Laboratory on Seed Germination:

The Effect of Light on Lettuce Seed Germination

Plant species occur on almost every continent, and the species of plants in any given ecosystem often defines what type of animals and other organisms can survive there. In each species, natural selection has resulted in evolution of seeds that germinate when conditions are most favorable for growth in that species' particular environment, and which remain dormant when conditions would not be favorable. Domesticated crops, however, have been artificially selected to germinate reliably when the farmer decides to plant them (or else he or she might not plant that variety again). Thus, most domesticated crops whose wild ancestors once had seed dormancy have lost it.

A case in point is lettuce (*Lactuca sativa*). The wild ancestors of lettuce were probably colonizers of disturbed areas in temperate climates of Europe and Asia. In a forest, the seeds that did best would lie dormant in the soil until a light gap opened above them. Buried seeds that were able to delay germination until the soil was disturbed, bringing buried seeds to the surface, also had a selective advantage. In addition, germination had to be timed so that the young plants could complete most of their growth in the cool, rainy weather of spring, not in the fall (when frost might kill a young plant) or in the summer (when it might be too hot and dry for optimal growth). Ancestral wild lettuce thus evolved a combination of light- and temperature-mediated seed dormancy.

The classic studies demonstrating that dormancy could be regulated by red and far-red light were performed using one of the last varieties of modern lettuce to retain ancestral seed dormancy, *Lactuca sativa* 'Grand Rapids' (Borthwick et al. 1954). Since then, under relentless selection for "uniform germination," most strains of Grand Rapids no longer show seed dormancy. However, one variety, Waldmann's Dark Green, still does (Neff et al. 2009). And we have searched far and wide to obtain this variety for your experimenting pleasure!

The following environmental variables may affect germination when lettuce seeds experience them singly, in combination, or in sequence. Check out the back counter of the lab to see what is available for you to use. You may go outside to collect leaves, leaf litter, pond/lake water, or other relevant materials. Think about what these various conditions might tell the seed about the suitability of the present (or future) environment for growth, and then, with your team, come up with a question and a hypothesis. Develop a rationale for your team's hypothesis, and then design an experiment to test your hypothesis. You may use up to 12 mini-germination chambers (a.k.a., petri dishes) per group. Each group should have a distinct question and design—please be sure to discuss it with your TA, and then the whole class, for helpful feedback.

Read on for more ideas and suggestions of variables you might consider.

<u>Light variables</u>:
Ambient light
Darkness (aluminum foil)
Cellophane light filters: red, blue, yellow, green

Far red light filters, made in two possible ways:
-red + blue cellophane (Takaki and Zaia 1984)
-lettuce leaves in plastic wrap (Neff et al. 2009)

Temperature variables:
Room temperature
Outside temperatures
Refrigeration at 4°C for one to several days (Margaris and Fiaku 1974, VanDerWoude and Toole 1980)
Freezing at below 0°C for one to several days
Heat shock at 30°C for 15-30 min (Takaki and Zaia 1984)

Nutrient and soil variables:
Inorganic fertilizer solutions (manufacturer's recommended maximum concentration = 0.12%)
0.05 M phosphate buffers, pH 6.0 – 8.0
NaCl solutions (as a starting point, the salinity of seawater is about 3%)
Brackish water from Lake Osceola
Fresh water from the microbiome pond
Organic compost tea
South Florida limestone soil tea
Slash pine needle litter tea from the remnant pine rockland (potentially allelopathic)
Brazilian pepper leaf litter tea from the remnant pine rockland (potentially allelopathic)
Mixed species leaf litter tea from the hardwood hammock microbiome

When contemplating your experimental design, consider what your treatments will be (including controls, if needed), and what your *experimental unit* (a.k.a., *unit of replication*) is. Different designs may suggest different controls. Possible designs using 12 dishes include 2 treatments with 6 replicates each, or 3 treatments with 4 replicates each. As in real life, there are tradeoffs in experimental design: more treatments (therefore with fewer replicates of each) means you can ask more complicated and interesting questions, while more replicates (of fewer treatments) means you may have a better chance of actually answering your question—by finding out whether the results of your treatments were statistically different. It's the adventure of science—choose your design wisely, execute it carefully, and above all, enjoy!

Helpful tips:

1. Hormones regulate seed dormancy, and in most seeds the hormones are in the tissues of the seed—not in the seed coat. The exception is some seeds of desert plants, which must be able to germinate immediately when substantial (and perhaps unpredictable) rainfall occurs. Abscisic acid (ABA) in the seed coat washes off, releasing the seed from dormancy (Koenig 1994). Lettuce seeds evolved in temperate, rainy climates, where rain is not a sufficient cue for good growing conditions—so there is no external ABA, and you don't have to rinse the seeds.

2. Place one 9 cm filter paper circle in the *bottom* half (not the lid) of each Petri dish. The paper is a little too big to fit exactly, so use a glass rod to nestle it as flat as possible in the dish bottom.
3. Place 50 dry seeds in each of your dishes, unless of course your hypothesis involves the effect of seed density on germination.
4. Add 5 mL of water or solution per dish, to moisten your dry seeds and begin the imbibing process. Seeds are sensitive to environmental cues only after imbibition (Finch-Savage and Leubner-Metzger 2006). (If you are using the thicker filter paper made from solvent saturation pads, use 7cc of water or solution per dish.)
5. Seeds begin to sense their light environment within minutes after they imbibe water. Therefore, apply any light treatments (using foil, cellophane, or wrapped lettuce leaf filters) immediately after moistening the seeds, or they may begin to respond to the ambient light, rather than to your intended treatment. For the same reason, do not remove the light treatments, once applied, for even an instant—unless that is part of the intended treatment.
6. You may be asking, "If I can't remove the foil or cellophane (or lettuce leaf), how will I record my germination data?" Good question! We will set up a special darkroom with filtered light (out of the sensitivity range of phytochromes), for you to safely look at your seeds next week!

Literature Cited:

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