

Laboratory: Environment and Development: The Effects of Environment on Seed Germination

by Dana Krempels

The seed is more than just a plant waiting to happen. It is a complex marvel of evolution, a miniature life-support system that responds to environmental cues in order to give the embryo nestled within the best chance of survival.

I. Characteristics and Classification of Plants

Plants share synapomorphies that set them apart from other organisms.

1. true tissues (of types unique to plants)
2. waxy cuticle (to prevent desiccation)
3. stomates (microscopic gas exchange pores)
4. apical meristems (permanent embryonic tissue for constant growth)
5. multicellular sex organs (male **antheridia** and female **archegonia**)
6. walled spores produced in structures called sporangia
7. embryo development inside the female parent
8. secondary metabolites (alkaloids, tannins, flavonoids, etc.)
9. heteromorphic **alternation of generations**

The most primitive plants do not produce seeds at all, but rather release **spores** into the environment where they grow into a second life cycle stage, the gametophyte. Seed plants still make spores, but the spores (of females) are enclosed in a **seed**, which affords protection against environmental challenges, as well as food for the growing plant embryo. Plants can be broadly classified as follows.

- A. Bryophyta – Non-Vascular Plants (mosses, liverworts and hornworts)
- B. Tracheophyta – Vascular Plants
 1. Seedless Vascular plants (ferns, club "mosses", horsetails, etc.)
 2. Seed-producing plants
 - a. Gymnosperms (cycads, ginkos, conifers)
 - b. Angiosperms (flowering plants)

Today, we will use seeds of flowering plants (Anthophyta) as our study organisms.

II. The Plant Life Cycle in a Nutshell

Not all plants produce seeds. All of the vascular plants (i.e., one that has xylem and phloem to conduct water and nutrients) you see outside are large, diploid organisms known as **sporophytes (2n)**. The vascular plant's sporophyte is the life cycle stage most familiar to the casual observer: large, leafy, and aesthetically pleasing.

The sporophyte contains special, diploid reproductive cells known as **sporocytes**. These are analogous to the diploid cells (oogonia or spermatogonia) in your own body that give rise to ova or sperm, depending on your sex. Like spermatogonia or oogonia, the sporocyte will undergo meiosis (sexual/reduction cell division) to produce a haploid cell. But in this case, it's not an egg or sperm. Sporophyte meiosis yields haploid cells known as **spores**.

A spore will undergo **mitosis** (asexual cell division) and grow into a haploid, independent individual known as a **gametophyte (n)**. It is the haploid gametophyte that will produce sperm (if male) or ova (if female), but these are produced via mitosis, as

the gametophyte is already haploid. Sperm and ovum from different individuals fuse to form the **zygote (2n)**, which grows into the next sporophyte generation. The cycle continues, as shown in Figure GR-1.

In more primitive vascular plants such as ferns, sporophytes release their spores into the environment where they grow into independent gametophytes (see Figure GR-1). Spores may be female (**megaspores**) or male (**microspores**), and the gametophyte that grows from each type will produce either **ova** (female) or **sperm** (male), depending on its sex. Some plants, such as the fern in Figure GR-1, produce spores that are neither male nor female; these grow into bisexual gametophytes that produce both ova and sperm.

In seed plants (Spermatopsida), the megaspore is not released into the environment. Instead, it is retained inside a specialized structure--the **ovule**--made of sporophyte tissues.

In a flower, for example, ovules are located in the female structure, the **pistil**. Male spores (called **microspores**) are formed in male structures. In flowers, these are the **anthers**. Each microspore will develop into a haploid male gametophyte, a pollen grain which will produce two haploid sperm via mitosis.

