


Seed viability testing guide for common wetland plant species

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Preface

Seeds are used in many aspects of plant research as well as in applications such as ecosystem restoration. However, not all seeds are living (i.e., viable). Non-viable seeds can result from a variety of factors such as failed pollination, fungal infection, insect damage, extreme weather events like historic drought, and inbreeding (Baskin and Baskin 2014; Rieger et al. 2014). Furthermore, seed viability can vary greatly across species and for individual species by patch, site, and year (Baskin and Baskin 2014; Rieger et al. 2014). In the wild and in storage (e.g., for restoration), seed viability declines with time, sometimes dramatically (De Vitis et al. 2020).

To successfully work with seeds, it is essential to know how many seeds are viable. Seed viability can be assessed through a few different avenues such as a basic cut test (i.e., is the embryo fully developed), germination assays, x-ray imaging, or the most common—a tetrazolium (TZ) test (Frischie et al. 2020). The first three options are imperfect—with a cut test, embryos that appear fully developed can still be non-viable; germination assay results can be skewed by dormant seeds or improper germination conditions; and x-ray imaging requires specialized equipment and training (Frischie et al. 2020).

This document focuses on best practices for TZ testing for a wide array of common wetland species that have been the focus of research and restoration in our region (the Intermountain West). These recommendations build on formal rules and practices developed by the AOSA (Association of Official Seed Analysts) and SCST (Society of Commercial Seed Technologists; Miller and Peters 2010). In some cases, pre-existing protocols were followed or adapted, and the most straightforward option was often chosen when multiple protocol options were available. For example, methods that were simple to follow and that led to more consistent results across observers were often chosen. In other cases, protocols were developed *de novo,* building on guidelines for similar species.

As information is developed for new species, the guide will be updated with new information (as indicated by the Edition number).

Acknowledgments

This guide was made possible by funding and resources from the Environmental Protection Agency; Utah Agricultural Experiment Station; Utah Department of Agriculture and Food; Utah Division of Forestry, Fire & State Lands; Utah Division of Wildlife Resources; and Utah State University Extension.

Recommended citation

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Background information

What is tetrazolium (TZ)?

TZ stands for 2,3,5-triphenyl tetrazolium chloride. TZ testing is a relatively quick way to determine seed viability for a seed lot, i.e., the portion of a seed lot that is alive.

The TZ staining reaction

When TZ solution enters living tissue, it is reduced to formazan, a pink-red compound, via H+ ions that are released by dehydrogenase enzymes that are present in respiring cells. Therefore, seeds that turn red when exposed to TZ are known to be respiring and are viable. Seeds that do not take on a red coloration are no longer respiring and are effectively dead.

Suggested reading

Pages 1–22 in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, cover the nature of the TZ test, history, seed structure, etc.

Supplies

The following supplies will be helpful when conducting TZ testing:

- Microscope and microscope lights for preparing and evaluating seeds.
- Square or rectangular plexiglass pieces to prepare and evaluate seeds under the microscope.
- Double sided tape (thin tape is useful for small seeds and thick tape is useful for large seeds) to keep seeds in place during preparation and evaluation.
- Precision knife with exacto blade replacements for cutting seeds during preparation and evaluation.
- Forceps (various sizes) for handling seeds.
- Petri dishes to hold the seeds during the TZ solution soak.
- Paper towels or filter paper for seed imbibition prior to soaking in the TZ solution.
- TZ powder to create the TZ solution.
- Deionized water for mixing the TZ solution.
- Handheld clicker (counter tally) for keeping track of viable and non-viable seeds when assessing the seeds under a microscope.

Seed viability assessment timeline

Here is some general guidance on the TZ testing timeline:

If possible, complete a germination test prior to a TZ test. Germination tests can be completed in Petri dishes under ideal conditions in growth chambers or greenhouses. Germinated seeds can be counted and removed once a week for a short period of time (~1–2 weeks) prior to conducting TZ tests on the ungerminated seeds that remain. Total viability proportion is germinated seeds + viable seeds (as determined by TZ) divided by the number of seeds assessed. At least three replicates of 50–150 seeds should be used in testing depending on the level of accuracy needed. A germination test prior to

TZ evaluation can save time if many or some of the species are expected to germinate readily. Conducting a germination trial also imbibes the seeds, meaning the seeds are already properly imbibed at the start of TZ testing.

If a germination test is not pursued, soaking the seeds in water for 24–48 hours before beginning initial cuts may facilitate cutting precision and imbibition of the TZ solution. Imbibition improves efficiency and may lead to more straightforward evaluation.

Storing and using TZ

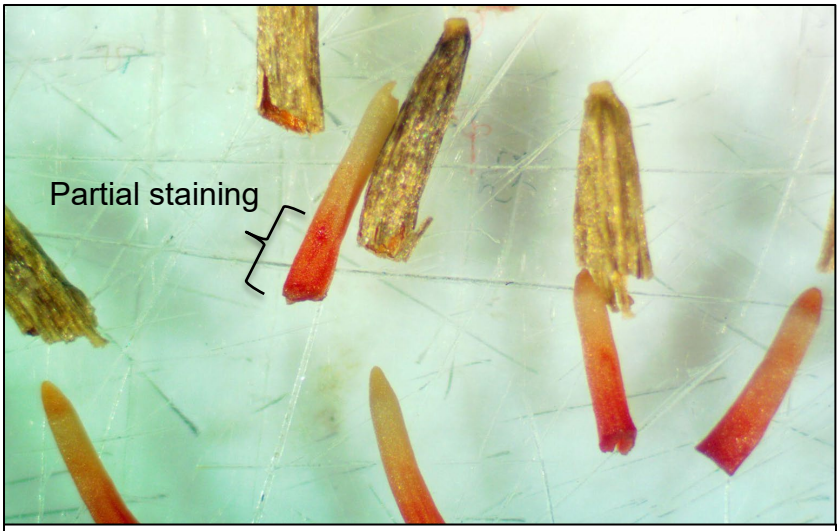
TZ should be stored under dark and cold (refrigerator) conditions to prevent degradation. Aluminum foil can be used to cover containers holding the solid or liquid TZ to minimize light exposure. For the protocols described in this document, the soak in TZ solution occurred at room temperature in a drawer that shielded the solution from direct light.

Mixing the TZ solution

- 1 gram of TZ powder dissolved into 100 ml of deionized water yields a 1% solution.
- Be sure to thoroughly shake the solution before using it.

TZ solution concentrations and soak time

- Information on many plant families and genera is in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition. Read about the suggested preparation and TZ concentration (typically 2–3 pages for each family).
- For species that lack a TZ protocol, using a 1% TZ solution for 48 hours is a good starting place.
- Try a few different cutting techniques and periodically check the progression of the staining (e.g., 12, 24, and 48 hours). Typically, you want to avoid cutting the embryo but sometimes this is unavoidable.
- Some species and seed lots will need more or less time in the TZ solution. However, if seeds are staining light pink or partially stained in a patterned way (darker closer toward the cut), this is evidence that they need more time in TZ or a higher TZ concentration.
- At right is an example of a TZ diffusion issue as opposed to non-viable seeds. These



Senecio hydrophilus seeds that show a staining pattern that points to a TZ diffusion problem.

seeds were soaked in 0.1% TZ for 24 hours and were stained dark pink only near the cut location.

- If this staining pattern is present, it is an indication that a longer time or higher concentration TZ is needed to ensure a more straightforward evaluation. A higher concentration and soaking time (1% for 48 hours) resulted in fully dark pink embryos (see *Senecio hydrophilus*, p. 21).

Viability evaluation

In general, a fully stained embryo is viable. However, some plant families have specific evaluation criteria (see the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, for information specific to plant families). Also, if other necessary tissues are damaged or absent, the seed can be deemed non-viable. From the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition (p. 1–18), "the following contribute to the classification of seeds as non-viable:

- Evidence of necrosis
- Half or more of the total cotyledon tissue in dicots non-functional
- Critical connective tissues damaged or decayed
- Flaccid tissues
- Pathogen invasion
- Mechanical breaks or bruises, especially in strategic locations that would impair growth and development"

A well-organized data sheet (see Appendix 1) can help with data collection and careful note taking. Many people find it useful to work in rows or groups of 10 seeds at a time when assessing viability. Counters can also be used to keep track of viable and non-viable seeds during the assessment. We suggest recording the characteristics observed that indicate a seed is non-viable. This helps people evaluate more purposefully and makes it easier to catch evaluation mistakes.

Seed handling tips

Here are some seed handling tips and tricks that are useful when conducting TZ testing:

- Embrace ambidextrousness. The dominant hand can cut or pierce seeds with a blade, while the nondominant hand can hold forceps to the seed to keep it in place.
- If seeds are flinging away and not staying in place when cutting, try a thick tape (i.e., thicker than regular double-sided tape). If seeds are falling apart when you try to move them with forceps, a thin double-sided tape (i.e., regular double-sided tape; less sticky) will release the seeds more easily.
- To remove seeds from the TZ solution, pour the seeds and the TZ solution onto a small stack of paper towels. This is easier than removing seeds with forceps when they are floating in the solution.
- Use white backgrounds whenever possible to keep track of seeds more easily. The contrast of the seed on a white background under the microscope makes it much easier to see the seeds.

- During preparation of seeds, seeds may stick to forceps when moving them into an empty Petri dish. Use an eyedropper to put one small drop of water in an otherwise empty Petri dish. To place the seed in the dish, let the water pull the seed off the forceps. This drop will likely evaporate before you add the TZ solution, so no need to worry about further dilution of the TZ solution.
- If static interferes with the handling of small seeds, wipe down tools and the workstation with a wet paper towel.
- Sharp blades are very necessary. If you are chopping, smashing, or imprecisely slicing seeds, use a new blade—it makes all the difference!
- After putting the seeds in TZ solution, lay a piece of paper towel the size of a Petri dish on top of the seeds/solution to force the seeds into solution and alleviate the issue of having seeds floating on top.
- Small seeds can be lined up on thin double-sided tape in preparation for the TZ solution. After cutting, remove the tape and stick it into a Petri dish before adding the TZ solution. This step saves time, as most of the seeds will stay on the tape even after 48 hours in solution.

How to use this guide

Below is an example of the content and format you will find on the following pages for each species.

1. Plant family that the species belongs to

2. Latin name for the plant species

3. Common name for the plant species

4. Percent (%) tetrazolium (TZ) solution to use; **Length of time** to soak the seeds in solution

We provide **images of the seeds** of the species with a scale bar in millimeters (mm) for reference.

5. Preparation: we describe how to prepare the seed prior to soaking in the tetrazolium solution. This step will involve puncturing the seed coat, or sometimes cutting off a part of the seed (without disturbing the embryo). This step may also involve saturating the seed in water (imbibing) prior to cutting or staining.

6. Evaluation: we outline the steps to evaluate the embryo for viability after the seed has soaked in the tetrazolium solution.

7. Notes: we provide additional tips and tricks for the species for successful viability evaluation.

*Throughout these pages, you will notice that we **bold and blue botanical terms** that we later define in the glossary.*

8. Figures illustrating seed preparation and evaluation

On the left side of Figure 1:

We provide hand drawn diagrams to illustrate the preparation stage. We indicate where and how to make the preparation cuts and a photo of an actual seed with that cut. The cuts are indicated with a **dashed line**. The scratch of a window is indicated with a **box with hatch marks**. In the diagram, we indicate the embryo (**E**), radicle (**rad**), and cotyledon (**cotyl**) where needed to illustrate the steps.

→

On the right side of Figure 1:

We have hand drawn diagrams and photos to illustrate the evaluation stage. We show the embryo (**E**) post-staining, and sometimes the seed coat (**SC**) where needed to illustrate the step.

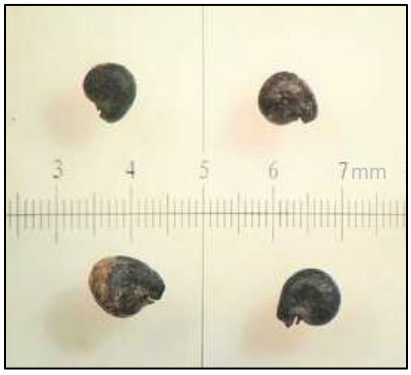
9. Additional photographs

In some cases, we also provide additional photos of seeds in varying levels of viability (and associated staining) to assist with interpretation of test results.

Aizoaceae

Sesuvium verrucosum

Verrucose sea-purslane



1% TZ; 48 hours

Preparation: Chip or scratch a "window" (W) into the center of the **seed coat** (SC) with an exacto blade (Figure 1). Try not to cut into the **embryo** (E) that curves around the edge of the **seed**.

Evaluation: Remove the embryo from the seed coat by pulling the embryo out of or chipping away the seed coat. Be sure to get a good view of the whole embryo and disregard any **artifact damage** (Figures 2–3). The **endosperm** in the center of the seed does not need to be stained pink for the seed to be **viable**.

Notes: **Imbibition** prior to creating the window cut does not seem to improve the technique since the seed coat is very hard.

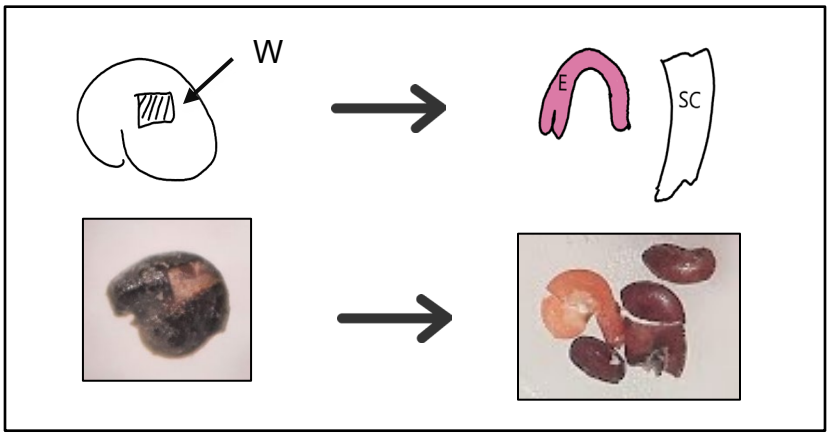


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Five viable seeds with minimal artifact damage. Embryos broke apart when removed from the seed coat, but it is still clear that the entirety of the seed is stained pink.

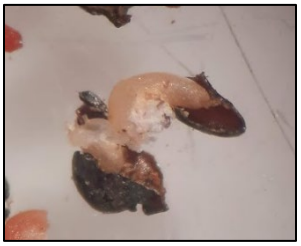
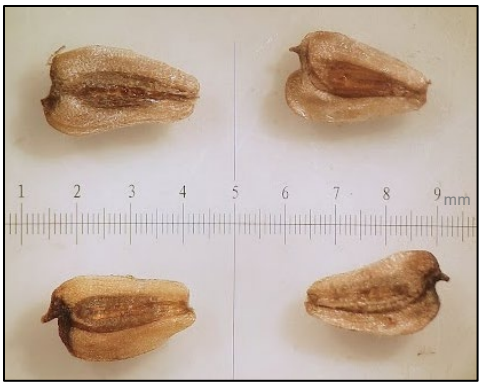


Figure 3. One **non-viable** seed. No visible staining.

Alismataceae

Sagittaria cuneata
Arumleaf arrowhead



1% TZ; 48 hours

Preparation: **Imbibe seeds** for 12–24 hours before preparation to make the **pericarp** and **embryo** less brittle. Leave the embryo inside the pericarp and pierce the embryo (E) through the pericarp (P) and **seed coat** (SC; Figure 1). This approach will avoid damaging the embryo and it stains well if you are careful to pierce through the seed coat.

Evaluation: Remove the embryo from the pericarp AND the seed coat (first remove pericarp, then the seed coat, which is right around the embryo) by cutting right at the curve of the embryo and gently squishing the embryo out of the seed coat (Figure 1, middle image). A fully pink stain is **viable** (Figure 2). Be sure to get a good view of the whole embryo and disregard any **artifact damage** which is common (Figure 3).

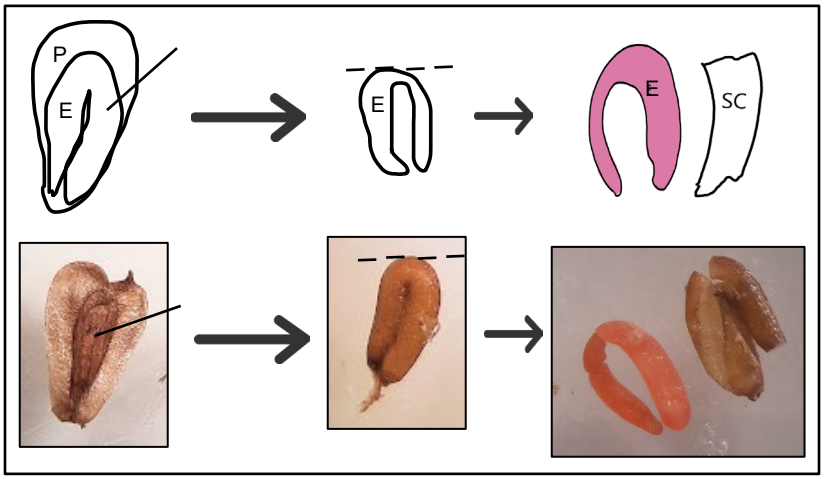


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Five viable seeds, most of which have artifact damage.

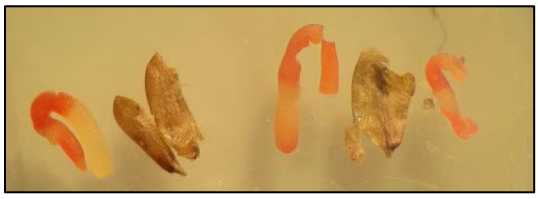
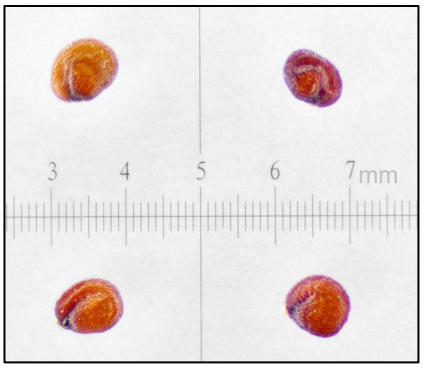


Figure 3. Two viable seeds (right) with artifact damage. One **non-viable** seed (left) where the artifact damage is visible and the other portion of the seed that wasn't damaged is still unstained.

