PROPAGATION OF PLANTS FROM SEED
Common Germination tests and factors that affect germination

An introduction to plants propagation laboratory exercises by:
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In this lab, you will be introduced basic application of common seed tests used to understand factors affecting seed germination

Lab Objectives:

Review seed germination test protocols
Review common factors in seed germination tests
Demonstrate basic steps in evaluating seed germination.

Seed Germination Tests

Obtain a pure and clean subsample of seed
Place seeds in controlled environment
Monitor and record germination data
Analyze and interpret data

Seed germination tests are used to describe germination requirements and when used in conjunction with viability tests, dormancy characteristics for a cohort of seeds can be determined. The first step in germination testing is to obtain a subsample of seed from a larger seedlot of interest, and when possible cull deformed individuals and surface sterilize seeds. Cleaned and graded stored seed of *Asclepias humistrata,* sandhill milkweed are shown here. This is a Florida native plant important for monarch butterflies. The seeds represent multiple populations from across a broad geographical area of Florida. The seeds were graded prior to storage and will need to be surface sterilized prior to use in germination experiments.

The second step of germination testing is to place seeds in a controlled environment. Typically, seeds are tested in a laboratory with highly regulated environmental conditions. For example, seeds can be placed within a petri dish and placed in germination chambers . DIY testing can also be accomplished in a home or garden setting, but you will have less environmental controls.
The third step of seed germination testing includes recording germination data and monitoring environmental conditions, especially moisture, and adjusting as necessary and culling seeds which are contaminated. Germination is typically defined as the protrusion of the radical from the seed coat and is collected throughout the experiment for 2-4 weeks or more. Emergence of seed from the soil line, rather than germination, can also be used in greenhouse or garden emergence experiments.

Germination is typically defined as the protrusion of the radicle through the seed coat followed by the emergence of the embryo and cotyledon(s). On the left, seeds of *Crocanthemum arenicola (*coastalsand frostweed), were placed in petri dishes on top of damp blotter paper for 48 hours prior to taking this picture where we see germination for

1 of the 5 seeds. The black arrow is pointing to the white tube shaped radicle.
On the right emergence of a single seedling of *Asclepias humistrata* can be seen after sowing within tree-tubes on top of a peat-based potting mix and lightly covering with fine vermiculite. After 4 weeks 1 of the 2 tree tubes had a seedling emerge.

In this picture 25 seeds of a FL native coastal dune species, *Oenothera humifusa* (Seabeach Eveningprimrose), were placed in germination boxes on top of damp blotter paper for 2 weeks. Determine the total number of germinated seeds and calculate the percent of seeds that have germinated. A total of 5 of the 25 seeds, or 20% of seeds germinated.

Unless seeds are dormant, they will germinate given the proper environmental conditions including moisture, oxygen, temperature and light. The two most common factors used in seed germination testing include temperature and light. For example, a standard horticultural germination temperature test may include 4 daily fluctuating temperatures. These temperature combinations may also be modified to simulate regional seasonal temperatures.

An additional temperature test is the use of a gradient table with temperature lanes that remain constant. This allows for the testing of multiple temperatures in minimal space compared to standard incubators

The length of daily light and its characteristics (quality, quantity, etc.) are often tested. Seeds may have improved germination in response to light, have germination inhibited by light, or germinate in light and dark equally. A common test includes testing an 8-12 hour photoperiod vs a 0 hour photoperiod (dark) which can be achieved by double wrapping germination container in aluminum foil shown on the left. Temperature and light can also be tested together to test for interactions.

In some cases, the seed may be sensitive to temperature in the dark where seeds do not germinate until a minimal temperature is met while seeds exposed to the light may not germinate above a given temperature threshold. This type of interaction will ensure the seed germinates readily in the spring as temperatures warm but germination in prevented for exposed seeds in the heat of summer when the seedlings may not survive the harsh summer temperatures.

Germination tests can be compromised by contamination from fungi, bacteria, and other microbes. This is why it is important to practice good hygiene when performing germination tests, including surface sterilization of seeds and the use of pure water in the form of distilled, deionize, or distilled and deionized water rather than tap water. Pictured here are 4 seeds of *Crocanthemum arenicola*, the two top seeds are covered with fungi while the two bottom seeds are not. If contamination is a persistent issue, despite surface sterilization, it may be overcome with hyper control of the germination environment and use of autoclaved germination paper, containers, and water. These procedures are typically for micropropagation, though the costs and expertise required are much higher than standard germination tests.

What can you conclude if there is low germination across multiple temperatures and light treatments, yet the seeds have high viability?
This could indicate that some of the seeds are dormant and will require an after ripening or another treatment to initiate germination. We will cover how to address this in a different section.

Lab concepts practice slides
You will be shown slides of seed experiment to help you practice the procedures described in this lab introduction. The correct answers are provided in the subsequent slides. You may wish to pause the presentation to allow you to evaluate the images and compute the answers before the results are presented in the next slide. Can you spot the seed or seeds with contamination?

The single contaminated seed is encircled here in black. What do you think the contamination is ? Most likely hyphae from a fungus.

Seed of a FL native coastal dune species, *Physalis angustifolia* commonly called coastal groundcherry, and placed in a growth chamber. After 1 week diseases was observed. Calculate the percent of total seeds that have diseases. Hint: each seed has an arrow pointed at it.

Answer = 44%. A total of 4 seeds have some sort of dark grey contamination on and around the seed coat and are circle here. Compare to the other 5 seeds with a golden appearance which are not contaminated.

Seed of a FL native sandhill species, *Eriogonum tomentosum* commonly called dogtounge or wild buckwheat, were collected in fall, air dried at room temperature, seeded at a rate of 2 seeds per cell in late December the following year. Seeds are sown within a peat based bagged mix and placed on an intermittent mist bench (misted for 10 seconds every 10 min from 9:00 AM to 5:00 PM). Pictured here 51 days after planting are emerged seedlings.

Calculate the emergence percentage. **Answer = 22/24 = 92%**. Note each seedling has an arrow pointed to it.

Lab Exercise
Your lab instructor will provide you with a data set from a seed experiment to test the effects of light and temperature on seed germination. Use the information provided to describe the light and temperature treatments and calculate the germination or emergence for each treatment. Depending on the treatments and the data set you may have to calculate this for multiple dates. Graph the data and evaluate the results of the treatments. In your lab report, draw conclusions on the optimum treatments for seed germination.