Supplemental Materials

Part I

Selected Personal Plant Prompts and Instructor Notes

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General Instructor Notes:

It is a good idea to order seeds for these projects at least 6 weeks in advance of the term in which you wish to use them. We have ordered seeds from a variety of seed companies, most recently Territorial Seed Company (territorialseed.com) and Johnny’s Selected Seeds (johnnyseeds.com). Both of these companies carry seeds for all the exercises described here.

These activities have been developed using six inch plastic round greenhouse pots and ProMix potting soil (with some exceptions, see below). Pots are filled with premoistened growing mix, placed in trays and subirrigated until seedlings are established. Subsequently, the trays are removed and plants are watered from above. The location of pots/treatments on benches should be randomized, especially as plants get larger and may begin to shade plants outside their pots. At least three replicates of each treatment are recommended; more are better if room is available; it allows for other factors like disease or inattention by students.

Midstream measurements of plant height keep students engaged as the experiment is proceeding, but students must keep track of WHICH individuals they measured each time. Given free rein, students often measure three “random” plants each time. Some have devised a system to tag individual plants with colored wires for tracking plants, instead of measuring each plant in the pot. When examining these data for the final report, encourage an analysis of growth RATE (change over a unit of time). Multiple statistical tests of the effects of treatments on plant size over time, although they provide good practice, are not advisable, statistically speaking; repeated measures analyses are more appropriate analyses. Graphing the data by hand over time might make a good substitute practice.

If you have the resources, other midstream measurements can be done in connection with classroom topics. We sometimes measure chlorophyll using a handheld Minolta SPAD meter, or take a variety of photosynthetic measurements using a Li-Cor 6400 Infrared Gas Analyzer. We also have a lab in which we measure water potential using a pressure bomb; students have done some of these measurements on their personal plants, but plan for this activity must be in place when planting experiments so that plants are available to be sacrificed.

Students generally count leaves and measure plant height as dependent variables. Leaf area is also a good variable to consider. An easy way to measure leaf area is to use a flatbed scanner and NIH’s shareware program called ImageJ (http://rsbweb.nih.gov/ij/index.html); make sure to use a reference ruler in the scanned images for interpretation.

Encourage students to harvest plants on an individual basis at the end of the experiment; multiple plants per pot, if not all of them. Aboveground dry biomass and leaf area are both excellent indicators of plant performance. If students place plants in individually labeled envelopes and put them in a 60°C oven for at least 72 hours, they will be sufficiently dry for good estimates of biomass. If they really want to keep their plants and use them for other purposes, you may also encourage students to destructively measure some plants and get regression factors to convert nondestructive measures into dry biomass estimates.

Depending on the design, analyses of data using block as a factor in an ANOVA test may be most appropriate; if the instructor does not have expertise in the field, this may be where teaming up with a statistician might prove fruitful.
Gardeners are often told not to plant carrots and dill next to each other. Why?

For centuries, gardeners have developed a great deal of common wisdom about how best to grow plants. The sources of some of these ideas are not always well known; last semester, one student told me that his grandmother always put egg whites on her plants because she said they grew better. She did this because her grandmother, and her great grandmother had always done it. We conducted an experiment in the greenhouse last year and found absolutely no effects of eggwhite addition on marigold growth in the greenhouse. This, of course, does not necessarily mean that egg whites didn’t impact the grandmother’s plants in her garden with her soil, but we only had one semester, one species, one environment; she had several generations of practice with a diverse array of garden plants.

One of the common practices gardeners engage in throughout the world involves plant placement in the garden. Some plants are said to do well together; eggplants and marigolds, beets and onions are common pairings. These are called “Companion plants”. The mutual benefits of these plants may include insect repellant properties or pollinator attraction.