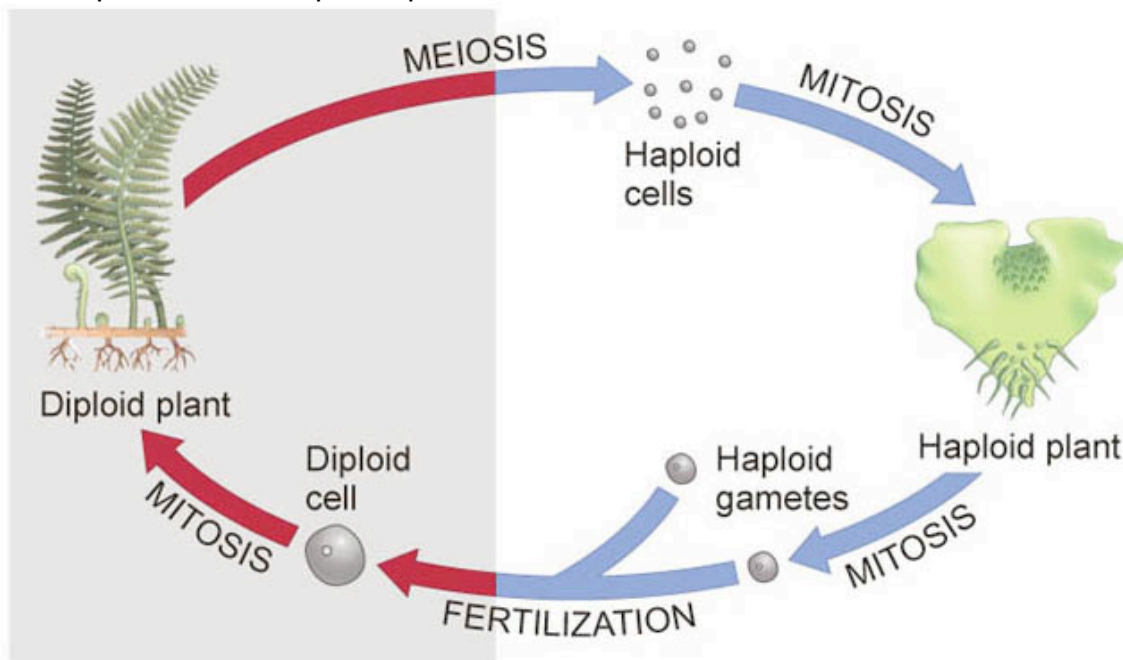


Figure GR-1. Alternation of generations, as exemplified by a fern plant. The cycle is the same in all vascular plants, although sporophyte (diploid) and gametophyte (haploid) are very different in appearance in the same species.

The female spore will still grow and mature into a female gametophyte, as shown in the plant life cycle in Figure GR-1. But it does so inside the ovule, and never becomes an independent plant, as the fern gametophyte does. The mature female gametophyte produces **ova**.

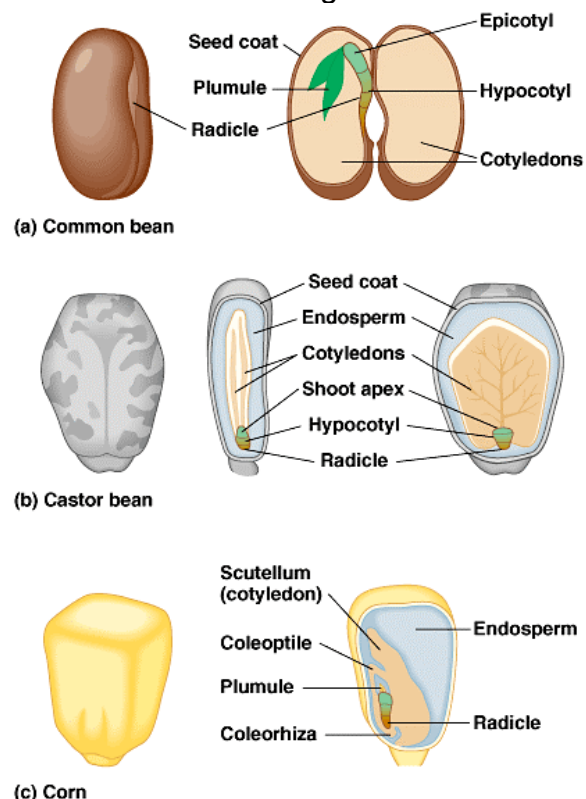
The sporophyte also produces male spores, and these will develop into male gametophytes called **pollen**. (As strange as it seems, a pollen grain is actually an individual, haploid male plant!) Each pollen produces two sperm via mitosis. Pollen are carried to the female gametophyte by wind, water, or animals (depending on the plant species), where they deliver their sperm. **Pollination** is the arrival of pollen at the female's containing structure (strobilus or flower stigma). **Fertilization** may not take place for days or even months afterward, and is the actual fusion of sperm and ovum nuclei to form the zygote inside the ovule. An ovule containing a zygote is known as a **seed**.