Amaranthaceae

Allenrolfea occidentalis
Iodine bush

1% TZ; 48 hours



Preparation: Chip or scratch a “window” (W) into the center of the **seed coat** (SC) with an exacto blade (Figure 1). Try not to cut into the **embryo** (E) that curves around the edge of the **seed**. Removing seeds from **utricles** before making preparatory cuts and placing them in TZ is suggested.

Evaluation: Remove the embryo from the seed coat by pulling the embryo out of or chipping away the seed coat. Be sure to get a good view of the whole embryo and disregard any **artifact damage**.

Notes: **Imbibition** prior to creating the window cut does not seem to improve the technique.

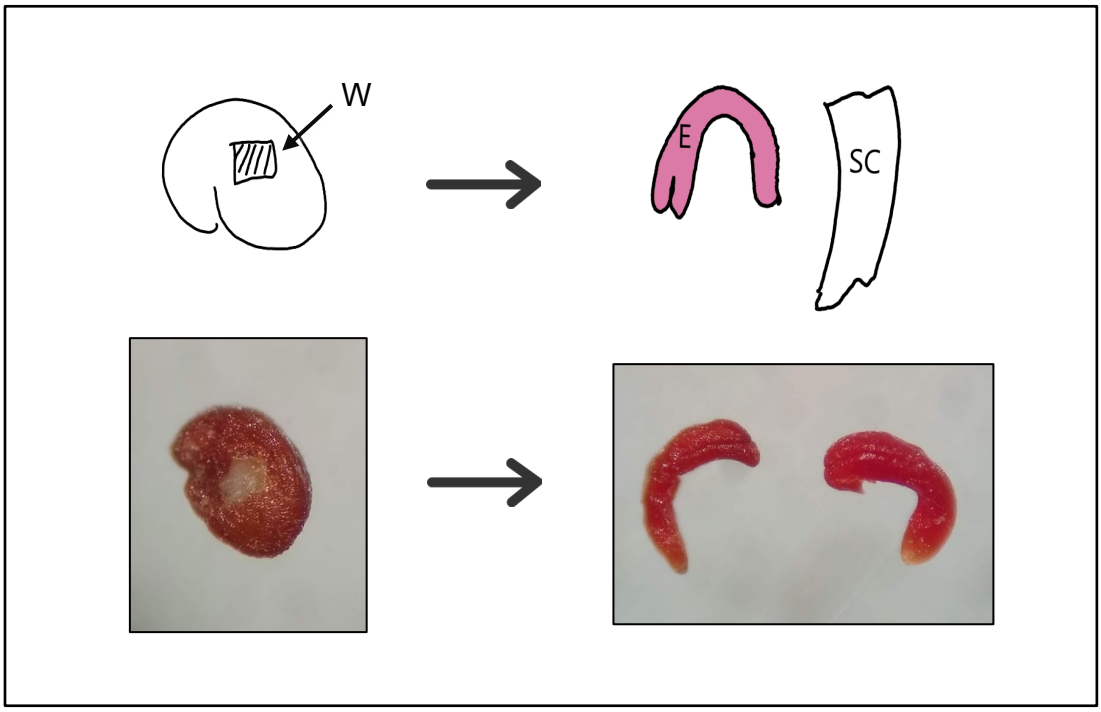
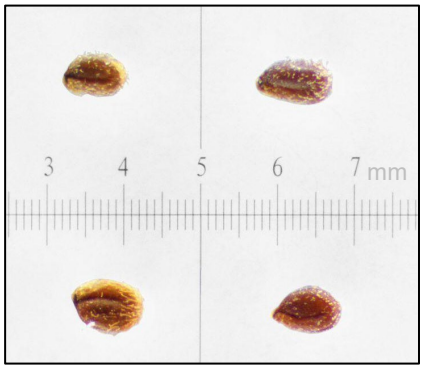


Figure 1. Images illustrating seed preparation and evaluation.

Amaranthaceae

Salicornia rubra
Pickleweed

1% TZ; 48 hours



Preparation: Imbibe for 24 hours before slicing through the seed coat between the embryo's (E) radicle (rad) and cotyledons (cotyl; Figure 1). Imbibition makes the seed softer, improving the precision of the cut. Removing seeds from utricles before making preparatory cuts and placing them in TZ is suggested.

Evaluation: Check to see if the full embryo is stained. Some green blotchiness is okay, but fully green embryos are non-viable (Figure 2; see the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, protocol).

Notes: 24 hours in 0.1% solution is not strong enough for a full pink stain.

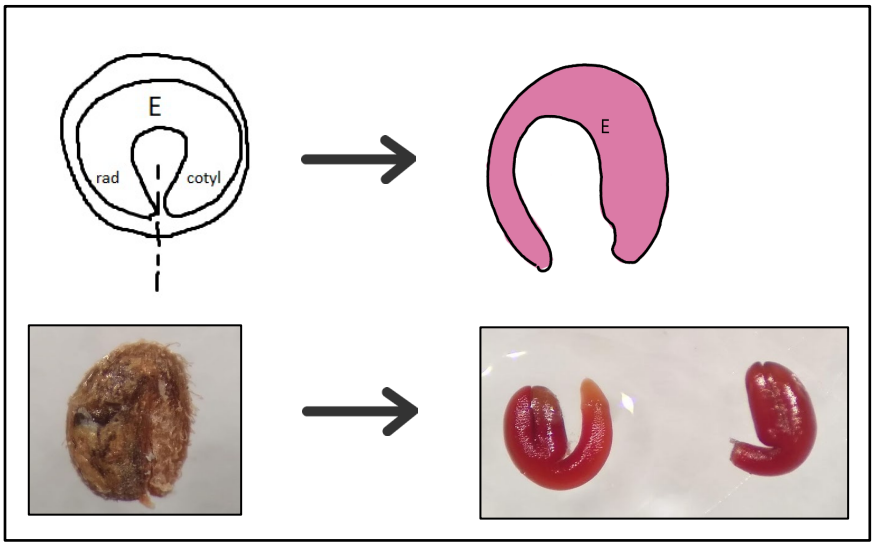


Figure 1. Images illustrating seed preparation and evaluation.

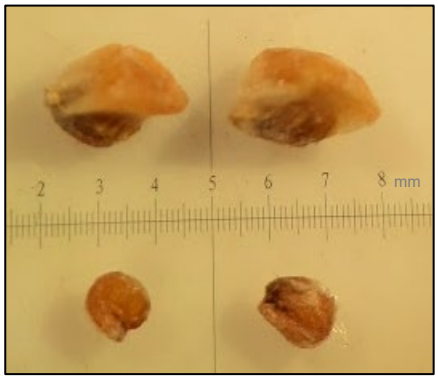
Figure 2. Two non-viable (green and turgid) seeds, left. Three viable seeds, right.

Amaranthaceae

Salicornia utahensis

Utah swampfire

1% TZ; 48 hours



Preparation: Imbibe for 24 hours before slicing through the seed coat between the embryo's (E) radicle (rad) and cotyledons (cotyl; Figure 1). Imbibition is necessary to remove any remaining chaff from the seed and makes the seed softer, improving the precision of the cut. Removing seeds from utricles before making preparatory cuts and placing them in TZ is suggested.

Evaluation: Check to see if the full embryo is fully stained (Figure 2). Some green blotchiness is okay, but fully green embryos are non-viable (Figure 3).

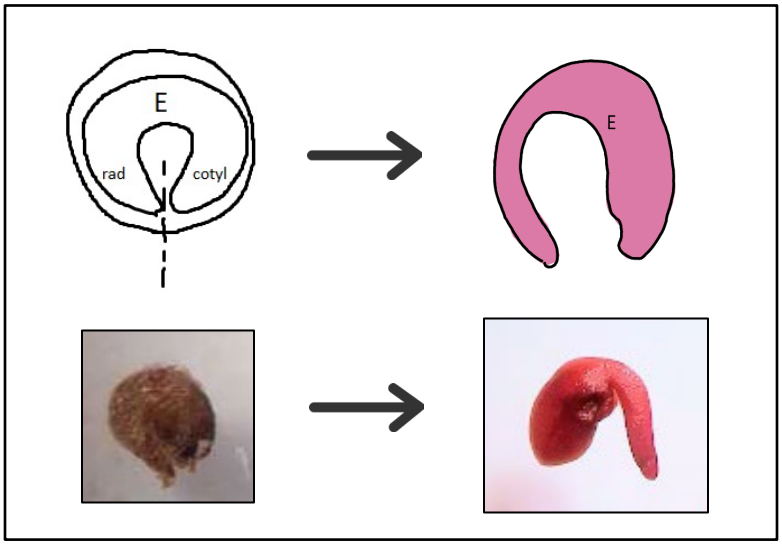


Figure 1. Images illustrating seed preparation and evaluation.

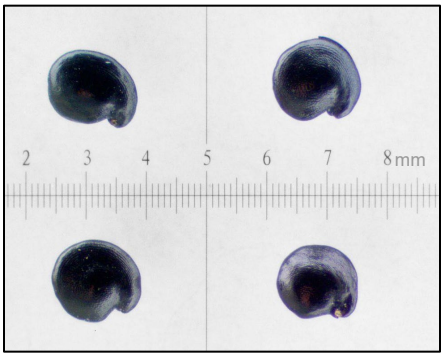
Figure 2. Three viable seeds that have very dark staining.

Figure 3. Four non-viable seeds with various conditions. One seed unstained and green (bottom right), another unstained and flaccid (top), and remaining two seeds contained no embryo (bottom left).

Amaranthaceae

Suaeda calceoliformis
Pursh seepweed

1% TZ; 48 hours



Preparation: Create a small slice in the **seed coat** (SC) where the **radicle** end of the **embryo** (E) meets the **cotyledon** end (Figure 1).

Evaluation: Use forceps and an exacto blade to chip away the seed coat or pull the embryo from the seed coat to get a good view of the embryo. The full embryo should be pink (Figure 2). Ignore any **artifact damage**.

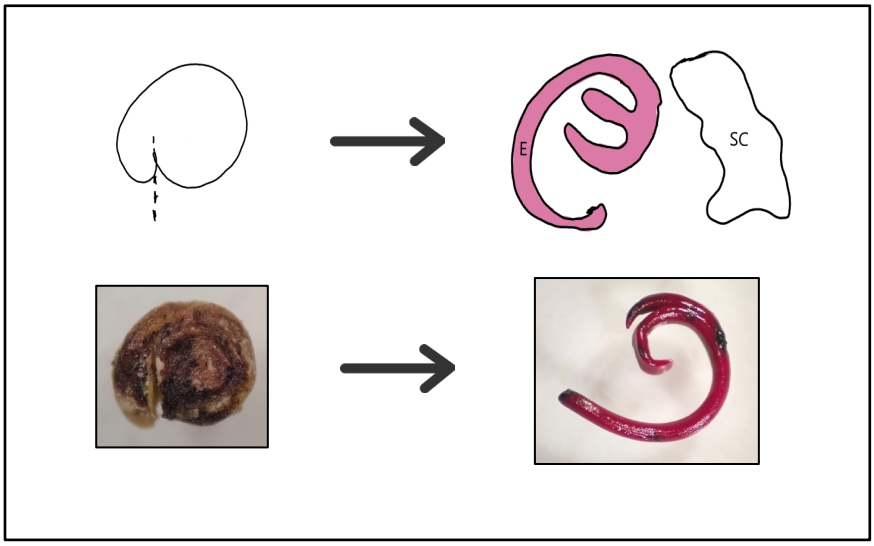


Figure 1. Images illustrating **seed** preparation and evaluation.

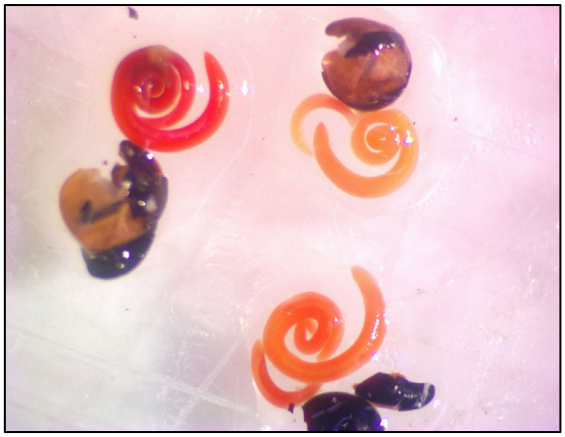


Figure 2. Three **viable** embryos ranging from light to dark pink.

Apocynaceae

Asclepias incarnata
Swamp milkweed

1% TZ; 48 hours

Preparation: Cut through the winged **seed coat** and a small part of the **cotyledon** side of the **embryo** (E). We find this diagonal cut to be easier than a **longitudinal** cut (Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.

Evaluation: Remove the embryo from the seed coat to evaluate viability. The **endosperm** and embryo will both stain pink, but only the stained embryo is needed for evaluation (Figures 2–3). Ignore any **artifact damage** at the cut location.

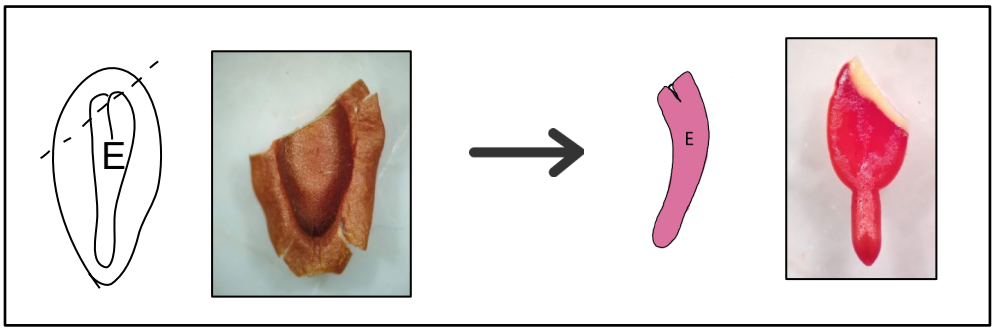


Figure 1. Images illustrating **seed** preparation and evaluation.

Figure 2. Six **viable** seeds (pink; left), one **non-viable** (green, right). Some viable seeds have acceptable artifact damage at the cut location.

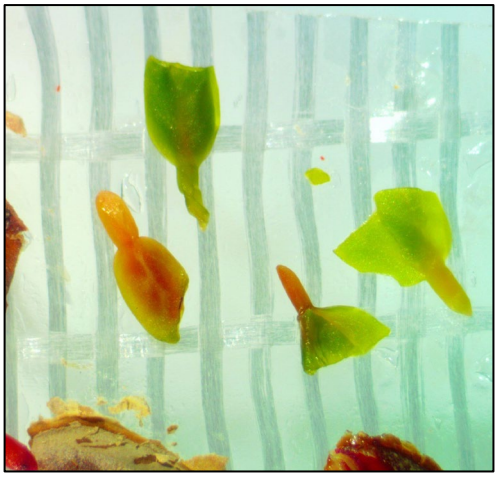


Figure 3. Four non-viable embryos (green or pink to blotchy green).

Apocynaceae

Asclepias speciosa
Showy milkweed

1% TZ; 48 hours

Preparation: Cut through the winged **seed coat** and a small part of the **cotyledon** side of the **embryo** (E). We find this diagonal cut to be easier than a **longitudinal** cut (Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.

Evaluation: Remove the embryo from the seed coat to evaluate viability. The **endosperm** and embryo will both stain pink, but only the stained embryo is needed for evaluation (Figures 2–4). Ignore any **artifact damage** at the cut location. Stained embryos may vary from dark pink to light pink when using 1% TZ and a 48-hour staining time, but a light pink stain is still considered to be **viable**.

Notes: 1% TZ for 24 hours led to confusion about diffusion of the chemical and color.

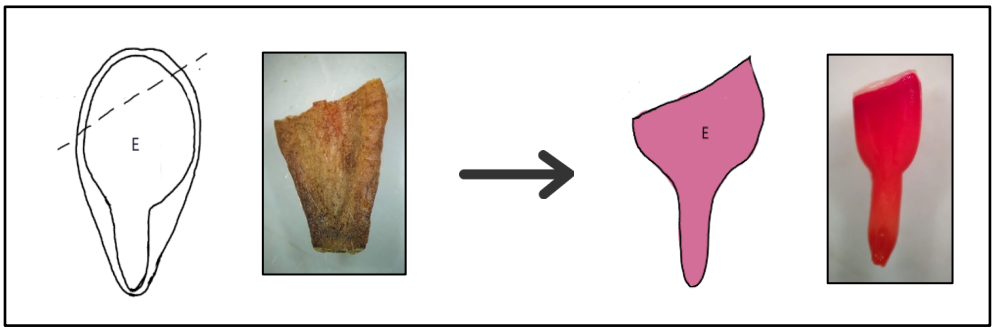


Figure 1. Images illustrating **seed** preparation and evaluation.

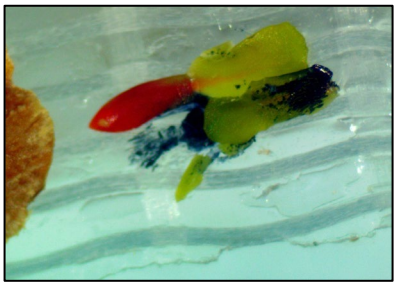


Figure 2. A **non-viable** seed where greater than half the cotyledons are not stained. Disregard black sharpie stain on green part.

Figure 3. A viable seed (top) next to a non-viable seed (bottom, **radicle** white).

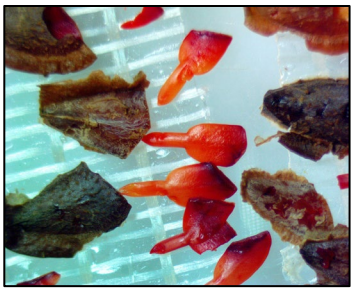


Figure 4. Four viable seeds (fully pink).

Asteraceae

Bidens spp.
Nodding beggartick

1% TZ; 48 hours

Preparation: Cut through the **pericarp**, membranous **seed coat**, and the very top of the **cotyledons** (Figure 1). Ignore small **artifact damage** which may be present as an unstained layer of cells at the site of cutting. Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining.

Evaluation: Bisect the **seed** and check both sides for a pink stain (Figures 2–4).

Notes: Preparing the seeds by scraping the pericarp to create a window led to a more subjective evaluation of the seeds due to a lighter pink stain. Using 0.1% TZ for 24 hours worked fine for some seed sources. However, different **seed lots** and sizes may need more time and a greater concentration, so we changed the protocol to 1% for 48 hours.