At the same time, other plants are negatively recommended as pairs. Carrots (Daucus carota) and dill (Anethum graveolens), are a plant pair to avoid, says the common wisdom. Both species are members of the plant family called the Apiaceae. Other members of this family include coriander or cilantro, fennel, parsley and parsnips. Because of their shared common ancestry, these are all smelly plants, and some produce compounds called furanocoumarins that are toxic to insects or even people, in high enough doses. Might this biochemistry be the source of the non-companion plants notion?

Activated carbon, which fish-lovers put in their aquarium filters, is very good at sopping up many toxic compounds in soil. If we add it to the soil, plants still produce the furanocoumarins, but the chemicals don’t have any effects on other plants because they are absorbed by the activated carbon.

Your job is to find out whether or not recommendation to avoid placing these carrots and dill together is due to the effects of these furanocoumarins. You will be provided with several pots, activated carbon, soil, and carrot seeds.

Resources:


Instructor notes on companion planting:

In mixed treatments, it is important to plant multiple individuals in mixed pots; at least four of each type. Initially, it is difficult to tell the difference between the two plants, but Dill produces featherier leaves. It also makes furanocoumarins while carrots do not, a fact that students should discover on their own through literature investigations.

Students have difficulty with the concept that activated carbon does not prevent the production of furanocoumarins; it merely reduces their effectiveness. Make sure to have some conversations on this matter.

You can purchase activated carbon in aquarium stores, but smaller pieces are more effective than larger ones; either have students grind up the carbon with a mortar and pestle or purchase ground up activated carbon. We use five grams per six-inch pot.

Activated carbon may also modify the availability of nutrients (Lau, J.A. et al. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytol.* 178 412-23 (2008)); you may wish to help students explicitly add this layer of complexity to their study.
Should I invest in bone meal for fertilizing plants at my organic cucumber farm?

Farmers are bombarded with advertising from seed vendors, fertilizer manufacturers, pesticide and herbicide makers, and a wide variety of other companies, seeking a piece of the farming “pie”. Organic farmers are not excluded from this marketing barrage, either. Farming organically essentially means avoiding synthetic pesticides and fertilizers, but there are plenty of naturally occurring products that are fair game for use in organic settings.

While plants get all the carbon they need from the atmosphere, provided they have enough light, they must get water and other mineral nutrients (like nitrogen, phosphorus and potassium) through their roots in the soil. The availability of these mineral nutrients can make a big difference for plants, influencing their growth and reproduction capabilities.

Phosphorus is an important element for plant growth. It’s in DNA and RNA, important molecules in all living things. Phosphorus is also a major component of ATP, an energy-shuttling molecule.

Phosphorus mainly comes from weathered rocks underneath the soil; it is in extremely limited supply in tropical regions, less so in temperate regions. Phosphate miners extract phosphorus from underground and process it for use in agricultural settings. It is also available naturally in the form of ground up animal bones, referred to as bone meal. Plant flowering has been linked to phosphorus availability; plants with phosphorus deficiency do not flower, but will flower when it is added.

Will adding bone meal make a difference for the productivity of cucumbers in your organic farm? Your assignment is to design an experiment to investigate how phosphorus influences plant vigor, especially rooting and flowering.

You will be provided with potting soil, bone meal, a natural source of phosphate, and cucumber seeds (*Cucumis sativus*). Read the label to figure out how much bone meal you need to mix in with your soil before planting the seeds. The rest is up to you.

References:


Instructor notes on phosphorus:

The bioavailability of phosphorus from bone meal is dependent on fungal activity – if you carry this one out with students, you may either wish to ensure that the potting soil you use has Glomus spores in it, or encourage an experiment using bone meal and mycorrhizal spores in a two-way design.

The literature recommends adding 60 g of bone meal per 2 L of soil; encourage students to do the math to figure out how much they should put in their soil before potting.

Make sure to get greenhouse cucumber seeds. (Diva at Johnny’s and Bella, Telegraph or Manny at Territorial). Although they are more expensive, they’ll fare better indoors than field cucumbers might and will not need hand pollination. Invest in some bamboo stakes, as well, for the twining vines. Students doing this project will need to be scrupulous to keep their plants from taking over other experiments on the bench.