III. Anatomy of the Anthophyte Seed

A seed is composed of the tissues of three generations of plants: the sporophyte that produced the ovule, the gametophyte that develops inside the ovule to give rise to the ova, and the embryo of the new sporophyte. The seed also contains a nutritive substance called **endosperm** upon which the growing embryo feeds as it develops. The anatomy of some typical anthophyte seeds is shown in Figure GR-2.

Seed characteristics vary with plant species, and have evolved different anatomies in response to natural selection and other evolutionary forces. For example, seeds of plants that grow into **epiphytes** (i.e., plants that grow on other plants, such as bromeliads, orchids, etc.) tend to be very small and easy for wind to carry to high places. They have comparatively little endosperm so as to reduce weight, and so have relatively little reserve energy to produce a sprout during germination.

Conversely, seeds of species that germinate deep underground (e.g., beans, corn, etc.) may have extensive reserves of endosperm, as the deeper a seed is buried, the more energy it must spend to grow a sprout that can break the surface before it can reach sunlight and make food of its own via photosynthesis.



Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

Figure GR-2. The anatomy of three different species of anthophyte seeds.

IV. Dormancy and Germination

Dormancy is the physiological condition of a viable seed that prevents germination, even in the presence of otherwise favorable germination conditions. During dormancy, the seed's metabolic rate is extremely slow, and it uses very little energy to stay alive.

Germination is defined as the reactivation of metabolic activity in the seed, an awakening from dormancy. It culminates with the emergence of the **radicle** (embryonic root) and finally, the **plumule** (first shoot). Germination can be divided into three main phases:

Phase I – Activation

This phase commences with **imbibition**, the uptake of water by the seed. This causes the seed to increase in volume. Respiration increases, and various enzymes involved in endosperm digestion and other functions are manufactured. The cells of the embryo begin to elongate, and the radicle, in particular, begins to lengthen.

Phase II – Digestion and Translocation

During this phase, metabolic activity increases dramatically. Protein synthesis begins, and stored reserve energy in the endosperm is metabolized. The synthesis of enzymes that promote a softening/loosening of cell walls begins, in preparation for further cell elongation and increase in volume. Nutrients from the endosperm (which is sometimes located in the cotyledons) are mobilized and transported to areas of growth.

Phase III – Seedling Growth

This phase is characterized by rapid cell elongation and cell division. At this point, the radicle emerges from the seed coat, and the embryo can now access water and nutrients from its environment for the first time via its own new root system. Then it's off to the races, and the new plant begins its growth in earnest!

V. Factors Affecting Seed Germination

As mentioned previously, most seeds undergo a period of **dormancy**, during which metabolic activity is minimal. This is maintained, in part, by a hormone, **abscisic acid** (ABA), which permeates the seed coat. In order for germination to commence, this water soluble ABA must be rinsed out of the seed coat. Hence, most seeds will not begin to germinate until they have been rinsed or soaked in water.

Oxygen, light, temperature, soil chemistry, humidity all may play a role in breaking dormancy of seeds, and the effect of any environmental stimulus on a seed depends on its own unique genes as determined by the evolutionary history of its ancestors and any new mutations it may carry.

Does it matter to a seed when and where it germinates? Can a seed's failure to detect environmental conditions result in a lower chance of survival, and its being dumped out of the gene pool? What environmental factors influence seed germination success, and how?

In today's lab, your team will consider these questions, as well as others that may not be mentioned here. In this section, various factors that may affect seed germination are described. Consider these factors, as well as the anatomy and natural history of the variety of seed species provided for you in the lab.

As you already know, there may be genetic variation not only among *different* species, but also among individuals of the *same* species. Don't forget this when you design your experiment. Following is a bit of background on some environmental factors that could affect dormancy and germination in various flowering plant species. Consider not only these factors, but the species of plant seed you've chosen as your subjects. Multiple factors may affect this phenomenon, and you must be aware of all of them to properly interpret your experimental results.

A. After-ripening

Some seeds will not germinate until they have undergone a process of **after-ripening**, defined as metabolic changes that must take place in a seed in order for it to overcome dormancy. For example, the seeds of some temperate species will not germinate until after they have been exposed to very cold temperatures. Other seeds must pass through an animal's digestive tract and become **scarified** (physically damaged) before they can take up water and become metabolically active. Still others must pass through fire, which is common in ecosystems where annual fires are part of the normal climate. Of what evolutionary significance might each of these after-ripening triggers be?