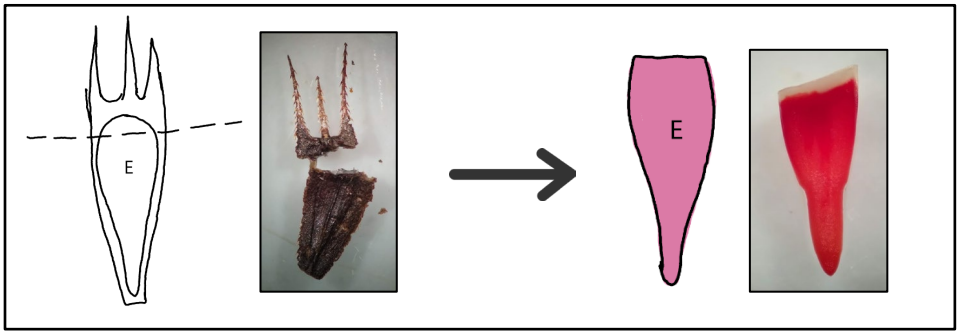


Figure 1. Images illustrating seed preparation and evaluation.

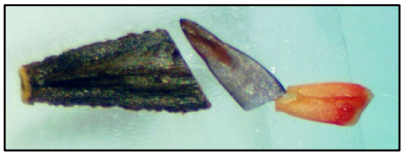


Figure 2. *Bidens cernua* **achene**. From left: pericarp, seed coat, embryo.

Figure 3. Three **non-viable** seeds, defined by a large portion of the cotyledon part of the seed unstained/white, or completely unstained.

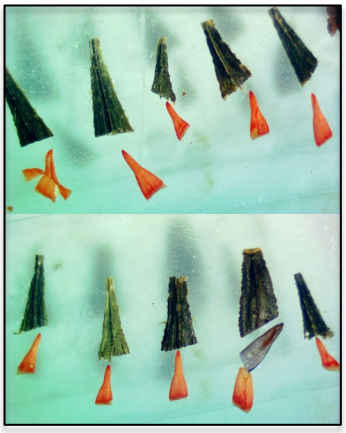


Figure 4. Ten **viable** seeds.

Asteraceae

Euthamia occidentalis

Western goldentop

1% TZ; 48 hours

Preparation: **Imbibe seeds** overnight. With a sharp blade, pierce the center of the **pericarp** into the **embryo** (E; Figure 1).

Evaluation: The embryo slides easily out of the pericarp. View the embryo and evaluate it for a full pink stain. Disregard any **artifact damage** at the pierce location.

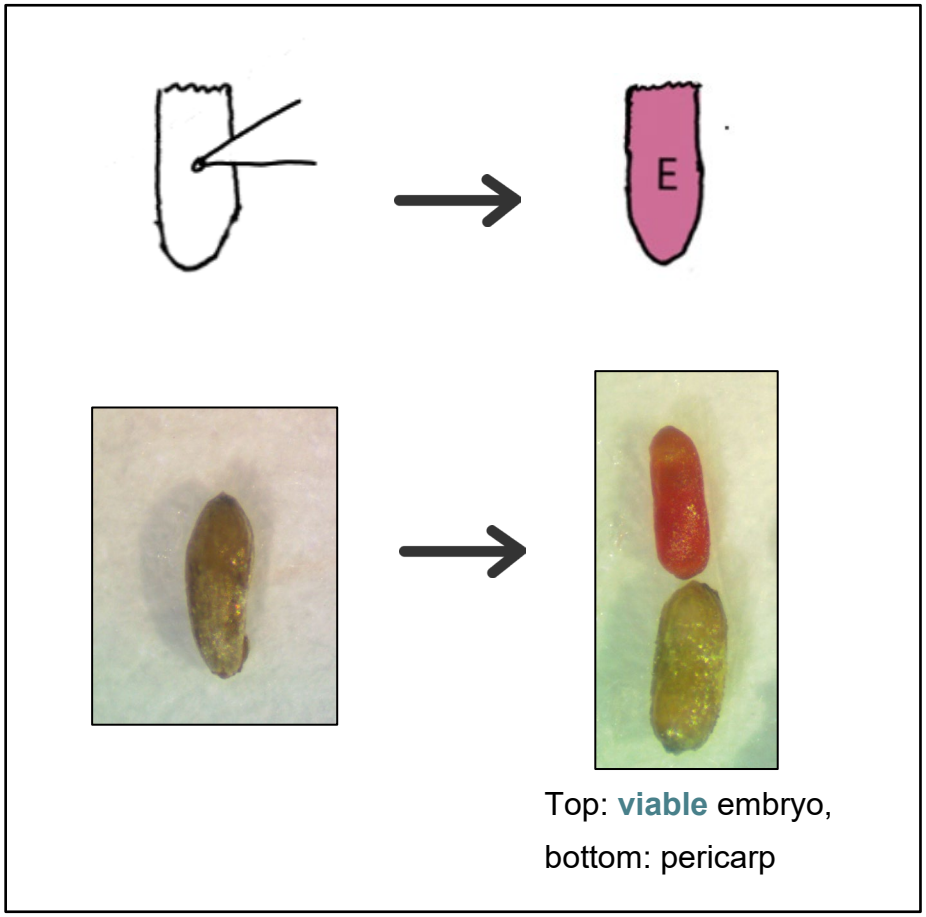


Figure 1. Images illustrating seed preparation and evaluation.

Asteraceae

Eutrochium maculatum
Spotted Joe Pye weed

1% TZ; 48 hours

Preparation: Cut the **seed** diagonally at the blunt end of the seed (**cotyledon** end). Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining (Figure 1).

Evaluation: The embryo slides easily out of the pericarp after soaking. View the full embryo and evaluate for pink stain (Figures 2–3).

Notes: Using a 0.1% TZ solution led to incomplete staining.

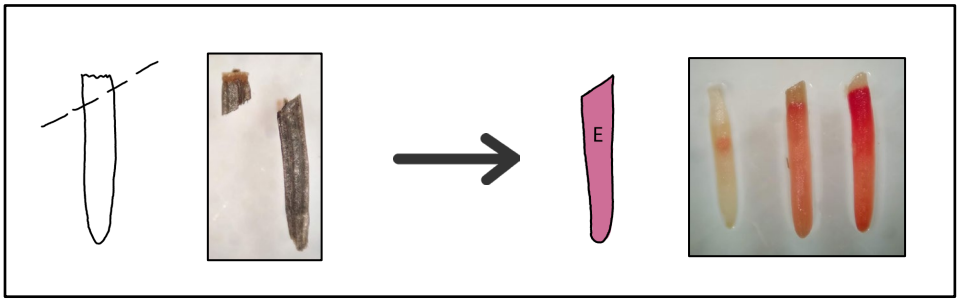


Figure 1. Images illustrating seed preparation and evaluation.

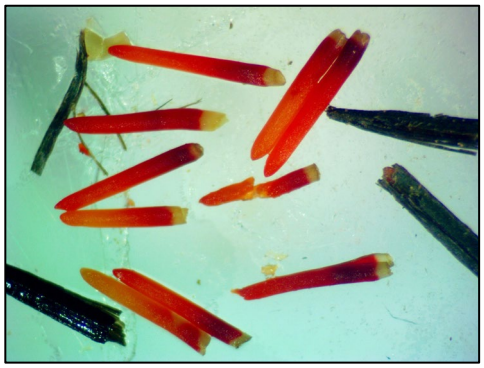


Figure 2. **Viable** embryos; disregard **artifact damage** which appears as an unstained sliver at the **cotyledon** end at the cut location.

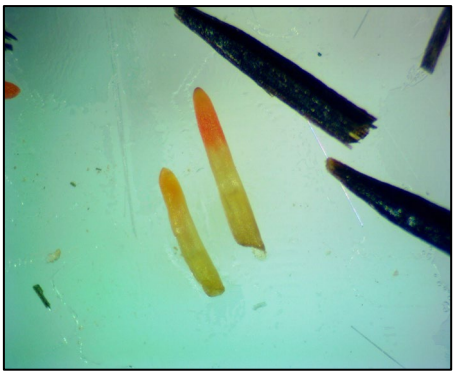


Figure 3. Two **non-viable** embryos.

Asteraceae

Grindelia squarrosa
Curlycup gumweed

1% TZ; 48 hours

Preparation: Cut the **seed** diagonally at the blunt end of the seed (**cotyledon** end). Discard the small portion that was cut off and keep the larger part of the **embryo** (E) for staining (Figure 1).

Evaluation: The embryo slides easily out of the **pericarp** after soaking. View the full embryo and evaluate for pink stain (Figures 2–3).

Notes: A straight, **lateral** cut (in contrast to the diagonal cut) led to embryos falling out of the pericarp during the TZ soak.

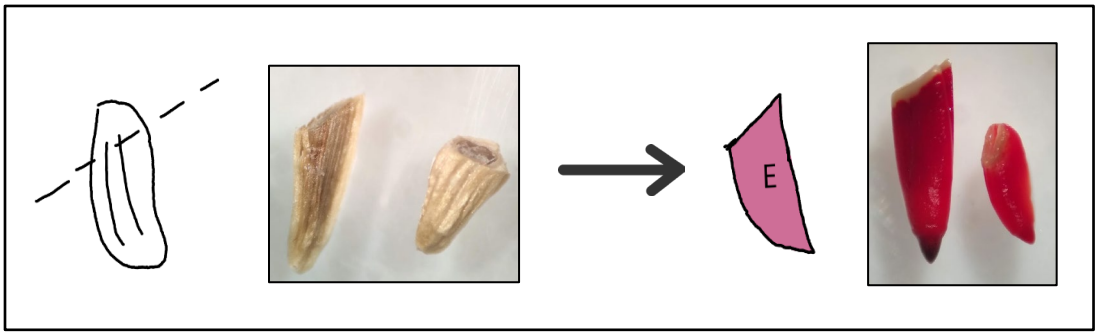


Figure 1. Images illustrating seed preparation and evaluation.

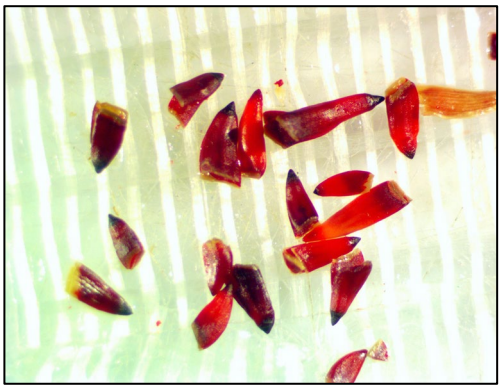


Figure 2. All embryos are **viable**. They are fully stained except for some acceptable **artifact damage** at the location of cutting.

Figure 3. These three seeds are **non-viable**. They are mostly white (left), mottled and bruised (middle), and white and brown at the **radicle** (right).

Asteraceae

Helianthus annuus
Common sunflower

1% TZ; 48 hours

Preparation: Slice through the **pericarp**, **seed coat**, and **embryo** (E) **laterally** or diagonally at the wider, **cotyledon** end (Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.

Evaluation: Using forceps, push the embryo out of the pericarp. **Viable** embryos will stain pink (Figures 2–3).

Notes: **Imbibition** is not very helpful.

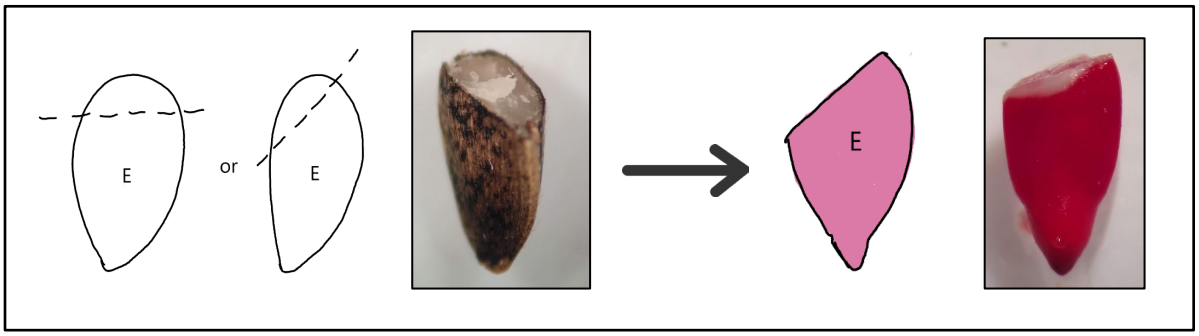


Figure 1. Images illustrating **seed** preparation and evaluation.

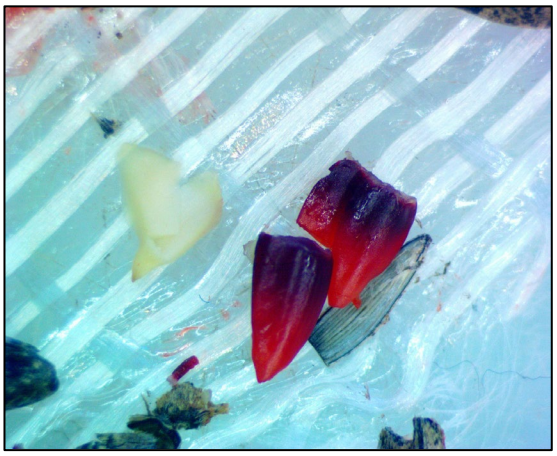


Figure 2. **Non-viable** seed at left (white); two viable seeds, right.

Figure 3. Many viable seeds.

Asteraceae

Helianthus nuttallii
Nuttall's sunflower

1% TZ; 48 hours

Preparation: Slice through the **pericarp**, **seed coat**, and **embryo** (E) **laterally** or diagonally at the wider, **cotyledon** end (Figure 1). Discard the small portion that was cut off and keep the larger part of the embryo for staining.

Evaluation: Using forceps, push the embryo out of the pericarp. **Viable** embryos will stain pink (Figure 2).

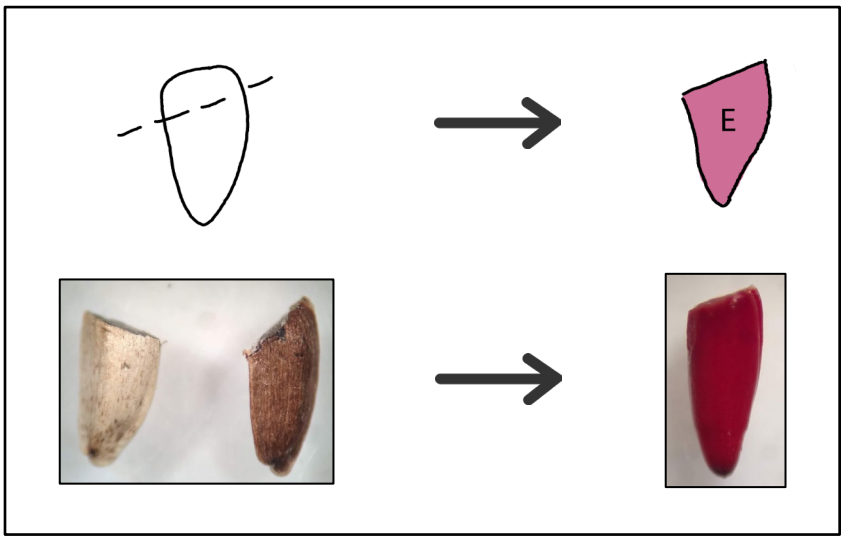


Figure 1. Images illustrating **seed** preparation and evaluation.

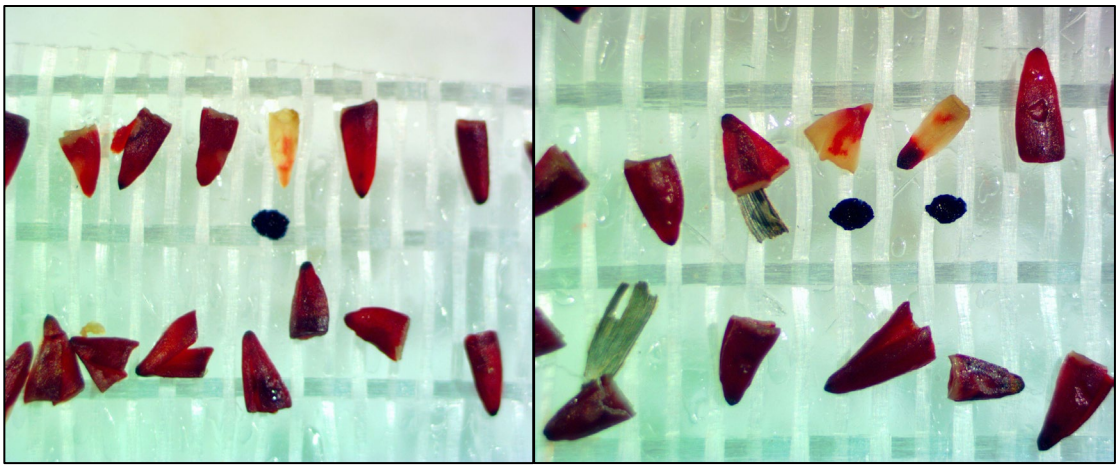


Figure 2. Black dots below embryos indicate **non-viable** seeds.

Asteraceae

Senecio hydrophilus
Water groundsel

1% TZ; 48 hours

Preparation: Slice through the **pericarp**, **seed coat**, and **embryo** (E) diagonally at the blunt, **cotyledon** end (Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.

Evaluation: Using forceps, push the embryo out of the pericarp. **Viable** embryos will stain pink (Figures 2–3).

Notes: Using a lower TZ concentration or a shorter staining time leads to diffusion issues, where the cotyledons are pink but the stain fades into a white **radicle**.

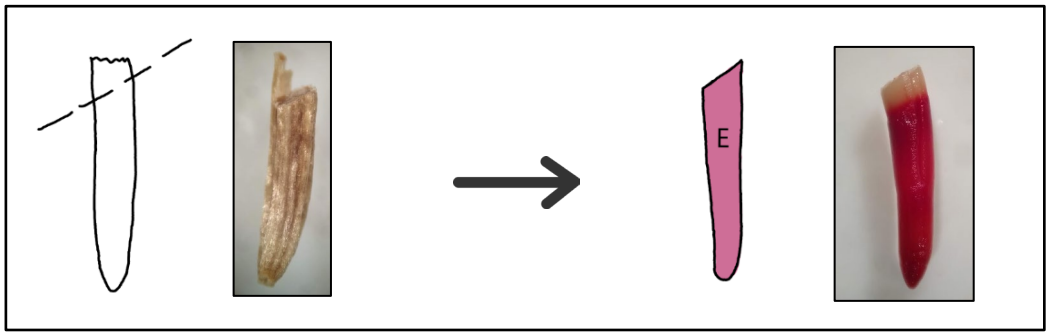


Figure 1. Images illustrating **seed** preparation and evaluation.