You do not need to plant more than one or two seeds per pot; the plants get large quickly.
My seed packet recommends sowing basil seeds at least 4-6 inches apart, but I can get more plants into the garden by planting them closer. Should I ignore the directions?

Competition occurs when there is not enough of a particular resource to go around for the organisms that need it. In the marketplace, businesses compete over customer dollars. In nature, animals often compete for food, territories, or mates. Competition only occurs when the resources are in limited supply.

Plants respond to competition in different ways than animals do. While animals can move around and seek resources elsewhere, plants cannot. They respond to their environment by growing differently. They may change the size and shape of their roots, their leaves or their stems.

While water and soil nutrients can be depleted by plants using them, light is a funny resource to think about competing for. It does not run out, yet plants can interfere with each other’s ability to obtain enough light to grow and reproduce. Growing tall and skinny might help a plant escape from competition from others, but this might be a costly tactic, since it takes calories to grow tall and skinny and renders them susceptible to being knocked over.

How do plants grow differently in response to competition? And does it matter if your objective is to get lots of leaves for making pesto or a salad with basil? Your assignment is to design an experiment to investigate why we might want to leave room between basil plants in a garden. You can grow basil seeds pretty close together – up to 50 plants in a 6 inch round pot.

You will be provided with pots, soil, and basil seeds (Ocimum basilicum). The rest is up to you. Be sure to include replication, and to make your independent variable replicates as consistent as possible.

References:


Instructor notes on plant density:

Students should use a range of densities and adjust replicate numbers. Many more pots of singletons are necessary than of high density plantings. Encourage students to ensure that their higher density plantings have the same numbers of plants. Students have used as many as 4 densities.

Because the number of plants involved varies by treatment (and thus variance), students may discover difficulties in finding statistical differences between their treatments, even though they are visually obvious. To quantify size distributions, some students have invented their own metrics that are very similar to coefficients of variance or Gini coefficients. Having them come up with their own metric is far more meaningful than if you provide a “professionally” invented statistic.

Some students may have heard about pinching apical meristems off basil plants, but are often not sure why they might want to do this – They may wish to add this as a layer of their experiment.
Farmers often rotate legume and grain plants on the same farmland in successive years. Why?

Crop rotation is an important tool for many farmers. It is thought that planting the same crop year after year on the same plot of land leads to decreased productivity. Because of this, farmers like to move their crops around, often planting grain in a field one year and legumes or beans in that same field the next.

Why beans and grains and not some other combination? Nitrogen (N) is the key – it's in every single protein in every living thing. The more N a plant has, the better it is at doing most of the jobs of being alive; that's why there's so much N in plant fertilizers. Some members of the legume family (like beans) are known to produce nodules on their roots that provide anaerobic (oxygen-free) environments. Some nitrogen fixing bacteria in the genus *Rhizobium* can only fix nitrogen in anaerobic conditions and these nodules on legume roots are perfect hideaways for these bacterial cells. The bacterial infection winds up "feeding" its legume host plant. Further, some of the N might also leak out of the legume plants and fertilize the soil where they grow.

Corn, barley and other grains are well known to pull lots of nutrients out of the soil; they deplete soil quickly and require a lot of fertilizers. They also are known to leave chemicals in the soil that can influence the performance of crops that come after them.

How much of an effect could this possibly have in one pot in the course of a semester? Does it really matter whether the first plant is a grain or a legume? Your assignment is to investigate how crop rotation between barley and mung beans might influence barley growth.