Cold - _____
- _____
- _____

Animal Digestive Tract Passage _____
- _____
- _____
- _____

Fire - _____
- _____
- _____
- _____

B. Light Environment

The seeds of some plant species may be sensitive to light, and have special photosensitive pigments that "tell" the seed whether it is close to sunlight or not. Short-wavelength light (e.g., violet and blue) penetrates deeper into soil (and other substrates, such as water) than long-wavelength light (red). To which wavelengths of light would you expect such seed pigments to be sensitive, short or long, and why?

- _____
- _____
- _____

Sensitivity to light keeps seed from germinating where they can't grow, such as under a canopy where there is not enough light, or if they have been buried too deeply in the soil. To detect light, any organism must have some sort of **pigment**, a light-absorbing substance. In this case, it must be a pigment that can trigger a biological reaction. As photosynthetic organisms, plants have a variety of pigments that function in various capacities in addition to photosynthesis.

Phytochromes

Phytochromes are proteinaceous molecules with a sulfur-linked (covalently bonded) pigment active group (the chromophore) consisting of a linear tetrapyrrole. The pigment absorbs primarily in the red and far-red region of the spectrum, and so appears blue-green to our eyes. The chromophore portion of the molecule can exist in one of two forms, Pr or Pfr, as shown in Figure GR-3.

The functional phytochrome consists of two identical proteins, each with a chromophore. One part of the protein acts as the photoreceptor, and the other as a

kinase, which triggers cellular responses. When the chromophore absorbs light, it isomerizes from one form to the other. This change in configuration results in a slight change in the kinase portion of the protein. The kinase is the biologically active region of the molecule, and its interaction with other biological molecules elicits a physiological response.

The more stable isomer is Pr, but it is not biologically active. In the presence of even a short flash of red light, this molecule isomerizes to the biologically active form, Pfr, which can trigger various metabolic responses in a plant, from blooming to inhibition of blooming to germination, depending on the activity of the kinase portion of the molecule. In darkness, or in the presence of far red light, Pfr returns to the more stable form, Pr. Given this information, consider what types of seeds might use phytochromes as receptors to relay information about light environment. Might there be a difference in light sensitivity among different species of seeds? Might different wavelengths (colors) of light have different effects on germination? Ask questions and make predictions.

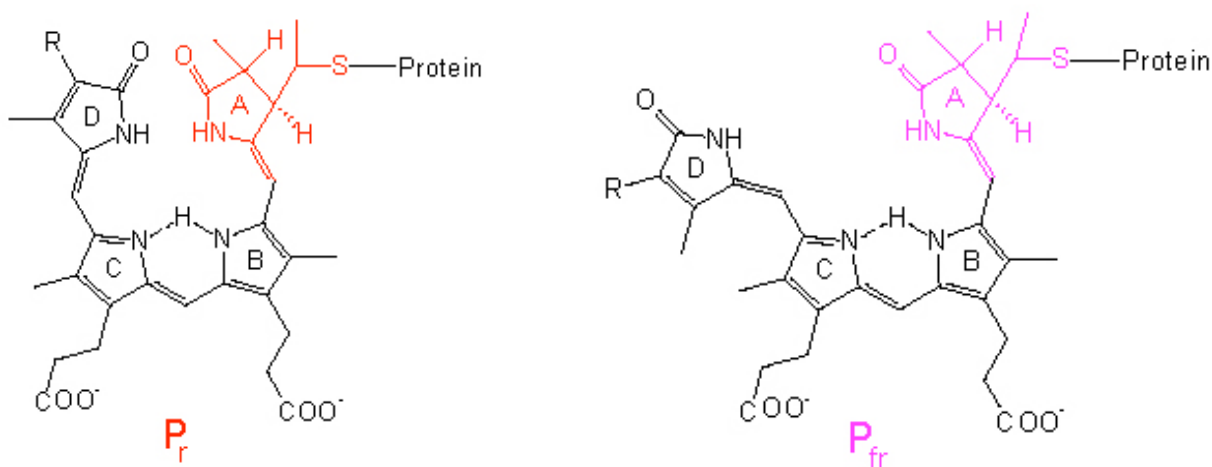


Figure GR-3. Phytochrome red (Pr, on the left) and Phytochrome far-red (Pfr) are isomers. In the presence of red light (~680nm), Pr isomerizes to the biologically active form, Pfr. In darkness or when exposed to far red light (~700nm or longer), Pfr changes back to the more stable isomer, Pr.

