Plant Propagation Lab Exercise
Module 2

PROPAGATION OF PLANTS FROM SEED
IMBIBITION AND SCARIFICATION

An introduction to plant propagation laboratory exercises by:
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In this lab, you will be introduced to the basic biology of a seed. The parts of the seed will be reviewed, and the role of each seed part explained. Imbibition of seeds will be introduced and the role of scarification discussed. Practice in identifying imbibed seeds and steps for evaluating the need for scarification treatments will be provided along with photos of seeds from scarification experiments to practice data collection, calculation and evaluation of scarification treatments.

The objectives of this lab are to: Review the basic structure of a seed. Review the process of imbibition and describe a basic imbibition test. Introduce methods of seed scarification Review photos of seeds to evaluate effects of scarification and the presence of imbibition. Evaluate imbibition and compare scarification treatments of a controlled experiment.

Imbibition is the first step in germination and is defined by a rapid increase in the uptake of water by a seed. A seed may imbibe and dry out several times before germination and certain seed pretreatments are only affective on imbibed seed (e.g. stratification). Additionally, an imbibed seed is often easily distinguished from a non-imbibed seed with an increase in size and/or a change in color of the outer surface. While seeds of most plants readily allow for the uptake of water, often within the first few to several hours, some seeds restrict the flow of water from outside of the seed to the inside of the seed. This specialized, water-impermeable seed coat is a mechanism of certain types of physical dormancy. Here we provide examples of *Crocanthemum arenicola* seeds with a small, dark, non-imbibed seed on the left and a larger (swollen) , lighter, imbibed seed on the right.

Scarification is the process of physical or chemical alteration of the seed coat and is used to overcome physical dormancy. Additionally, scarification can also enhance germination of seeds that imbibe but have some type of tissue that inhibits germination, like some tissues surrounding the caryopsis in Poaceae. Scarification can be tricky since many of the treatments have the potential to damage seeds if misapplied . The key to successful scarification is to reach a "sweet spot" where enough of your seeds have been scarified but your treatment is not harsh enough to shatter or kill the seeds. Nonetheless, scarification is principally achieved via three methods including mechanical scarification, chemical scarification and high-temperature scarification. **Mechanical scarification** is achieved by abrasion or nicking the seed coat and is commonly achieved with tumblers or drums that spin and are lined with sandpaper as is shown in the top picture. On a smaller scale mechanical scarification can also be accomplished by rubbing seeds together between sandpaper or grabbing seeds with forceps and individually puncturing seed coats. **Chemical scarification** is achieved by immersing seeds into an acid, typically sulfuric acid, for a certain amount of time. Similarly, **high temperature scarification** can be achieved by exposing dry or moist seed to high temperatures. For all scarification types, the duration and intensity of treatments will vary by species and other factors.

A common way to test for a water impermeable seed coat or to test the effectiveness of a scarification technique is by conducting an imbibition test which quantifies the uptake of water over time for a seed by weighing seeds immersed in water over a period of time. Here we present data from an imbibition test on two seed lots of a Florida native plant, *Lupinus diffusus*, collected from wild populations in Milton and DeFuniak Springs, Florida. Half of the seeds were nicked with a razor blade (i.e. scarified) on the distal end while the other seeds were not nicked with a razor blade to serve as a non-scarified control treatment. Seeds were weighed at 0.25, 0.5, 0.75, 1, 2, 3, 6, 12 and 24 hours after immersion and a fresh weight increase percentage was calculated by multiplying 100 times the quotient of the weight increase divided by the original weight (**Weight increase = 100 * (original weight– new weight)/original weight**).

Here we describe how to use visual cues to calculate the percent of imbibed *Crocanthemum arenicola* seeds. These seeds swell and appear lighter brown in color when imbibed. Here are 15 seeds (3 rows of 5 seeds) 24 hours after scarification by rubbing seeds between two pieces of sandpaper and placement on moist blotter papers within a petri dish. What is the total number of imbibed seeds? What is the percentage of imbibed seeds?

A total of 12 of the 15 seeds, or 80% have imbibed. Imbibed seeds are circled here. Do you think this was an effective scarification technique?

Here we have seeds of *Lupinus diffusus* following a period of time in an electric seed scarifier. What is the total number of imbibed seeds? What is the percentage of imbibed seeds?

8 out of the 19 seeds, or 42% of seeds are imbibed.

How do we handle the occurrence of fungi and bacterial contamination of our seed tests?

First you must recognize what is present on your seeds, so you know how to approach seed

cleaning prior to initiating your seed trials. The Photo on the left shows a mixture of various fungi. Fungi are usually hairy looking and have a fibrous or filamentous appearance. Spots appearing wet and sticky are most likely bacteria.

Intense contamination of seeds during testing may require simple washing under clear water prior to placing seeds in petri dishes. Difficult to clean seed may require a surface sterilant such as alcohol or the presence of a fungi or bacterial inhibitor in the solution or on the blotter paper.

Lab Exercise

Evaluation of imbibition in response to scarification

• Seeds of Virginia Saltmarsh Mallow (*Kosteletzkya pentacarpos* (L.)Ledeb.) were subjected to 4 scarification treatments.

- Non scarified control
- Sandpaper abrasion – seeds rubbed between two pieces of sandpaper
- Razor blade seed coat penetration – seeds nicked on the distal end with a razor
- Submersion in hot water – seeds submerged in boiled water for a period of 10 min after removal from heat

• Seeds were placed in petri dishes on moist paper towels and allowed to imbibe. Each treatment is presented in comparison to the non scarified control seeds.

• Evaluate the seeds for imbibition and calculate the imbibition percentage.

• Prepare a short lab report and discuss your results. Also provide your impression of the ease or difficulty of evaluating the imbibition of this species.

Review the seeds in the four petri dishes and calculate the % imbibition for each of the 4 scarification treatments.