You will be provided with barley (*Hordeum vulgare*) and mung bean (*Vigna radiata*) seeds and a number of pots. Your first task is to create (at least) two kinds of soil; one that has had mung beans growing in it, and one that has had barley growing in it (what might a third kind be?). What are you going do with the plants once the soil has been "created"? Consider that you will need time for both species to grow and want to be comparing the growth of plants of the same chronological age. Remember to include replication in your plan and to quantify both your independent and dependent variables. For the purposes of the rest of your personal plant work, two of you should select barley, and two should select mung beans.

References:


Instructor notes on crop rotation:

This experiment yields incredibly clear results, regardless of design. In response to this prompt, students have often devised two-way experiments, with three soil types and two species, yielding six treatments that need, at minimum, three replicates.

Ensure that students create at least two, if not three types of soil (planted in barley, planted in mung beans, planted with nothing) and that the numbers of plants are consistent across the pots by species – this may require overplanting and thinning over several weeks. Plant at least fifty seeds of each species.

The first crop should be harvested after about five weeks, carefully removing all surviving plants (just cutting them at the soil surface may result in resprouting). Some very thorough students establish plant-less pots and treat them the same as their planted pots for the first five weeks. Barley exudes alkaloids like hordenine and gramine that can affect subsequent plant growth in interesting ways. Some bean seeds are pretreated with rhizobium spores; use your judgment as to the use of these.
Is it worth paying extra for potting soil with mycorrhizal fungal spores in it?

Plants get all the carbon they need from the atmosphere, provided they have enough water and light, but they must get other mineral nutrients (like nitrogen, phosphorus and potassium) through their roots in the soil.

Sometimes these mineral nutrients are hard to come by because they’re locked in organic (carbon-bound) forms or complex molecules that plants can’t get at.

Fungi (we know them as yeast or mushrooms) mainly exist as tiny chains of cells called hyphae that live in between soil particles. They and their spores are all around us all the time. Fungi are very good at breaking down organic molecules and living tissues, extracting these useful mineral nutrients, sometimes at very high rates, often liberating more of these minerals than they themselves need.

At least 75% of land plant species have developed ways to tap into this excess mineral supply using fungi, inviting them to infect plant roots and exchanging photosynthesized sugar for these useful mineral compounds. In many cases (especially forest trees and most orchids), the plants are utterly dependent on these fungi; they could not live without them. These fungi also expand the “reach” of plants; sometimes even doubling the amount of soil surface plants are able to exploit. These mutually beneficial relationships between plants and fungi are called mycorrhizae.

At our garden supply center, there are two kinds of potting soil; one with no ingredients added ($28), one with mycorrhizal spores added ($30). I can also buy a pound of mycorrhizal spores for $20.

Your assignment: Design an experiment that will reveal the amount of growth improvement provided by these mycorrhizal fungi – is it worth it to the host plants to share sugar in exchange for mineral nutrients? And is it worth it to invest the extra money in spores or in potting soil containing fungi?

You will be provided with mycorrhizal fungi and chive seeds (Allium sp.). The mycorrhizae must be mixed in with the soil at the time you plant the experiment; make sure that liquid draining out of pots with mycorrhizae in them cannot mix with liquid draining out of non-mycorrhizae pots. The rest is up to you.

References:


Instructor notes on mycorrhizae:

Although chives are not very exciting to watch grow, they respond a great deal to the availability of mycorrhizae. Consider that soilless mixes with and without *Glomus* spores are widely available – invest in a bale of *Glomus*-free soil and keep it away from any other potting soil you may use. Potting soil with these spores are also common in the marketplace, as well as potting soil with fungal suppressants in the form of bacteria (biofungicidal *Bacillus subtilis*).

Adding mycorrhizal granules will require that students calculate the volume of their pots, add the granules early in the experiment, and keep the pots away from control pots, especially if subirrigation is used. We have not ventured to compare the performance of plants growing in packaged mycorrhizal soil with plants growing in sporeless soil with granules added.

If students are interested and microscopy expertise is available, it is possible to stain roots and look for endomycorrhizae. Try [http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm](http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm) for a start.