C. Temperature Environment

Different plants have different responses to temperature depending on their evolutionary history and where they have evolved. **Cool temperature tolerant** plants are found primarily in temperate regions (between 30° – 60° latitude, north and south). Optimum temperature for growth ranges between 77° - 86°F, although various species can tolerate a range of 40° -104°F. Cool temperature tolerant species include domestic cultivars of *Brassica oleracea*, such as broccoli, cabbage, turnips, cauliflower, etc. as well as many temperate grasses.

Cool temperature requiring species require temperatures lower than 77°F. Seeds from these species will generally not germinate if temperatures are too warm, but require after-ripening triggered by a period of very cold temperatures (40° F or lower). Examples include lilacs, freesias, apples.

Warm temperature requiring plants tend to occur in the tropics (between 0° – 23.5° latitude, north and south) and subtropics (between 23.5° – 30° latitude, north and

south). These species require temperatures of at least 50° - 60° F in order to germinate. Examples include beans, eggplant, corn, tomatoes, and peppers.

Alternating/Fluctuating day-night temperature plants. Some seeds require a drastic temperature differential in order to break dormancy. Such species as conifers, and wild roses need at least an 18° F (10°C) difference between day and night before they can germinate.

D. Soil Environment

Soil must be of adequate hydration, oxygen levels, proper particle size, and tolerable chemical environment in order for seeds in the soil to germinate. Depth of planting can have marked effects on seed germination success, as oxygen and light decrease with soil depth. Soil particle size can also affect these factors: the smaller the soil particle size, the less passage of light there will be through the soil.

Acidity or alkalinity of soil can affect germination success or germination rate. Soil that is hypertonic also can inhibit germination, as high osmotic potential in the seed's environment will prevent water from moving into the seed. Too much fertilizer can thus inhibit seed germination, as can living in a very dry environment, such as a desert.

Soil water must be present in the appropriate amount. Too little water will prevent proper metabolism, whereas too much can literally drown the seeds, which die of anoxia (lack of oxygen).

You can probably think of other variables of soil condition that might affect germination rate or germination success. Consider every possible option before you decide on the question your team will answer.

E. Competition, Secondary Metabolites and Allelopathy

When resources are limited, and there is competition for them between members of the same species or different species, might the density of individuals in the system play a role in germination success? Are some species better than others at surviving crowding? By what mechanism? Faster germination time? Or perhaps even via inhibition of the germination and/or growth of other individuals or species?

Competition for light, space, and nutrients in the plant world can be ferocious. But how do you fight when you're (literally) rooted to the spot? As you might already have guessed, plants are the greatest purveyors of chemical warfare.

Metabolites are compounds made via metabolic reactions. **Primary metabolites** are those found in all cells, and are necessary for normal cellular function and energy transduction. These include things like biological macromolecules (nucleic acids, proteins, lipids, carbohydrates) and simple sugars.

Secondary metabolites are complex chemical compounds that are NOT found in every cell, and not found in every species of plant. Once thought to be waste products of metabolism, they are now known to serve many vital plant functions, such as

- * analogs to animal neurotransmitters (albeit a lot slower)
- * natural antibiotics or anti-fungals
- * deterrents against herbivores
- * allelopathy
- * attracting pollinators
- * attracting seed dispersers
- * protecting young plant parts from harmful UV radiation

Like animal hormones, plant secondary metabolites are usually produced in a specific location of the plant, and then transported for storage (usually in vacuoles) to another part of the plant. Production of the compounds often follows a Circadian rhythm, with concentration varying in a diurnal cycle, seasonally, or even with environmental influence, depending on the plant producing it and the specific compound being produced. The three major types of plant secondary metabolites are **alkaloids**, **terpenoids**, and **phenolics**.

Allelopathy is defined as the inhibition of growth in one species of plants by chemicals produced by another species. This is one important function of plant certain secondary metabolites. It's more common to find plants with allelopathic compounds in areas where evolution has driven fierce competition for space and resources. Plants that have evolved in geologically old biomes (where there has been ample time for mutation and natural selection to operate), and in the tropics (where growth, reproduction, diversity and abundance of species are at their maximum) are the most common players in this plant drama.

