heading out to the garden, your instructor will tell your group which plants to measure: record the plot number and plant ID for your assigned plants in **Table 2** and highlight them on the garden map (**Fig. 3**). Also note how many values you need to record for your assigned trait. Each group will have two clipboards for data recording. Depending on your assigned trait, you may need to bring outside with you special equipment for measurement (**Table 1**).

**Table 1.** Data to be collected and equipment to be used by each lab section in fall 2021.

Trait	Instructor (Lab sections)	Special equipment to bring outside	Special equipment to leave in the classroom
Insect counts	Lauren (1), Ellis (11, 32)	InsectaZookas with charged batteries, collection cups	
Plant height	Rachel (2, 36), Brian (10, 31), Brynn (14, 18)	Meter tape	
Leaf area	Jack (3, 23), Kate (19, 28), Tyson (6), Jaden (15)	Digital calipers with working batteries	
Seed pods	Ellie (7, 22), Luke (37)		
Herbivory	Callie (4, 21), Elizabeth (16, 24), Luke (27)		
Seeds within pods	Tyson (8), Layne (12, 20), Ally (29, 35), Jaden (33)	Labeled collection tubes	White paper
Leaf toughness	Marilyn (5, 9), Megan (13, 25), Grayson (17, 30)	Labeled envelopes	Penetrometers and rigs

Name:

Group:

Lab section:

3) Use **Table 2** to record measurements made while in the field. If a plant cannot be measured for some valid reason, record “NA” for the measurement, and make note of the reason in the notes column.

**Table 2.** Trait measurements.

Plot #	Plant ID	Measurer initials	Trait value(s) (units:   ):	Notes

4) Once your group is finished with measurements, you need to share your data with your GTA. During class, your GTA will add your data to the official fall 2021 Science Garden data sheet for use in future years of BIOL 1615.

**Methods:** Part 2. Data analysis & scientific write-up

5) Before class, watch the second Week 5 video for an example of this week’s data analysis. The video in Canvas will go through the following methods with a trait, the mean proportion of ladybugs present, that was measured in Sept 2019 and Oct 2020; you will not use this trait in your analysis.

6) If you borrow a class laptop, the student password is: 1615lab

7) You will analyze previous years’ data for your class’ assigned plant trait (**Table 1**). The amount of data available for each trait is in **Table 3**.

**Table 3.** The amount of data that exists for each trait.

Trait (units)	Dates measured (number of times measured)
<i>Example: proportion of ladybugs</i>	Sept 2019, Oct 2020 (2)
Plant height (cm)	Sept 2019, Oct 2020 (2)
Leaf toughness (g)	Sept 2019, Oct 2020 (2)
Leaf area (mm <sup>2</sup> )	Sept 2019, Oct 2020 (2)
Herbivory	Sept 2019, Oct 2020 (2)
Insect counts: Total insects	Sept 2019, Oct 2020 (2)
Seed pods	Sept 2019, Oct 2020 (2)
Seeds within pods	Sept 2019, Oct 2020 (2)

8) This week's data tables have already been made for you. They are waiting for you in the RStudio Cloud: Week 5 project. You will use the first data table for calculating population means and standard errors and for graphing. You will use the second data table for performing the ANOVA. They are called:

**sciencegarden-data-fall20-datesincol-sgonly.csv**  
**sciencegarden-data-fall120-datesinrows-sgonly.csv**

9) Follow the R script pre-loaded for you in this week's RStudio Cloud project (week5-fa21.R) to visualize differences in your assigned trait among the six alfalfa populations in the Science Garden. Specifically, you will graph the mean trait values (and standard errors) across the populations and across time. You can record the population means and standard errors in **Table 4** below.

**Table 4.** Results. Mean trait values (units: ) and standard error (SE) per population and ANOVA p-values.

	ALP	APLL	AWFS	BST	VIC	VUH
Sept '19 trait mean:						
Sept '19 trait SE:						
Oct '20 trait mean:						
Oct '20 trait SE:						
Pop p-value						
Plot p-value						
Date_collected p-value						
Pop:Plot p-value						
Pop:Date_collected p-value						
Plot:Date_collected p-value						
Pop:Plot:Date_collected p-value						

10) Next, to test whether there are significant differences among the trait means across the populations when compared to the within-population trait variation, you will conduct a statistical test called an ANOVA (analysis of variance). Specifically, an ANOVA tells you whether at least one population is significantly different from the rest. We are most interested in significant differences among the six populations, but we will also be able to test for significant differences among the plots, across time, and the interactions between and among the three factors. (Note: our model will use fixed effects, not random effects; we could be treating some of these as random effects but will count them all as fixed effects here.)

The p-value you will receive for the population effect after running the ANOVA is the probability of observing a difference in the means as big as the one you observed by chance. It indicates whether we should view the populations as random samples or whether there are true differences among them. Traditionally, a p-value of 0.05 or lower indicates there are true differences among the populations. You can record your ANOVA p-values in **Table 4**.

11) Interpret the results. Does your p-value indicate there are true differences among populations (specifically, this means there is a difference between at least one of the populations and the others)? If your population ("Pop") effect has a p-value less than or equal to 0.05, refer to the R script for an additional test to run, called Tukey's HSD, to give you evidence for which populations are significantly different from each other. Tukey's HSD looks at the difference in trait means between all pairwise comparisons of the populations. (Note: you can get a significant overall ANOVA p-value but no significant pairwise differences with Tukey's HSD. There is an adjustment for the multiple comparisons in Tukey's HSD that can account for this difference in results.)

12) **In-class assignment.** You will now write about your results scientifically, following the lab report format we will use for our upcoming biodiversity project. On the Canvas Week 5 page, there is a link to a google doc template you can make a copy of and type into. You will only write **the Results and Tables & Figures sections** of a lab report this week. In general, results are simply your findings. A results section of a scientific paper is strictly for narrating your findings, without trying to interpret or evaluate them. This is often done using text, tables, and figures.

You will receive credit for including each of the following correctly:

- Results: A description of the differences among the alfalfa populations found in your plot (1 pt)
- Results: A comparison of your population means (with SEs) from Sept '19 and Oct '20; you must include the values (1pt)
- Results: A description of your ANOVA population p-value; you must include the p-value but do not include the R table output (1pt)
- Results: A description of your Tukey's HSD results, if applicable; do not include the plot (if not applicable, say this in your write-up; 1 pt)
- Tables & Figures: Your plot made with R that includes all of the mean and standard errors per population and year, axis labels, axes, and a legend (1 pt)
- Tables & Figures: A short informative caption below the plot (1 pt)
- Writing quality: Grammar and spelling mistakes do not hinder the meaning of the report (1 pt)

Save your document as a pdf and upload it to Canvas. Your GTA will provide timely feedback to help you make progress towards our course learning objectives.

14) **Group table clean-up.** If you borrowed a class laptop, delete your files. Clean the laptop lightly with isopropyl alcohol as instructed at the cleaning station. Use Clorox spray and paper towels to wipe down your group table. Thank you!

**Reference:**

Putnam, D., Russelle, M., Orloff, S. Kuhn, J., Fitzhugh, L., Godfrey, L., Kiess, A., Long, R. (2001). Alfalfa, wildlife and the environment: the importance and benefits of alfalfa in the 21<sup>st</sup> century. *California Alfalfa and Forage Association*: Novato, CA.