Figure 2. Six viable, fully stained seeds. Darker staining on one end is not an issue.

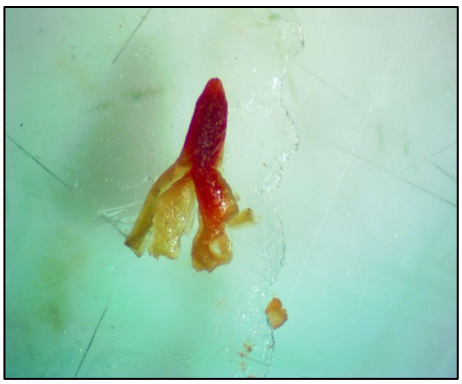
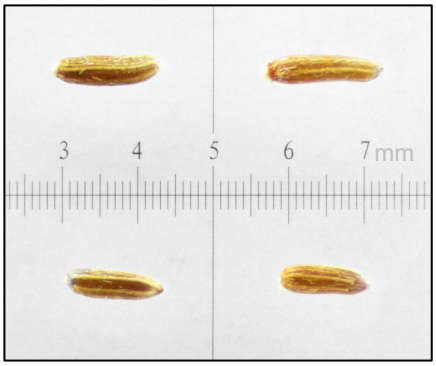


Figure 3. One **non-viable** seed.

Asteraceae

Solidago canadensis
Canada goldenrod

1% TZ; 48 hours



Preparation: Imbibe for 24 hours then pierce through the center of the pericarp and embryo (E) with a sharp blade (Figure 1). Use the impalement of the seed on the blade to transfer it to a Petri dish.

Evaluation: The embryo easily slides out of the pericarp after soaking. View the entire embryo to evaluate. Viable seeds are entirely pink (Figure 2). Disregard any artifact damage at the location where the embryo was pierced.

Notes: For this species, because the seed is oval shaped, it is hard to cut laterally across the cotyledon end.

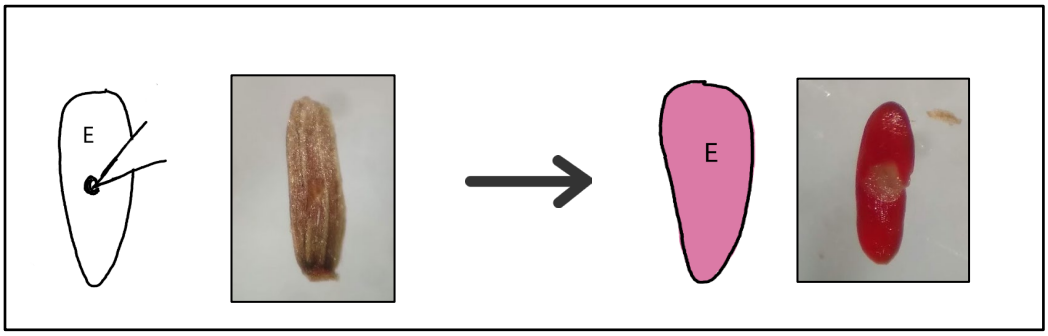


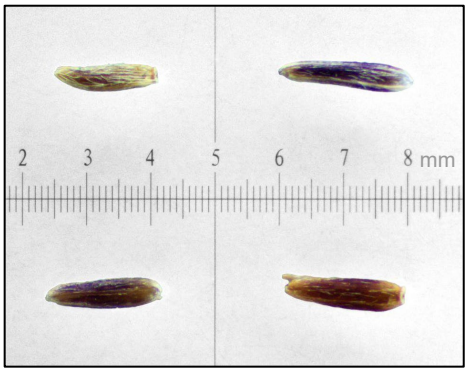
Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Four viable seeds on the left (pink) and two non-viable seeds, right (clear).

Asteraceae

Symphyotrichum ciliatum
Rayless alkali aster

1% TZ; 48 hours



Preparation: Slice through the **pericarp**, **seed coat**, and **embryo** (E) diagonally at the wider, **cotyledon** end (Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.

Evaluation: The embryo will easily slide out of the pericarp after soaking. View the entire embryo and evaluate it for a full pink stain.

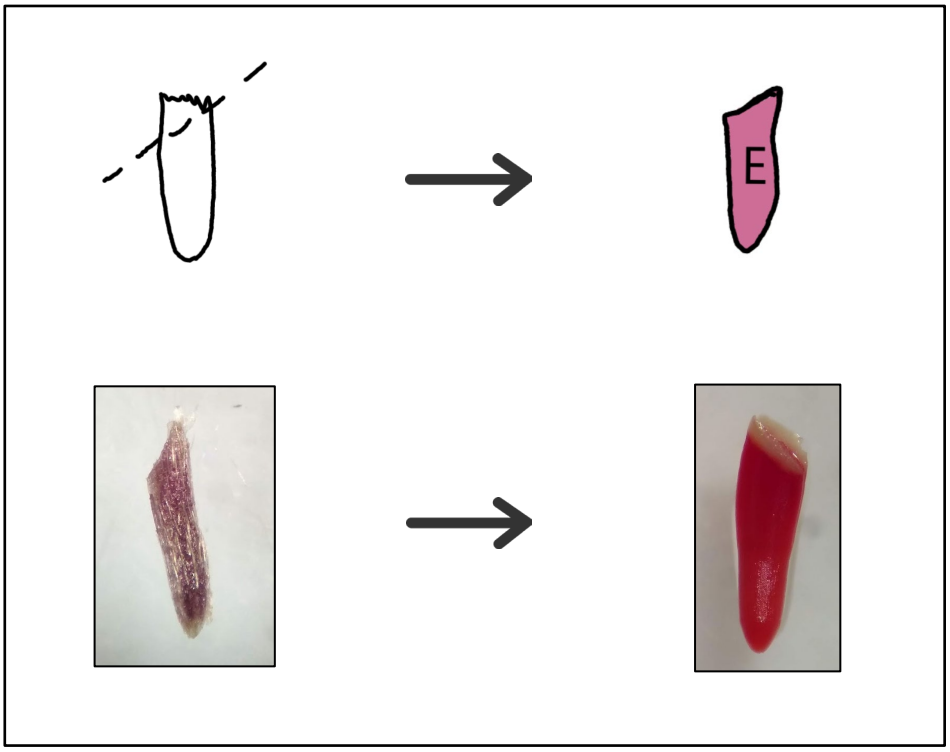


Figure 1. Images illustrating **seed** preparation and evaluation.

Capparaceae

Cleome serrulata
Rocky Mountain beeplant

1% TZ; 48 hours

Preparation: **Imbibition** of **seed** on a moist media before cutting makes it slightly easier to cut the **seed coat** (SC) but is not necessary. Prepare the seeds by scraping the seed coat with an exacto blade to create a "window" (W; Figure 1). A larger window leads to greater imbibition of the TZ solution, so make the initial cuts large and uniform. However, if too much of the seed coat is removed, the **embryo** (E) will fall out of the seed coat.

Evaluation: Cut in half upon **scoring**. White seed coats do not always mean **non-viable** embryos are inside. Non-viable seeds often have flaccid embryos that fail to totally fill the seed coat. Some **viable** embryos are pink in the middle and more orange on the ends (Figure 2). Non-viable embryos may appear white or yellow.

Notes: Using 0.1% TZ for 48 hours resulted in a lighter pink stain of viable seeds.

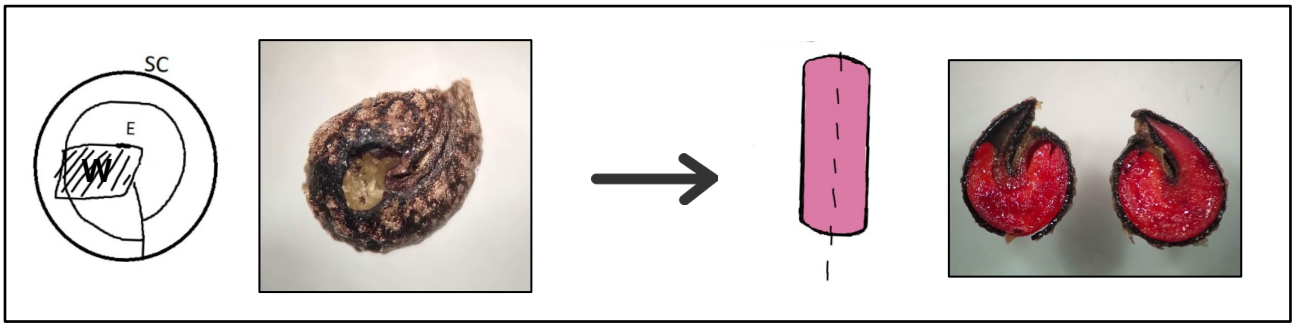


Figure 1. Images illustrating seed preparation and evaluation.

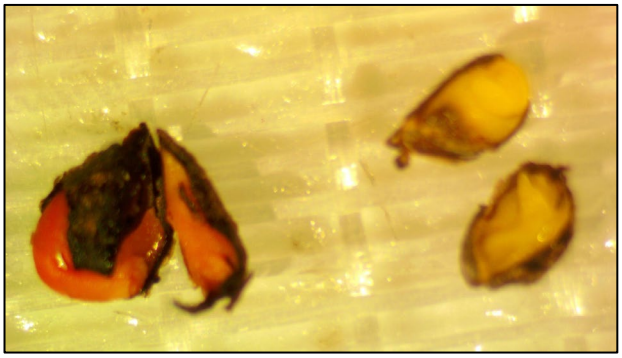
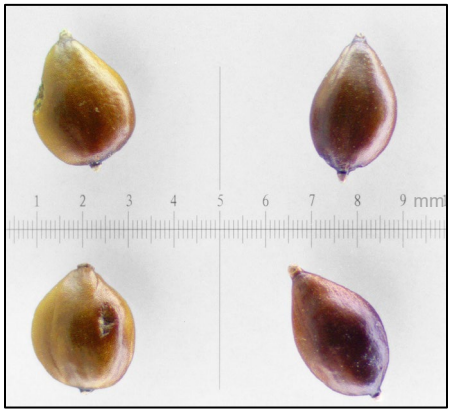


Figure 2. Viable (pink) left; non-viable, right (clear/yellow).

Cyperaceae

Bolboschoenus maritimus

Alkali bulrush



1% TZ; 48 hours

Preparation: **Imbibe** the **achenes** overnight. Slice diagonally through the thick **seed coat** and white, chalky **endosperm** near the widest part of the **seed**. Discard the small portion that was cut off and keep the large part that contains the **embryo** (E) for staining (Figure 1). The embryo is basal, located at the narrow end of the seed.

Evaluation: Bisect the achene and view both sides of the embryo. A full pink stain (including light pink) is **viable**.

Notes: Some **seed lots** may take longer to stain if they have deeper physiological dormancy. See Cyperaceae protocol in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, for more information.

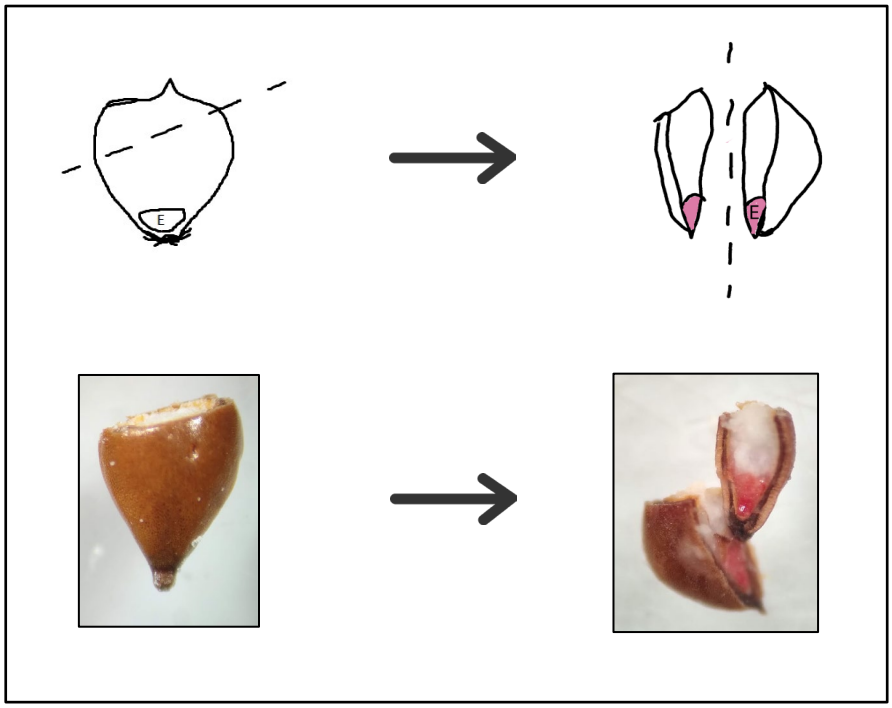


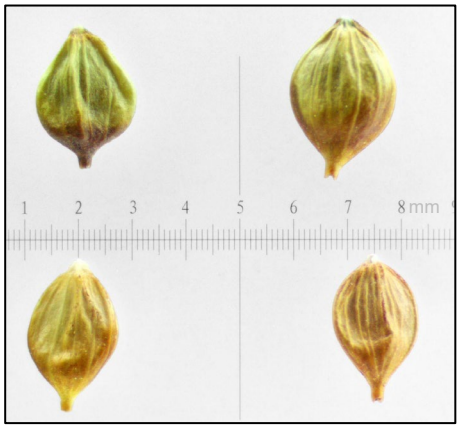
Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Carex nebrascensis

Nebraska sedge

1% TZ; 48 hours



Preparation: **Imbibe** the **seed lot** overnight to soften the **seed coat**. With the **perigynium** on (papery coating around the **achene**; unique to *Carex*) it's hard to tell where the achene actually is, so remove the perigynium before slicing through the seed coat and **endosperm** on the side of the achene that is far from the **embryo** (E). Imbibition followed by rubbing the **seeds** on paper towels helps to remove the perigynium. Discard the small portion that was cut off and keep the large part that contains the embryo for staining (Figure 1).

Evaluation: Bisect the achene and view both sides of the embryo. A full pink stain (including light pink) is **viable** (Figure 2).

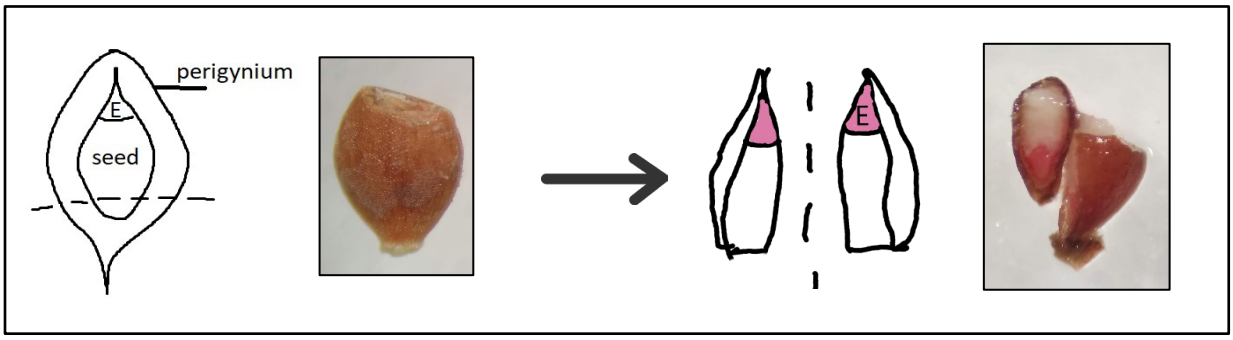


Figure 1. Images illustrating seed preparation and evaluation.

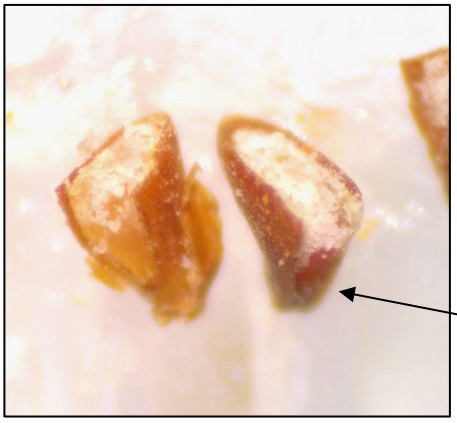
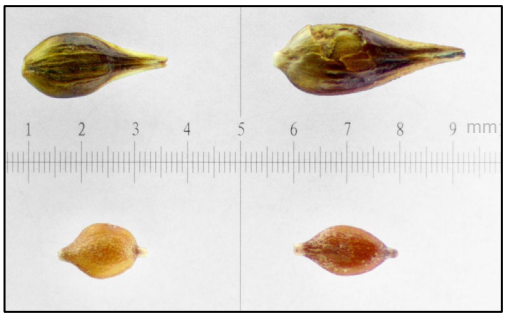


Figure 2. Left: **non-viable** seed (clear basal embryo); right, viable seed (fully pink basal embryo).

Cyperaceae

Carex praegracilis
Clustered field sedge

1% TZ; 48 hours



Preparation: **Imbibe** the **seed lot** overnight to soften the **seed coat**. With the **perigynium** on (papery coating around the **achene**; unique to *Carex*) it's hard to tell where the achene actually is, so remove the perigynium before slicing through the seed coat and **endosperm** on the side of the achene that is far from the **embryo** (E). Imbibition followed by rubbing the **seeds** on paper towels helps to remove the perigynium. Discard the small portion that was cut off and keep the large part that contains the embryo for staining (Figure 1).

Evaluation: Bisect the **seed** and view both sides of the embryo. A full pink stain (including light pink) is **viable**.

Notes: Using 0.1% TZ for 24 hours resulted in a very light pink embryo stain.