Florida is relatively young, geologically speaking. Native plant species here have not been evolving and competing nearly as long as those in areas such as the Amazon Basin, Australian forests, or African habitats. So when species from those localities are imported here (accidentally or on purpose) and then escape cultivation, there can be disastrous consequences, especially if the escaped species are allelopathic.

Three such examples form a cohort of Public Enemy Number One, One, and One in southern Florida: the **Australian Pine** (*Casuarina equisetifolia*), the **Cajeput** or **Paperbark Tree** (*Melaleuca leucadendra*), and the **Brazilian Pepper** (*Schinus terebinthifolius*). Wherever these species are introduced into native Florida ecosystems, they tend to out-compete native species. They simply take over, creating vast stands of a single species (a **monoculture**) and severely restricting food and shelter for native wildlife. How do these **invasive exotic** plants do it? Might allelopathy be playing a role?

We'll let you ask (and maybe answer) that question.

VI. Asking a Question and Designing an Experiment

Now that you have read and considered some of the factors that can affect seed germination, it's time to put your knowledge to work.

A. Experimental Design

1. Decide on the environmental variable you want to examine.
2. Choose the parameter of germination you are going to use to gauge the factor's effect. Here are a few suggestions, but feel free to choose something relevant that's not on this list.
 - a. time to emergence of radicle (this will happen first, but might require a stereoscope to see)
 - b. time to emergence of plummule
 - c. number of seeds successfully germinated over selected time intervals
 - d. seedling survival over selected time intervals
 - e. your choice here!
3. Decide whether to test the effect of changing a variable on a single species, or whether two different species have different responses to a particular variable.

4. Design appropriate treatment and control groups, being very specific about what you will hold constant, and what you will intentionally vary.
5. Decide what type of statistical test you will use to analyze your data. If the data will be non-parametric, be sure to use an appropriate non-parametric test. If the data will be continuous/parametric, also be sure to use the right test. If you're not sure about this, refer to **Appendix I** of your online lab manual for a quick primer!
6. When you ask your question, be sure it's relevant and not trivial. Consider the evolutionary history (and artificial selection, if applicable) of your chosen species. Why is this question important to ask, and what will the answer tell you?

What is your question? _____

What is your null hypothesis? _____

What is your alternative hypothesis? _____

What is your prediction and why? _____

What seedling parameter will you measure? _____

How often will you record data? _____

What environmental conditions will you record and keep constant in your system? _____

What environmental factor will you vary, and how? _____

Which statistical test will you use to analyze your data? _____

When all teams have a good idea of the hypotheses they want to test, and the experimental design they'll use, your TA will allow each team about five minutes to explain its idea to the class for critique and revision. This is a very important step, as there may be pitfalls in your experiment or design that others might see that you didn't, because you're a bit closer to the material.

Before you leave lab today, your team should devise an appropriate data collection sheet, properly labeled, so that you will have a template ready to go when you begin to record your data. Use any software you deem appropriate, even if it's just to create a grid for your raw data.

Be sure to use appropriate units of measure, indicate the time/day of your recording, and every bit of information that might be relevant when it comes time to analyze your results and put them together into a Power Point presentation for your lab colleagues to watch and critique. The Seed Germination Symposium will take place after spring break. Refer to the online syllabus for specific dates.

B. Materials

Various supplies have been provided that you can use to design and execute your experiment. It might be wise to check to see what is available, and then meet with your team to decide on a question, a prediction, and an experimental protocol inspired by the materials we have on hand.

These basic supplies will be available, but feel free to supply or request others (if you give us at least a week's head start).

1. Seeds from plants of various species (front TA desk)
2. Petri dishes
(Each group should take no more than 6 petri dishes, half for treatment and half for control. You can, of course, place more than one seed per dish!)
3. filter paper to fit the petri dishes, or plain old paper toweling
4. Tape and wax pencil markers to label your experimental vessels
5. organic potting soil
6. silica sand
7. light sources (though sunlight would be best, if you can manage this)
8. various colors of cellophane to act as light filters
9. compound stereoscopes
10. blenders
11. distilled water
12. beakers, graduated cylinders, and other glassware
13. cuttings from *Casuarina*, *Melaleuca*, and *Schinus* (refrigerator)

If you think you can put any materials from outside in the arboretum or native biome to good use, then go for it! Be creative and questing!

C. Setting up Your Seeds

For those of you not well versed in how to sprout seedlings (shame on your second grade teachers!), here's a brief primer. Depending on the size of your seeds, you will place 10 – 30 seeds per petri dish on wet filter paper, and watch their progress over the course of your experiment.

1. Select the species (or two) of seed you wish to use for your experiment.
2. Count the appropriate number of seeds of each, and bring them to your station.
 - a. The number of seeds you use and the number you place in each petri dish will depend on the question you're asking and other aspects of your experimental design. But unless you're testing the effects of different population densities, you should—of course!—keep the number of seeds per container constant.
 - b. Don't take more than you need, and be sure to return the rest of the seeds to the TA desk when you are finished. **DO NOT MIX UP DIFFERENT SPECIES OF SEEDS!**
3. Before a seed will germinate, it must be soaked in water to wash the ABA out of the seed coat and allow the seed to imbibe water to initiate its metabolism.

Once you soak your seeds, they will become metabolically active relatively soon. So whatever manipulation you intend to do must be done promptly—especially if it involves brief exposure to light.
4. Line the bottom of your experimental containers with clean, wet filter paper. Place your soaked seeds, evenly spaced, on the filter paper. (If you are manipulating only the spacing or number of seeds, you may modify this step. But then keep all other variables constant.)
5. When you moisten the substrate, be sure to use exactly the same amount of water for each container (unless that is the variable you are manipulating).
6. Introduce your single environmental variable.
 - a. For example, If you're testing the effects of allelopathy, for example, instill your leaf extract of whatever species you're using (*Casuarina*, *Melaleuca*, *Schinus*, or other) onto the plate. Make extract by macerating **leaves** from your chosen species in the blender with water (it's a good idea to weigh the leaves and measure the water, of course!), and if you want to remove particulate matter (which isn't necessarily required; that depends on your team's experimental design), strain your blender mix through a small piece (don't waste!) of cheesecloth available at the blender station. **WASH AND RINSE THE BLENDER VERY THOROUGHLY WHEN YOU HAVE FINISHED, OR YOUR TEAM WILL AUTOMATICALLY LOSE TEN POINTS! DO NOT PUT ANYTHING EXCEPT SOFT PLANT PARTS AND WATER/SOLUTION IN THE BLENDERS. THEY ARE NOT APPROPRIATE FOR ANYTHING ELSE!**
7. Cover the petri dish with the lid and tack it shut with masking tape. **DO NOT** seal the dish! The seeds need oxygen to survive, and you don't want to suffocate them.
8. Label each dish appropriately with masking tape and wax pencil. Be sure you know which is treatment and which is control. Be sure your team's name is on all the dishes!
9. You may either take the dishes home with you for easier monitoring (this is actually preferable, as you'll be able to maintain the dishes in a small location and take data at convenient intervals, at your convenience), or leave them in the lab on a tray marked with your lab section and TA's name. If you are leaving your plates in the dark, an ideal spot is one of the drawers at your station. Just be sure to put a label on the drawer saying **DO NOT OPEN** if your experiment is light sensitive, and maybe even put a strip of tape over the drawer to discourage accidents!

10. You probably do not need to open your experimental vessels as you collect data, as the petri dishes are translucent, and the seeds should be visible easily through the plastic. By opening the dishes, you will introduce possible error into your system.

D. Data Collection and Analysis

Be sure your team agrees in advance on exactly what type of data are to be collected, when, and by whom. If more than one person is collecting data, agree on a technique that will minimize variation and human error due to sampling technique.

Be sure your samples are well marked and placed in a location where they will not be disturbed. If your seeds are going to be germinating in the dark (which is fine, as long as you're not using light as a variable), then use one of the drawers at your lab station. If a drawer is already in use by another team, choose a different drawer. Clear out any debris (inconsiderately left by other lab students), place your samples, and clearly label the front of the drawer with tape with your team's names and lab section. Assign one or more teammates to check the progress of your experiment, as dictated by your experimental design. Labs are open every day from about 9am – 9pm, and it's okay to come into a lab that's in session as long as the TA isn't in the middle of giving a lecture or a quiz. (If lab is bustling, come on in and check your samples as often as necessary.)

Most of all: Ask important questions, and have fun with this.

The Kindergarten Wall

*Of all you learn here, remember this the best:
Don't hurt each other and clean up your mess.
Take a nap every day, wash before you eat,
Hold hands, stick together,
Look before you cross the street.
And remember the seed in the little paper cup:
First the root goes down,
and then the plant grows up!*

--John McCutcheon