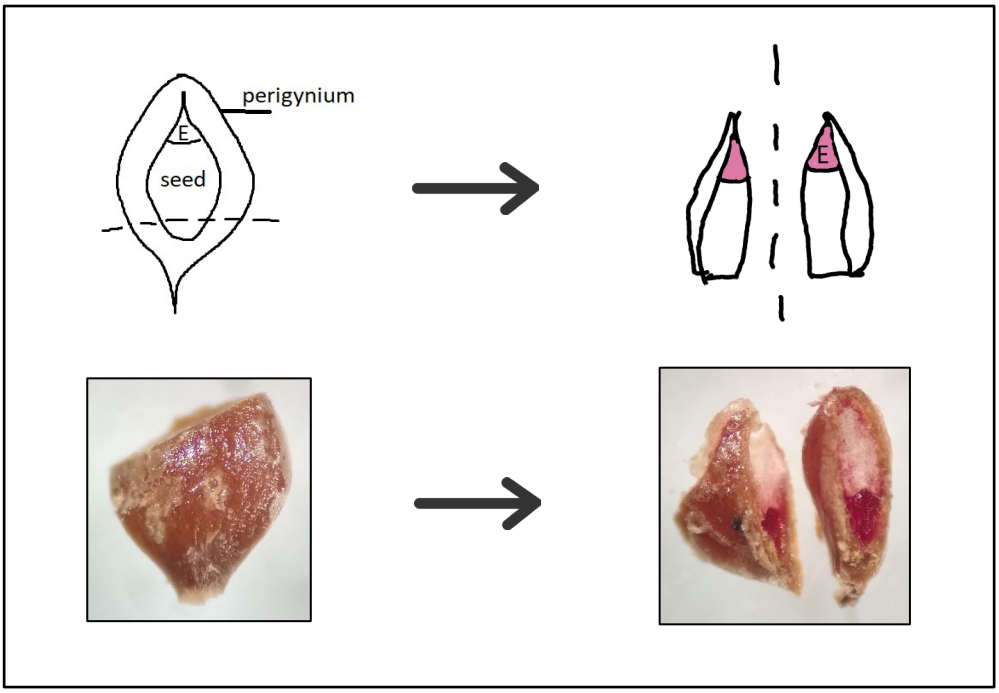
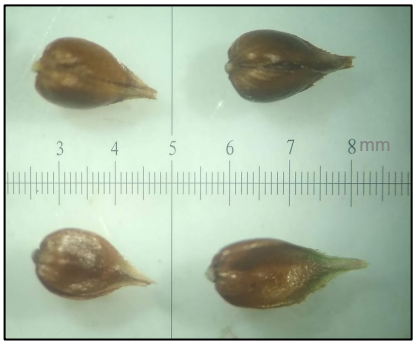


Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Carex simulata
Analogue sedge

1% TZ; 48 hours



Preparation: Imbibe the seed lot overnight to soften the seed coat. With the perigynium on (papery coating around the achene; unique to *Carex*) it's hard to tell where the achene actually is, so remove the perigynium before slicing through the seed coat and endosperm on the side of the achene that is far from the embryo (E). Imbibition followed by rubbing the seeds on paper towels helps to remove the perigynium. Discard the small portion that was cut off and keep the larger part that contains the embryo for staining (Figure 1).

Evaluation: Bisect the seed and view both sides of the embryo. A full pink stain (including light pink) is viable.

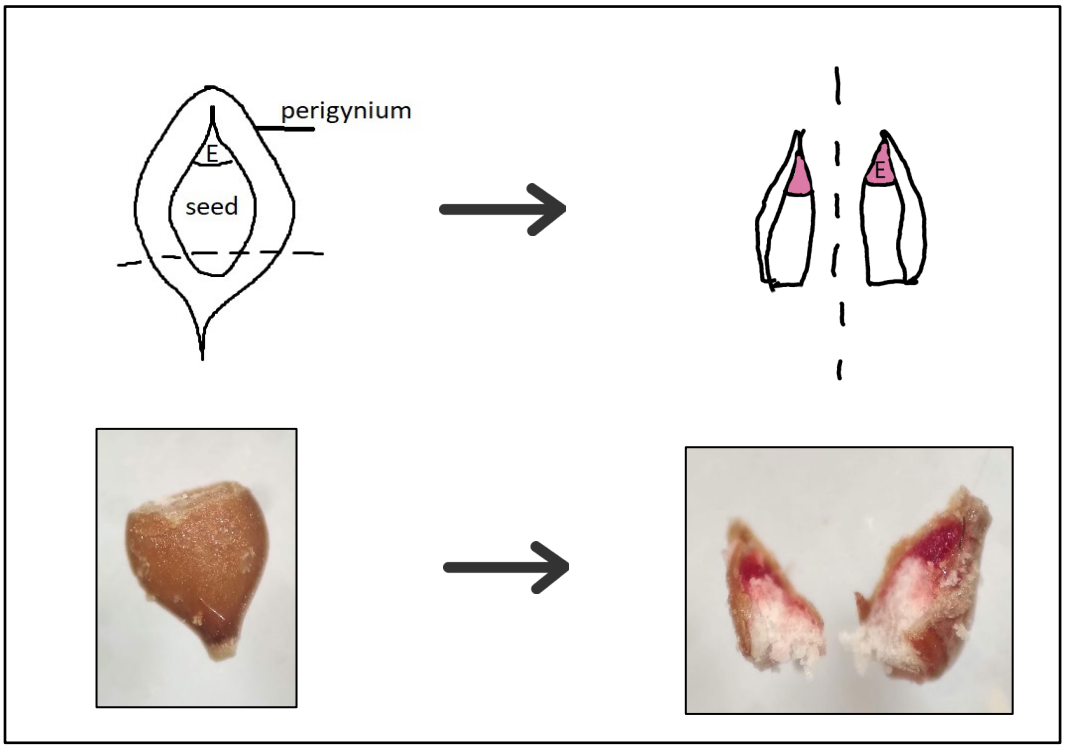
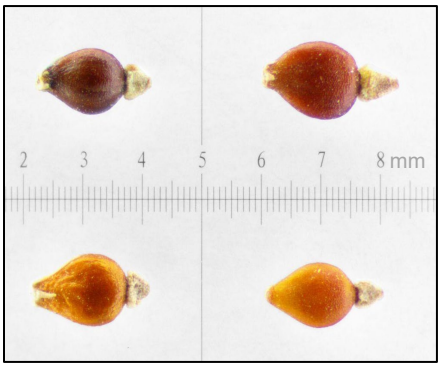


Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Eleocharis palustris
Common spikerush

1% TZ; 48 hours



Preparation: Slice diagonally through the **seed coat** and white, chalky **endosperm** near the widest part of the **achene**. The **embryo** (E) is basal, located at the narrow end of the **seed**. The **tubercle**-side of the achene (the right side of the achenes in the photo to the right) will be discarded during preparation, keeping only the larger part that contains the embryo for staining (Figure 1).

Evaluation: Bisect the seed and view both sides of the embryo. A full pink stain (including light pink) is **viable**.

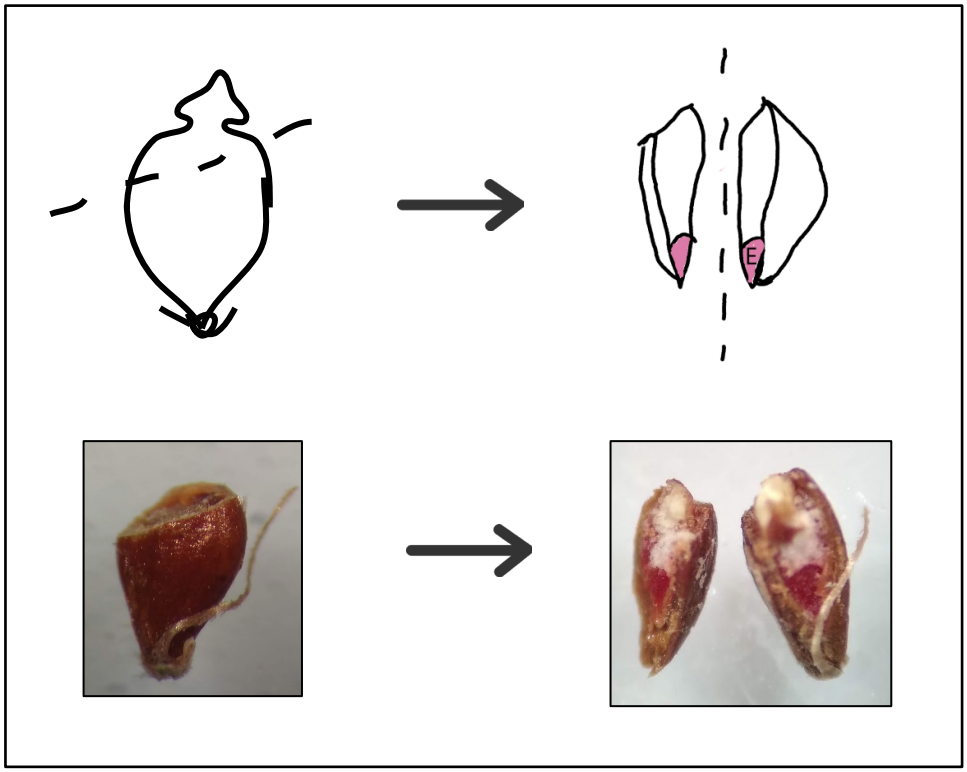
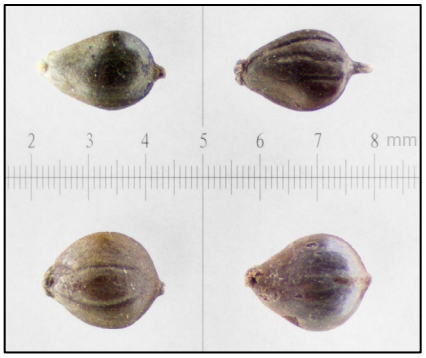


Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Schoenoplectus acutus
Hardstem bulrush

1% TZ; 48 hours



Preparation: Imbibe the achenes overnight. Place achenes flat side down on the tape and slice diagonally through the thick seed coat and white, chalky endosperm near the widest part of the seed. Discard the small portion that was cut off and keep the large part that contains the embryo (E) for staining (Figure 1). The embryo is basal, located at the narrow end of the seed.

Evaluation: Bisect the achene and view both sides of the embryo. A full pink stain (including light pink) is viable.

Notes: Some seed lots may take longer to stain if they have deeper physiological dormancy. See Cyperaceae protocol in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, for more information.

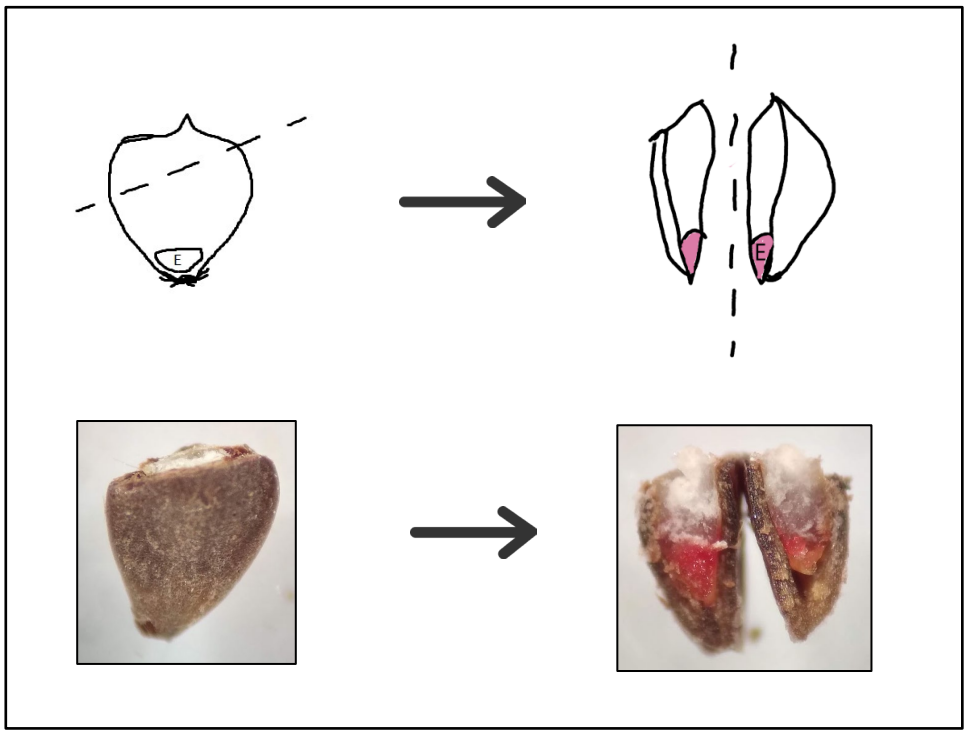


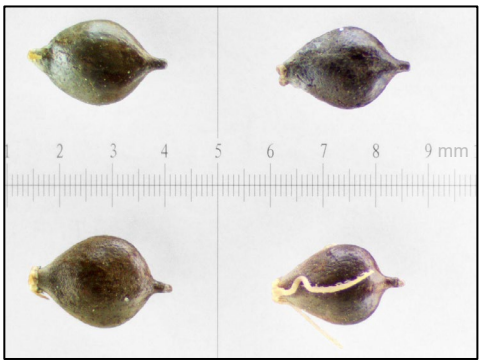
Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Schoenoplectus americanus

Chairmaker's bulrush

1% TZ; 48 hours



Preparation: Imbibe the achenes overnight. Slice diagonally through the thick seed coat and white, chalky endosperm near the widest part of the seed. Discard the small portion that was cut off and keep the large part that contains the embryo (E) for staining (Figure 1). The embryo is basal, located at the narrow end of the seed.

Evaluation: Bisect the achene and view both sides of the embryo. A full pink stain (including light pink) is viable.

Notes: Some seed lots may take longer to stain if they have deeper physiological dormancy. See Cyperaceae protocol in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, for more information.

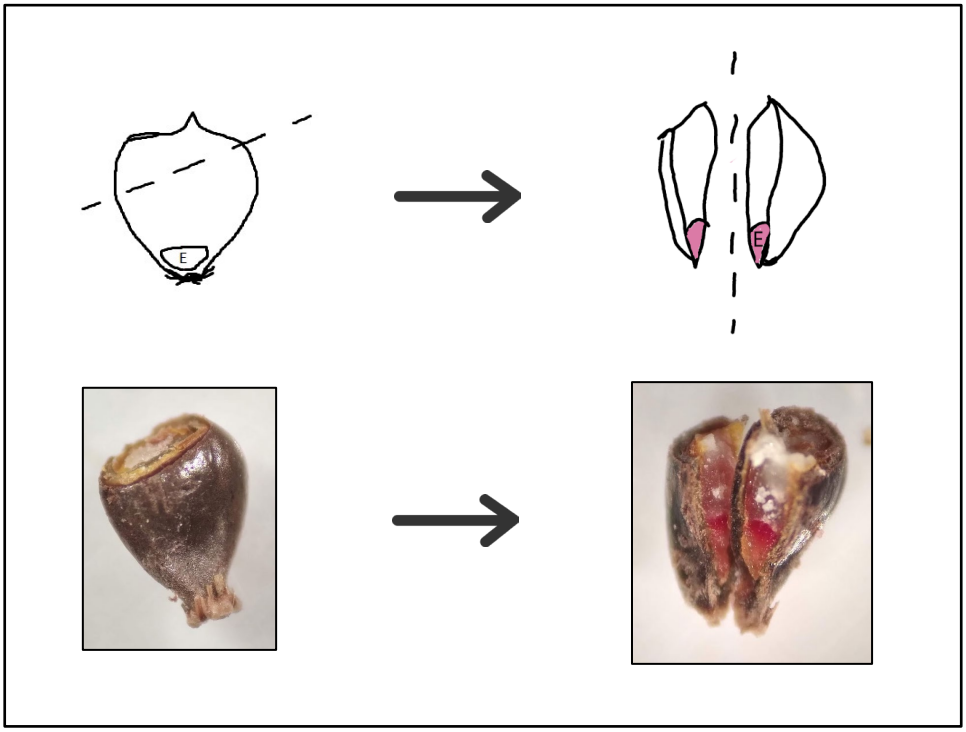
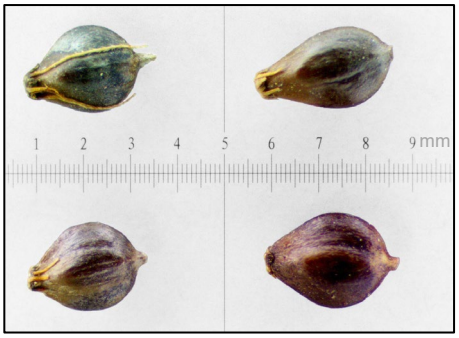


Figure 1. Images illustrating seed preparation and evaluation.

Cyperaceae

Schoenoplectus pungens
Common threesquare bulrush

1% TZ; 48 hours



Preparation: Imbibe the achenes overnight. Slice diagonally through the thick seed coat and white, chalky endosperm near the widest part of the seed. Discard the small portion that was cut off and keep the large part that contains the embryo (E) for staining (Figure 1). The embryo is basal, located at the narrow end of the seed.

Evaluation: Bisect the achene and view both sides of the embryo. A full pink stain (including light pink) is viable (Figures 2–4).

Notes: Some seed lots may take longer to stain if they have deeper physiological dormancy. See Cyperaceae protocol in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, for more information.

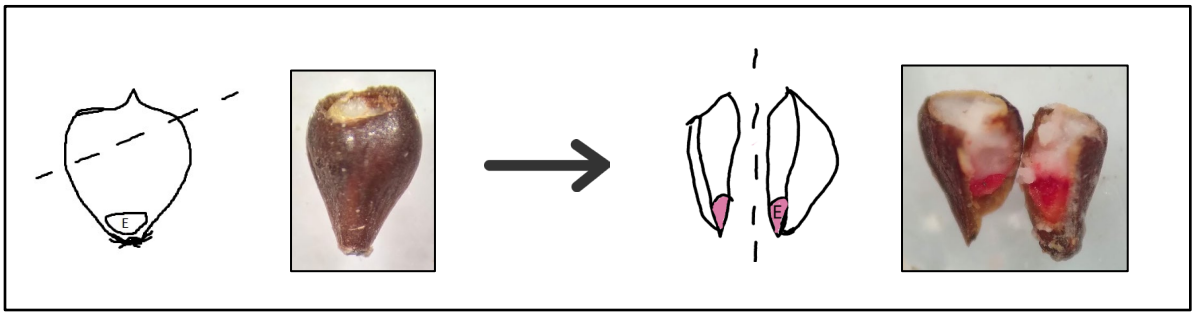


Figure 1. Images illustrating seed preparation and evaluation.

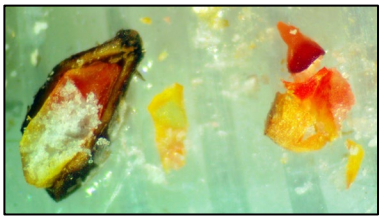


Figure 2. Two viable seeds.

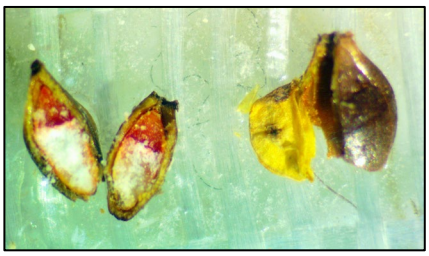
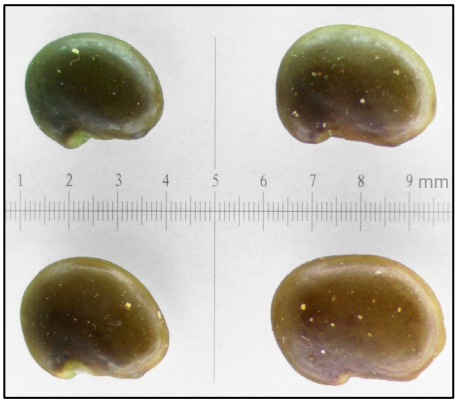


Figure 3. Viable embryo, left; non-viable embryo, right.

Figure 4. Viable seed, left. The embryo has been removed from the seed coat. Right, non-viable seed (no fill and no stained embryo).

Fabaceae

Glycyrrhiza lepidota
Wild licorice



1% TZ; 48 hours

Preparation: **Seed coat** is hard enough to prevent **imbibition** of TZ solution or water, so scarification is needed before imbibing with water. With an exacto blade, saw through the tough seed coat on the round, curved end of the **seed** (away from the **radicle**) and be sure not to scratch the **embryo** (E) itself (Figure 1). Overnight, imbibe seeds on a moist paper towel and then soak the seeds in TZ for 48 hours.

Evaluation: To evaluate, remove the seed coat and look at the whole embryo. **Viable** seeds may have a radicle that stains darker pink than the rest of the embryo.

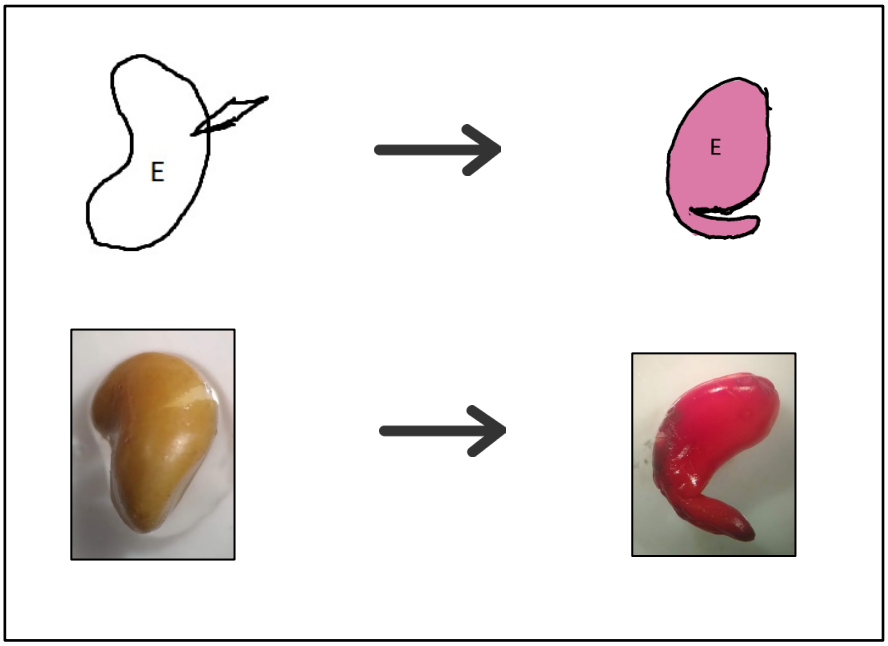
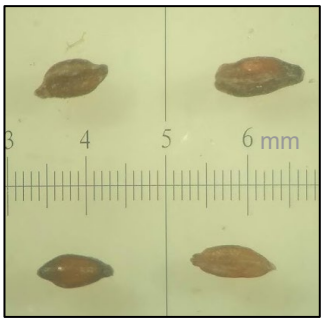


Figure 1. Images illustrating seed preparation and evaluation.

Frankeniaceae

Frankenia salina
Alkali seaheath

1% TZ; 48 hours



Preparation: **Imbibe seeds** overnight. Pierce the center of the seed with a sharp exacto blade (Figure 1).

Evaluation: Bisect the seed to evaluate a fully stained **embryo** (E, Figure 2). **Viable** seeds will be entirely stained pink.

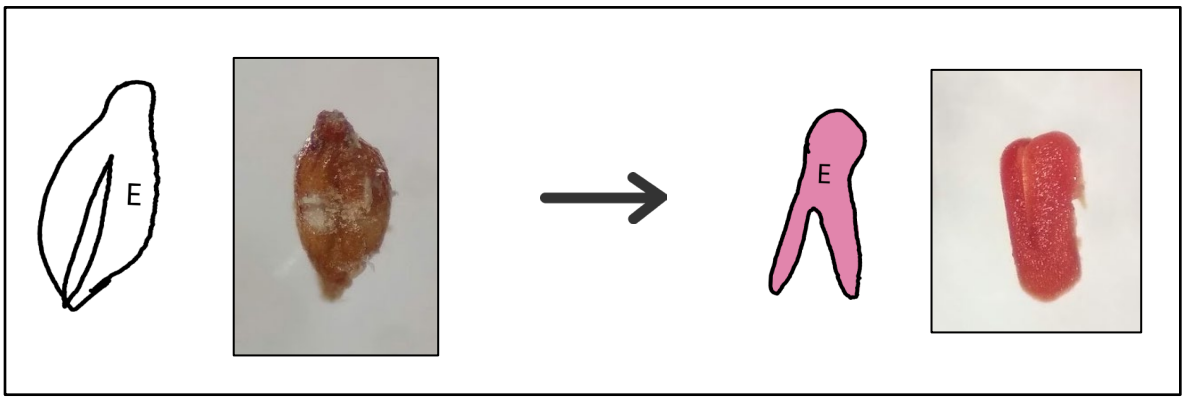


Figure 1. Images illustrating seed preparation and evaluation.

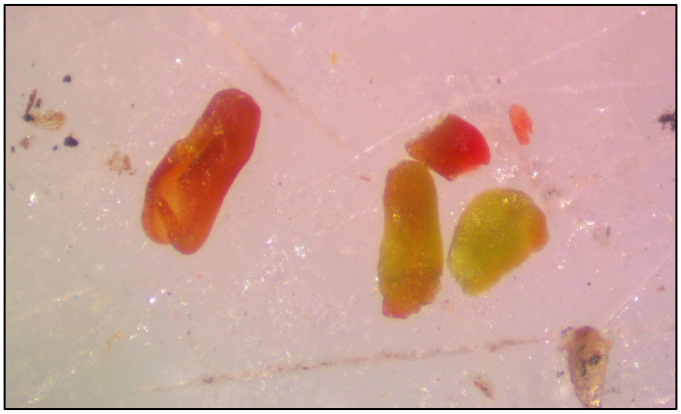
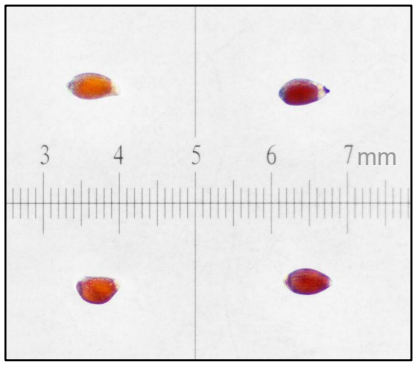


Figure 2. One viable seed (left) and broken-up, **non-viable** seed (right).

Juncaceae

Juncus arcticus
Mountain rush



1% TZ; 48 hours

Preparation: **Imbibe seeds** on a moist medium for 24 hours. Stick seeds to a small piece of thin, clear, double-sided tape (Figure 5). Use a sharp exacto blade to pierce the center of the seed through the **seed coat** and **endosperm** (Figures 1–2). The tape that the seeds were prepared on can be transferred to the TZ solution.

Evaluation: Remove tape from TZ solution and place it under a microscope. Be sure to find and evaluate any seeds that are unstuck from the tape and floating in the TZ solution. Bisect seeds and evaluate the basal **embryo** (E) for pink stain (Figures 3–4).

Notes: Some seeds may fall off the tape when soaking in the TZ solution, but most seeds stay stuck to the tape if agitation is minimal.

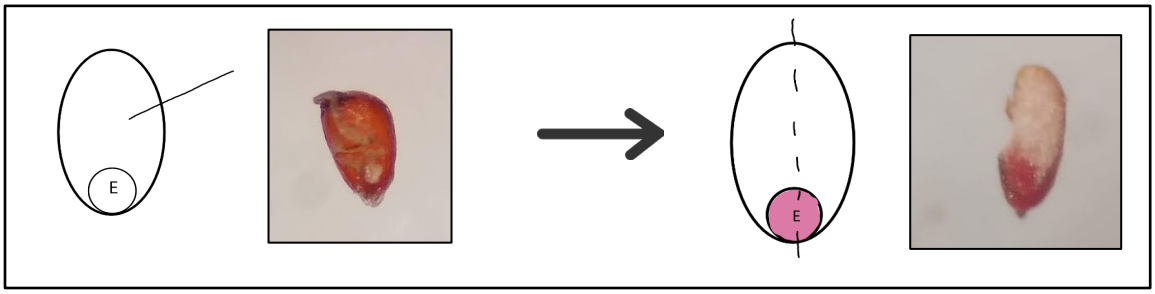


Figure 1. Images illustrating seed preparation and evaluation.

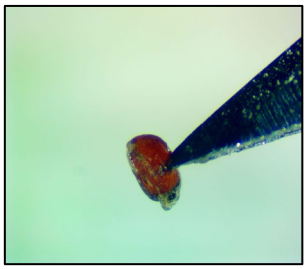


Figure 2. Imbibing the seed first allows for piercing through the center with a sharp exacto blade.

Figure 3. Three **non-viable** seeds (left), three **viable** seeds (right).

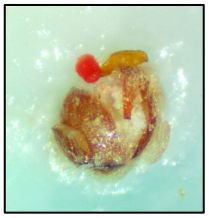


Figure 4. Viable seed with pink embryo.

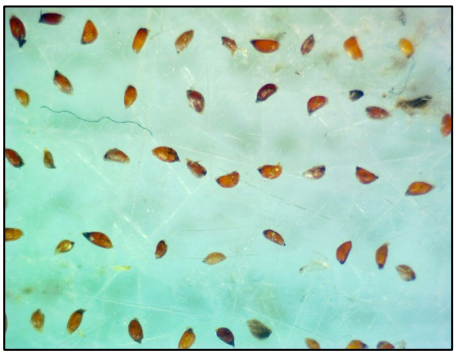
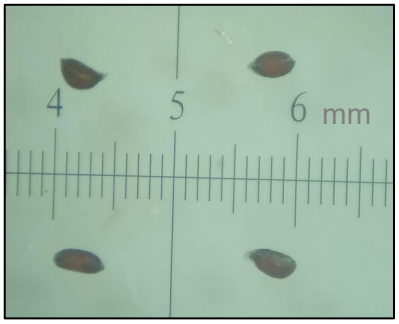


Figure 5. Organizing seeds in rows of ten on thin double-sided tape facilitates piercing and evaluating for this small-seeded species.

Juncaceae

Juncus gerardii
Saltmeadow rush



1% TZ; 48 hours

Preparation: **Imbibe seeds** on a moist medium for 24 hours. Stick seeds to a small piece of thin, clear, double-sided tape. Use a sharp exacto blade to pierce the center of the seed through the **seed coat** and **endosperm** (Figure 1). The tape that the seeds were prepared on can be transferred to the TZ solution.

Evaluation: Remove the tape from the TZ solution and place it under a microscope. Be sure to find and evaluate any seeds that are unstuck from the tape and floating in the TZ solution. Bisect seeds and evaluate the basal **embryo** (E) for pink stain (Figure 2).

Notes: Some seeds may fall off the tape when soaking in the TZ solution, but most seeds stay stuck to the tape if agitation is minimal.

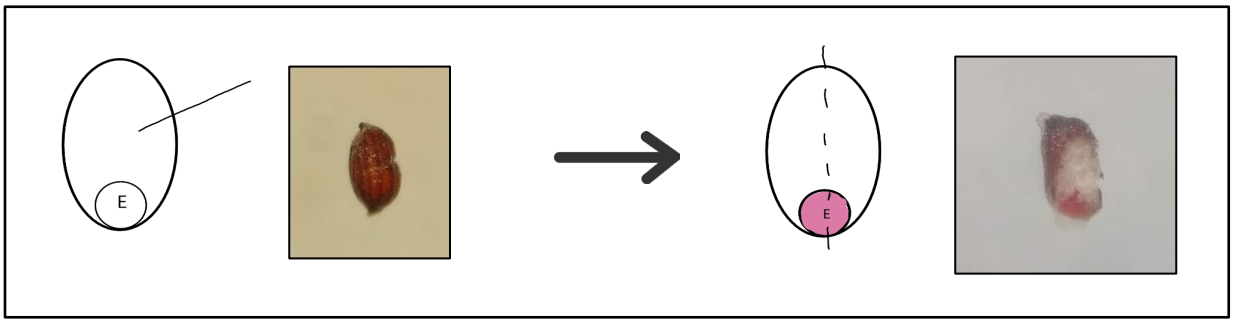


Figure 1. Images illustrating seed preparation and evaluation.

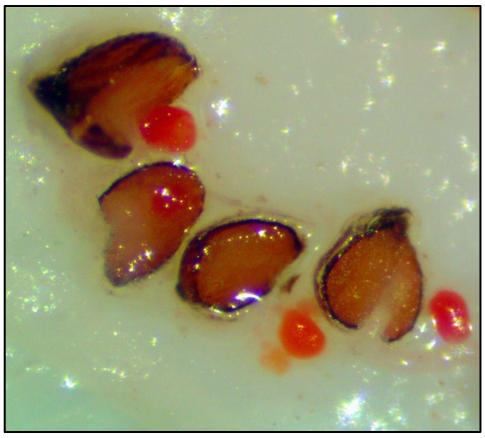
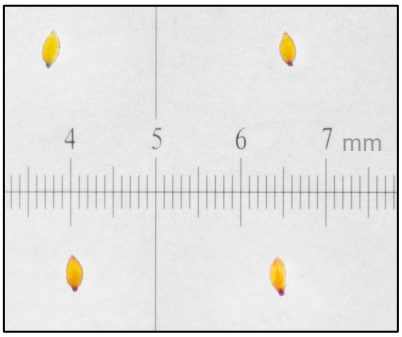


Figure 2. Four **viable** seeds.

Juncaceae

Juncus torreyi
Torrey's rush



1% TZ; 48 hours

Preparation: Imbibe seeds on a moist medium for 24 hours. Stick seeds to a small piece of thin, clear, double-sided tape (Figure 2). Use a sharp exacto blade to pierce the center of the seed through the seed coat and endosperm (Figure 1). The tape that the seeds were prepared on can be transferred to the TZ solution.

Evaluation: Remove the tape from the TZ solution and place it under a microscope. Be sure to find and evaluate any seeds that are unstuck from the tape and floating in the TZ solution. Bisect seeds and evaluate the basal embryo (E) for pink stain (Figure 3).

Notes: Some seeds may fall off the tape when soaking in the TZ solution, but most seeds stay stuck to the tape if agitation is minimal. Exposure at 0.1% for 24 hours yielded light pink seeds so a higher concentration is better. Post TZ soaking, embryos will glow pink through the seed coat, but should still be bisected and the embryo should be further evaluated to confirm.

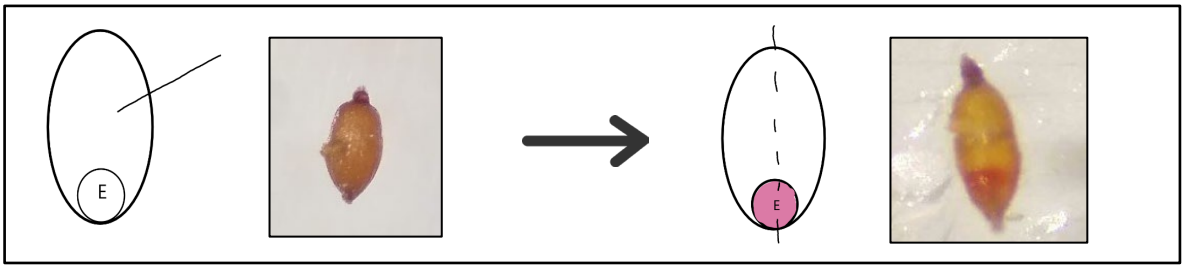


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Use thin double-sided tape to organize seeds and facilitate preparation.

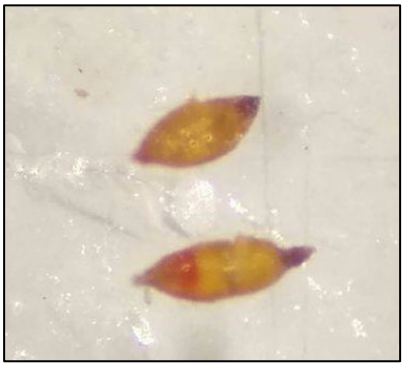
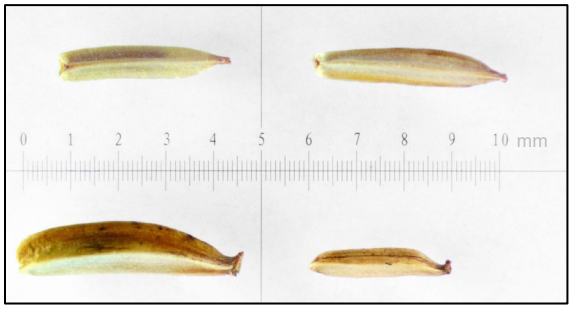


Figure 3. Non-viable (upper; no parts glowing pink) and viable (lower; with glowing pink embryo on left side) seeds.

Juncaginaceae

Triglochin maritima
Seaside arrowgrass

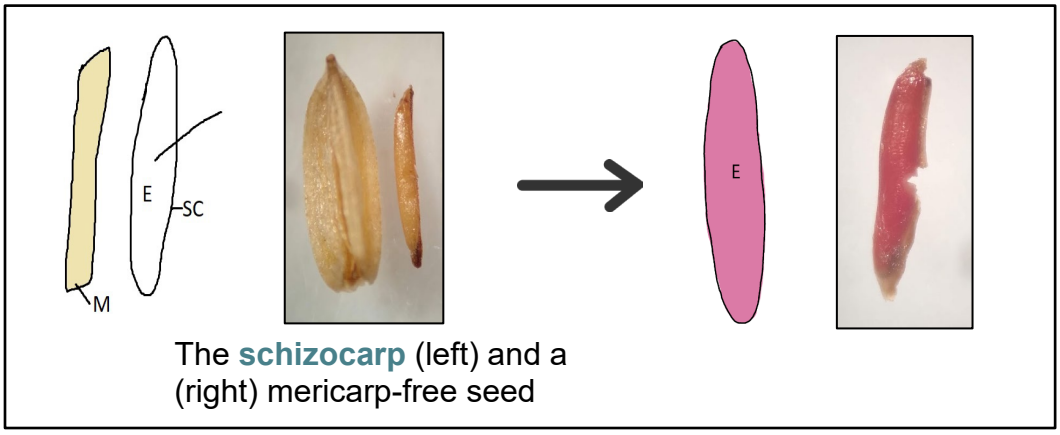
1% TZ; 48 hours



Preparation: Imbibe on a moist medium for 24 hours to make the seed easier to handle and minimize artifact damage. Remove seed from the mericarp (M) and pierce the center of the seed coat (SC) and embryo (E) with a sharp exacto blade (Figure 1).

Evaluation: View the full embryo. The viable stained embryo will glow through the membranous seed coat (Figure 2).

Notes: Cutting the seed at the top of the cotyledons in preparation for the TZ solution did not work as well as piercing due to artifact damage.



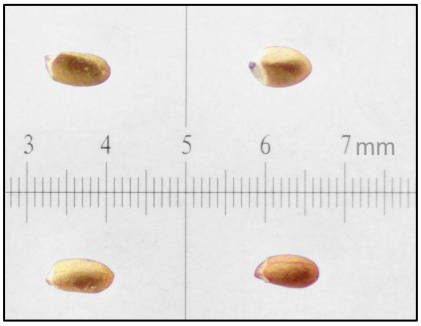
The schizocarp (left) and a (right) mericarp-free seed

Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Three viable seeds, glowing pink through the seed coat. Some discoloration will occur from piercing; disregard it if the whole seed glows pink otherwise.

Lamiaceae

Mentha arvensis
Wild mint



1% TZ; 48 hours

Preparation: Slice through the round, **cotyledon** end of the **seed**, through the **pericarp** and **seed coat**. Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining (Figure 1).

Evaluation: Remove the pericarp and seed coat to view the full embryo. A fully pink stain indicates a **viable** seed.

Notes: Sometimes the seed coat will turn blue. A 24-hour **imbibition** prior to making preparatory cuts did not hurt the staining and evaluation process, but it is probably not necessary.

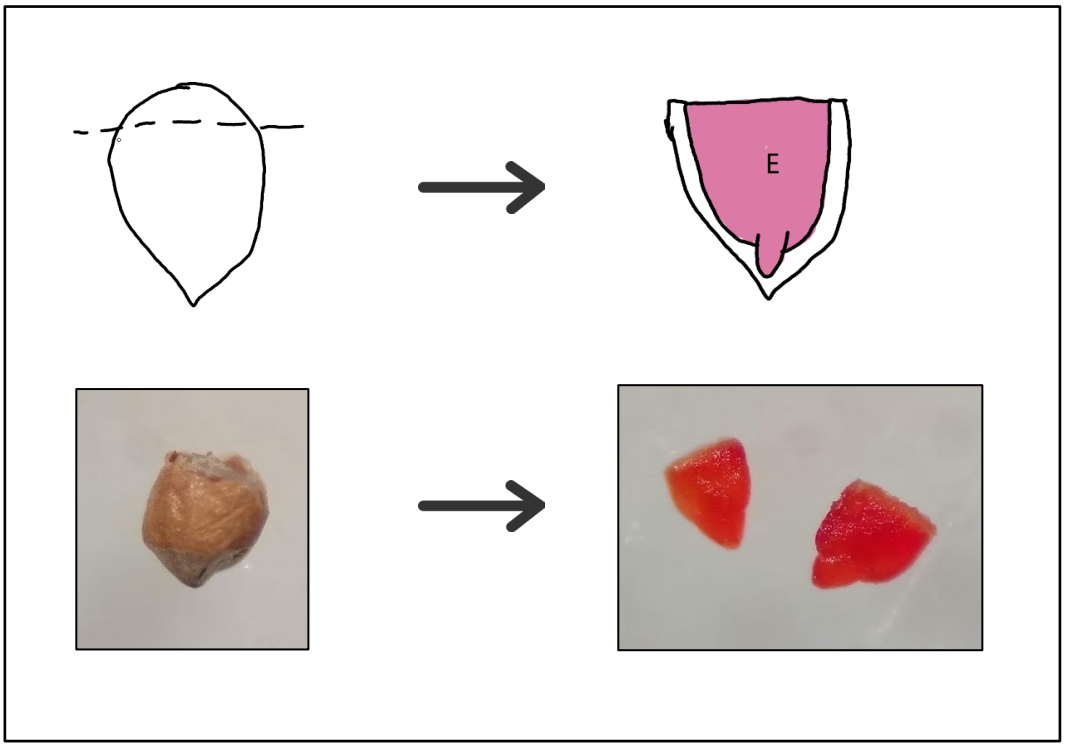
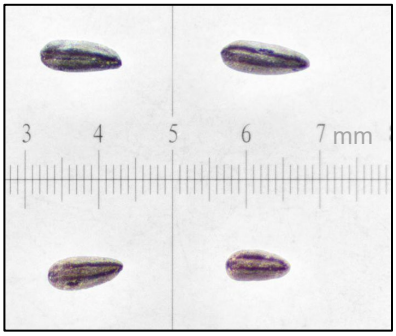


Figure 1. Images illustrating seed preparation and evaluation.

Onagraceae

Epilobium ciliatum
Fringed willowherb

1% TZ; 48 hours



Preparation: Pierce the center of the **seed coat** and **embryo** (E; Figure 1).

Evaluation: Cut **longitudinally** and evaluate. Some **seeds** are still light pink (**viable**) even using a 1% solution and a 48-hour soaking time (Figure 3). **Non-viable** seeds will have a clear embryo (Figure 2).

Notes: Using a 0.1% TZ solution and a 24-hour soaking time resulted in a very light pink stain.

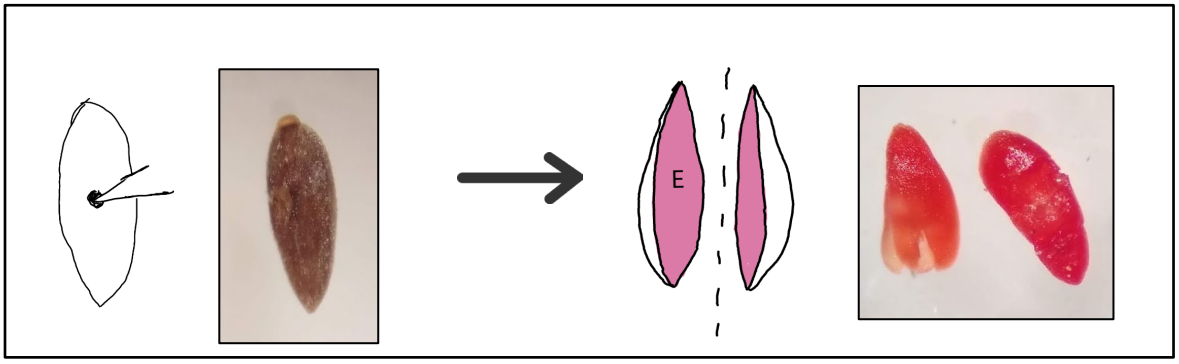


Figure 1. Images illustrating seed preparation and evaluation.

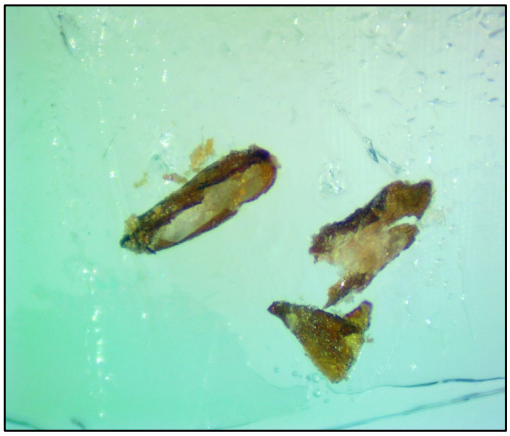


Figure 2. Two non-viable seeds. Non-viable seeds have clear embryos and are typically less filled than the viable seeds.

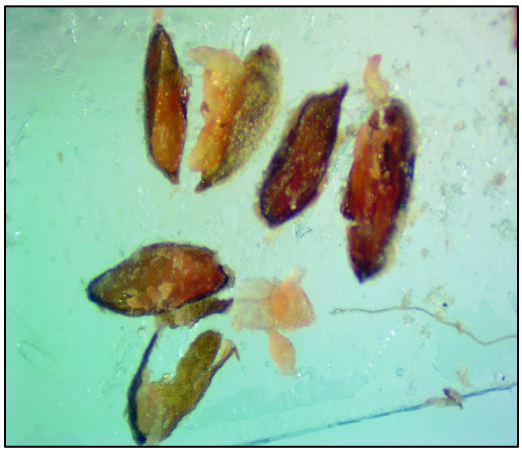


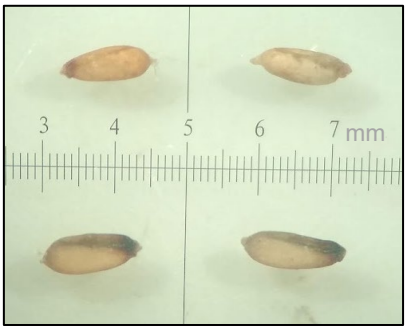
Figure 3. Three viable seeds—filled and pink.

Poaceae

Crypsis schoenoides
Swamp pricklegrass

1% TZ; 48 hours

Preparation: Slice off a piece of the **seed coat** and **endosperm** on the side of the **seed** that is farthest from the **embryo** (E; Figure 1). Discard the small portion that was cut off and keep the large part of the embryo for staining.



Evaluation: The embryo may glow through the seed coat. Bisect **longitudinally** to evaluate. The embryo will be fully pink.

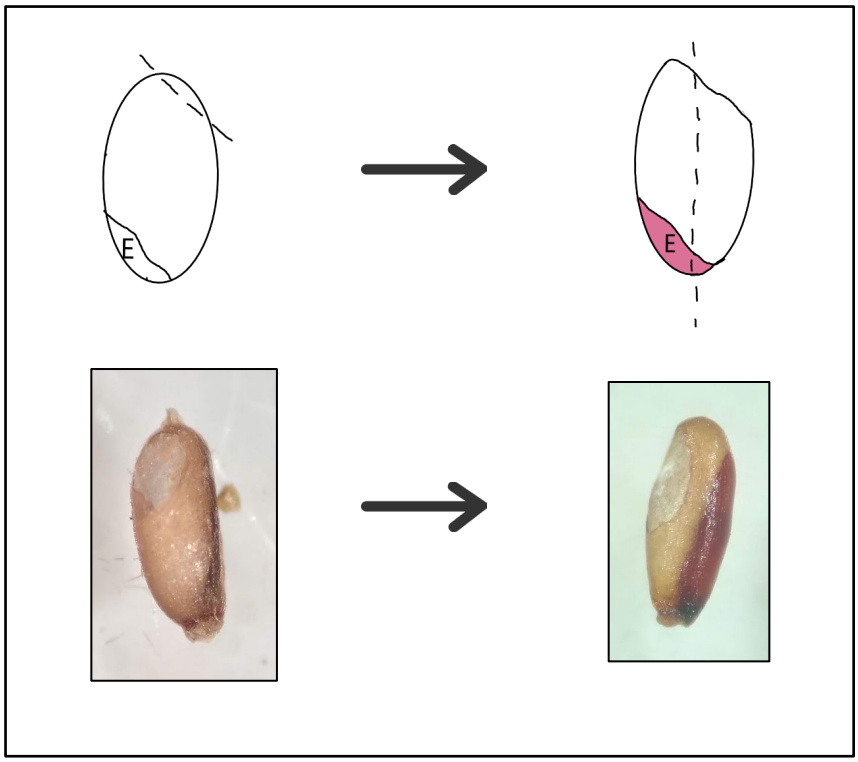


Figure 1. Images illustrating seed preparation and evaluation.

Poaceae

Distichlis spicata
Saltgrass

1% TZ; 48 hours

Preparation: Remove the **seed** from the **palea** and **lemma** (golden coverings, top right photo) if still attached, by agitating the seeds out using gloved hands or with forceps under the microscope. Typically, the **embryo** (E) is on the less sharply pointed side, but it can be hard to tell. Slice through the brown **seed coat** and white **endosperm** in the middle of the seed (the part that juts out). Discard the small portion that was cut off and keep the large part of the embryo for staining (Figure 1).

Evaluation: Turn the seeds so that the cut location is facing up, then bisect the seed **longitudinally** through the cut area and check both halves. A fully pink embryo (even a very pale pink) is **viable** (Figures 2–3).

Notes: Our lab used a 0.1% TZ for 24-hour soaking time for several years prior to 2022, but embryos stain a darker pink when a 1% TZ solution and 48-hour soaking time is used, leading to a more objective evaluation.

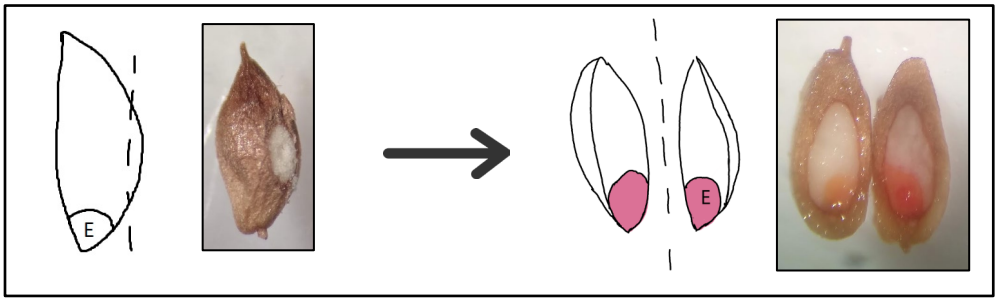


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Four viable seeds.

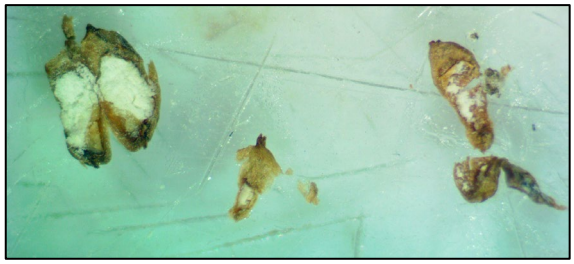


Figure 3. Three **non-viable** seeds. Left: clear embryo; middle: stunted seed with endosperm but no embryo; right: seed with minimal endosperm and no embryo.

Poaceae

Leymus triticoides
Beardless wildrye

1% TZ; 48 hours

Preparation: Keeping the **seed** in the **chaff**, make a small **longitudinal** cut through the chaff and the **endosperm** on the side furthest from the **embryo** (E; Figure 1). Try to keep the seed intact.

Evaluation: Bisect longitudinally to evaluate. **Viable** embryos will be fully pink (Figure 2).

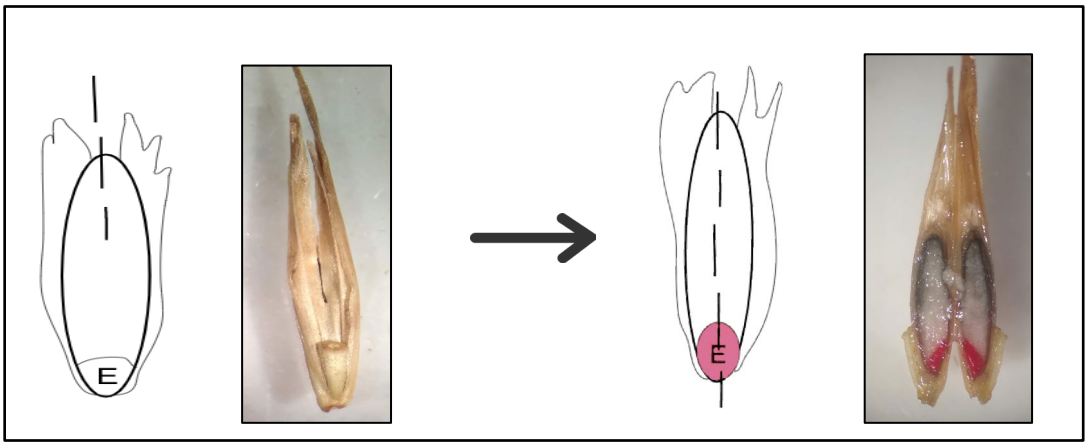


Figure 1. Images illustrating seed preparation and evaluation.

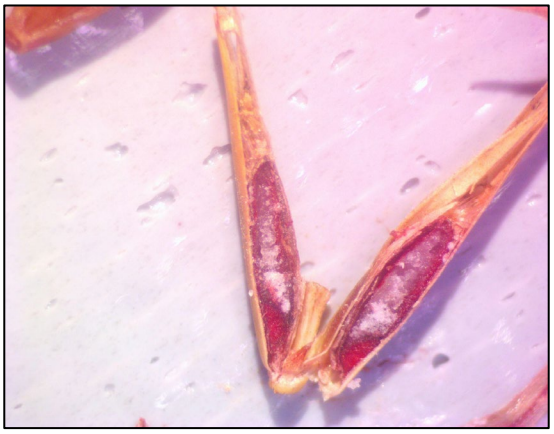
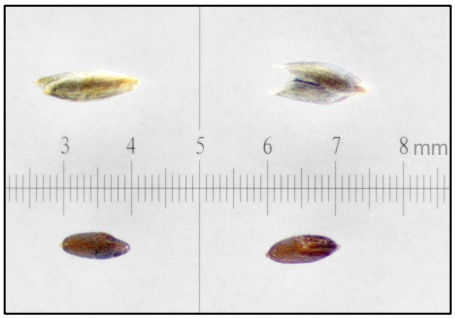


Figure 2. One viable seed with the entire embryo stained pink (bisected).

Poaceae

Muhlenbergia asperifolia
Scratchgrass

1% TZ; 48 hours



Preparation: With forceps under the microscope, remove the **seed** from the **chaff**. Cut off the top end of the seed, opposite the wrinkly **embryo**-end (E) of the seed (Figure 1). Discard the small portion that was cut off and keep the large part for staining.

Evaluation: Bisect **longitudinally** to evaluate. **Viable** embryos will be fully pink (Figures 2–3).

Notes: These small seeds hold up well in the solution and most viable seeds stain a dark red.

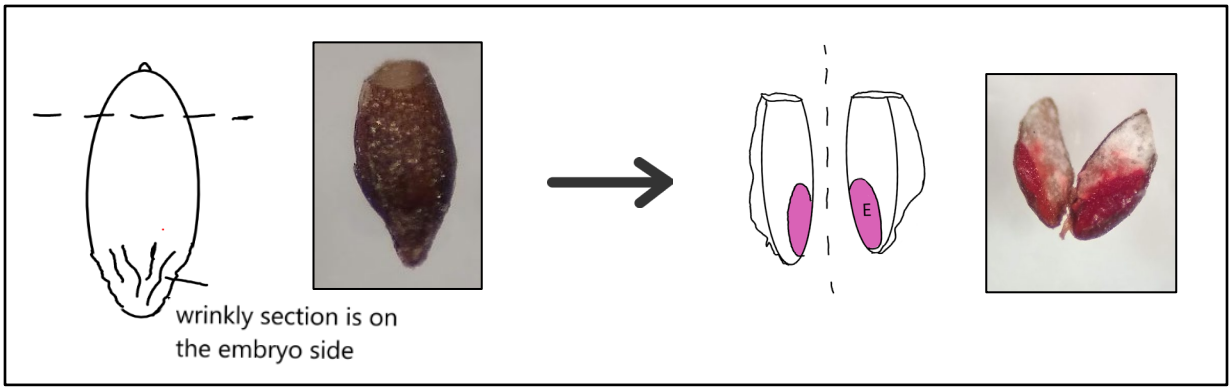


Figure 1. Images illustrating seed preparation and evaluation.

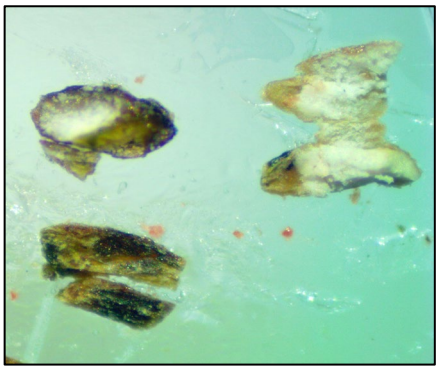


Figure 2. Three **non-viable** seeds—seen as undifferentiated and crispy seed without embryo (bottom), seed with green turgid embryo (top left), or green/clear flaccid embryo (top right).

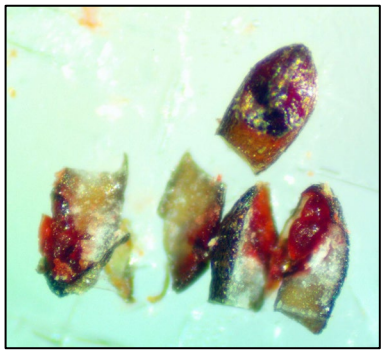


Figure 3. Three viable seeds—two are bisected (bottom), one is not (top) but the embryo glows through.

Poaceae

Phragmites australis
Common reed

1% TZ; 48 hours

Preparation: Imbibe overnight. The embryo (E) is on one end of the seed. Pierce the center of the seed with a needle or exacto blade (Figure 1).

Evaluation: After 48 hours, bisect longitudinally and check for a pink embryo.

Notes: We found that 0.1% TZ for 24 hours leads to more light pink stained seeds.

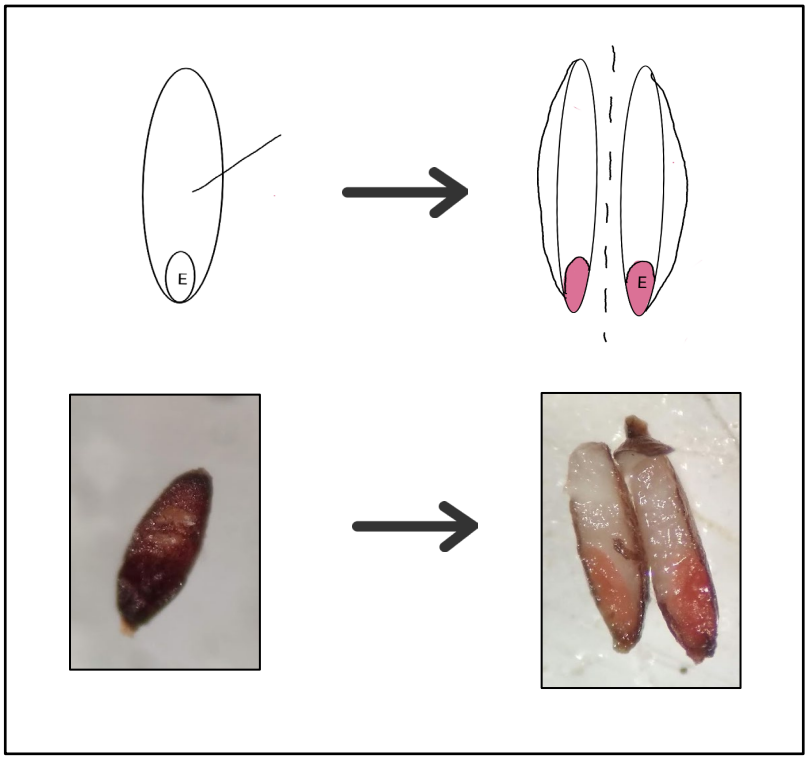
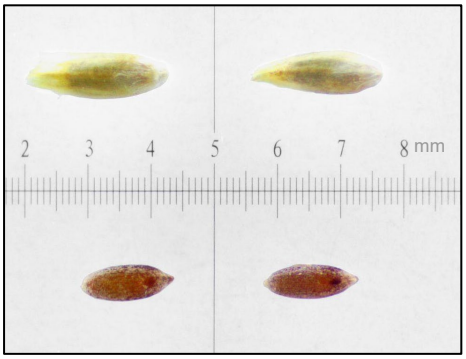


Figure 1. Images illustrating seed preparation and evaluation.

Poaceae

Puccinellia nuttalliana
Nuttall's alkaligrass

1% TZ; 48 hours



Preparation: Rub **seeds** out of their **chaff**. Gently scrape or saw into the seed (through the **seed coat** but leave seed intact) above the **embryo** (E; Figure 1). The embryo is located at the bottom (dark and pinched part of the seed). Tip: to keep seed intact, use a sharp blade to saw gently rather than pushing down into the seed.

Evaluation: Bisect to view the full embryo and evaluate (Figures 2–3). The **embryo** (E) will be fully pink if **viable**.

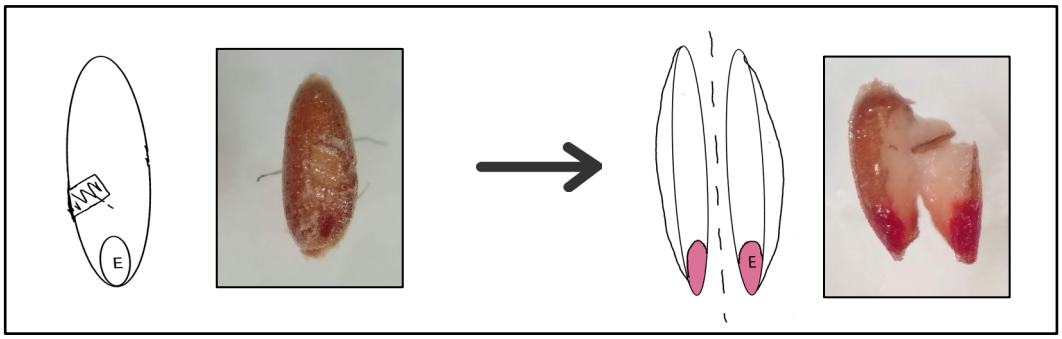


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Four viable seeds with fully stained embryos.

Figure 3. Two **non-viable** seeds with unstained embryos.

Poaceae

Sporobolus airoides
Alkali sacaton

1% TZ; 48 hours

Preparation: Pierce the **endosperm** in the middle of the **seed** (Figure 1).

Evaluation: Bisect **longitudinally** to evaluate (Figure 1). The **embryo** (E) will be fully pink.

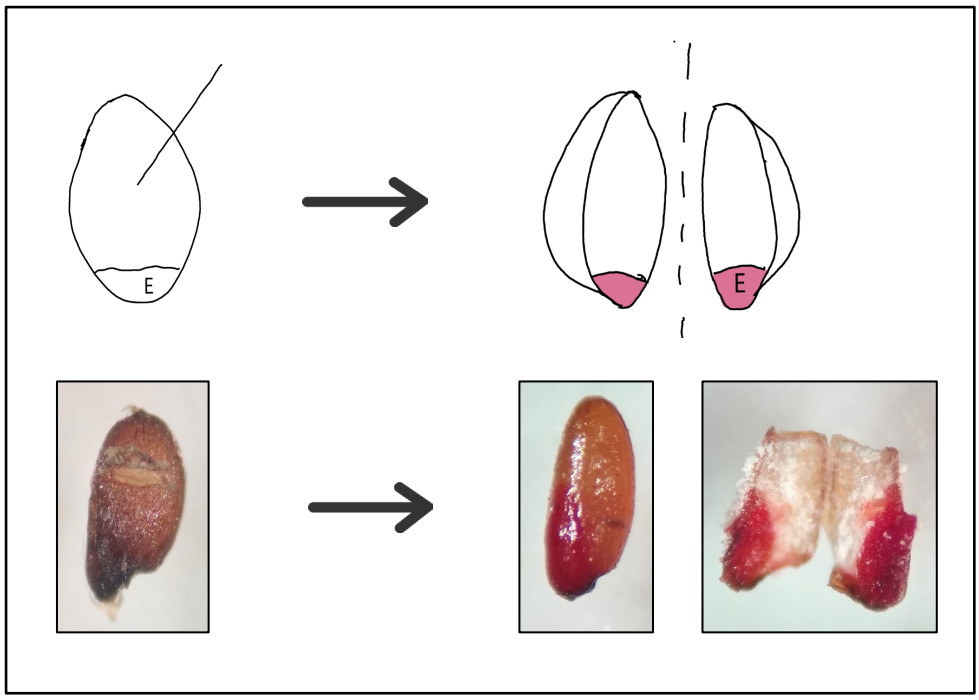
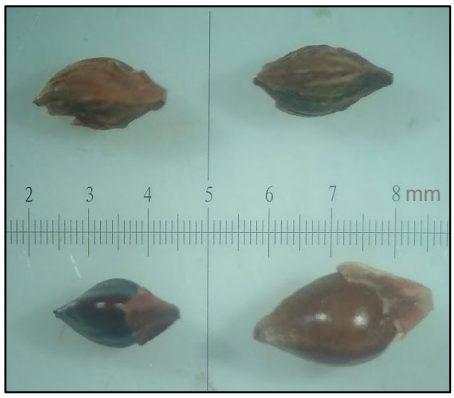


Figure 1. Images illustrating seed preparation and evaluation.

Polygonaceae

Polygonum ramosissimum
Bushy knotweed



1% TZ; 24 hours

Preparation: Using an exacto blade at an angle, shave off parallel to the flat face (Figures 1–2). This species stains very quickly.

Evaluation: Bisect the **seed longitudinally** to evaluate. **Viable** seeds will have bright pink **embryos** (E; Figures 3–5).

Notes: In the 2021 collection of this species, there were two seed morphologies in our **seed lot**—a rounder light brown morph and a darker brown trigonal morph (below). We were unsure if this was two species or just two different morphs of the same species.

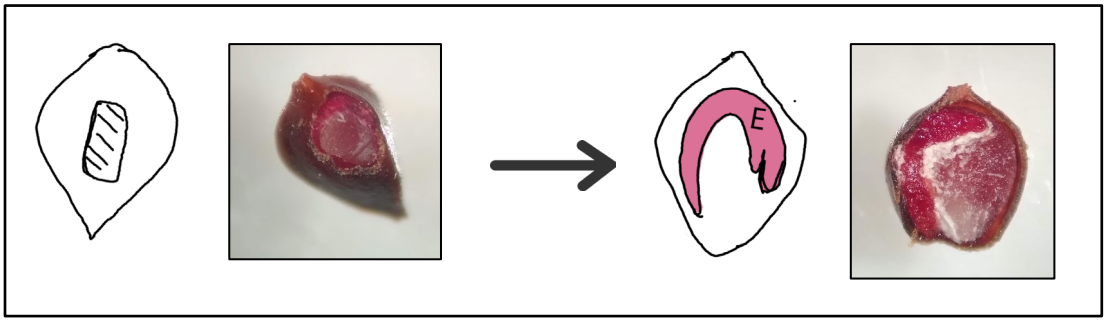


Figure 1. Images illustrating seed preparation and evaluation.

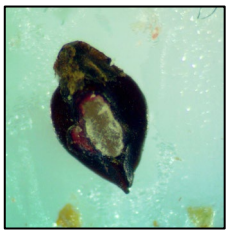


Figure 2. Turn the seed so that it is sitting on its flattest face. Shave off one face (parallel to the flattest face). Pictured: darker brown trigonal morph.

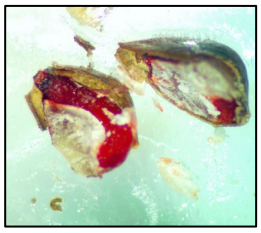


Figure 3. Viable seed, bisected.

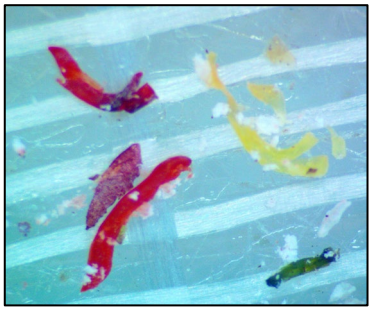


Figure 4. Left, viable (two stained embryos); right, **non-viable** (two unstained embryos).

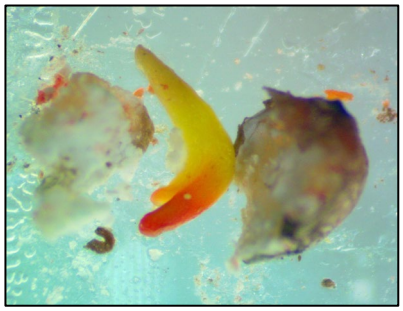


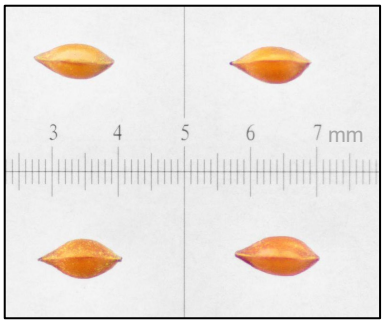
Figure 5. Non-viable seeds marked by pale embryo.

Polygonaceae

Rumex maritimus

Golden dock

1% TZ; 48 hours



Preparation: **Seeds** are football shaped. Shave off one of the three angles with an exacto knife (Figure 1). A thicker double-sided tape helps to hold seeds in place. Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining.

Evaluation: Bisect the seed **longitudinally** to evaluate. Even after a 48-hour stain, some **viable** embryos are light pink (Figures 2–3).

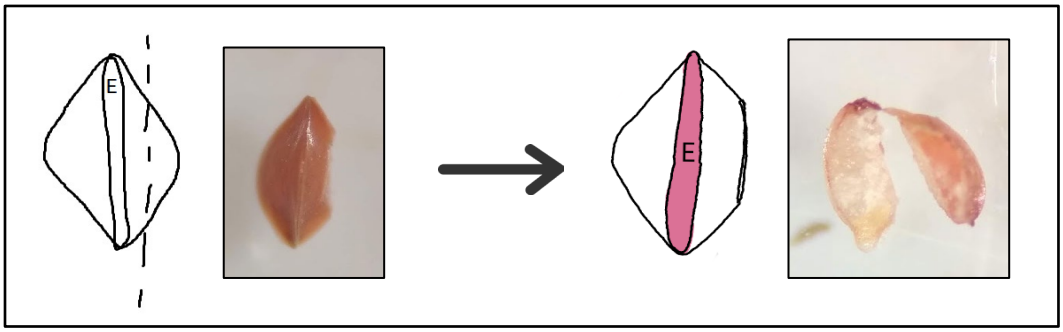


Figure 1. Images illustrating seed preparation and evaluation.

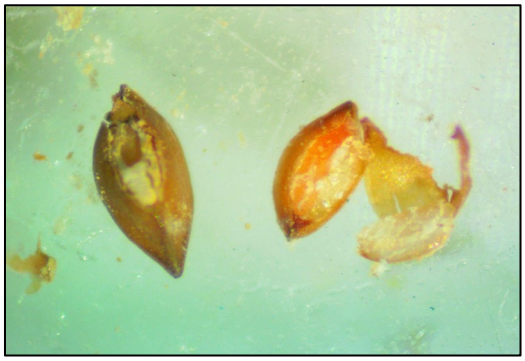


Figure 2. Left, a seed with one angle cut off (pre-TZ preparation). Right, a viable seed.

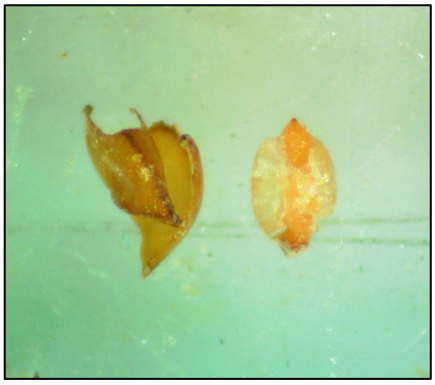
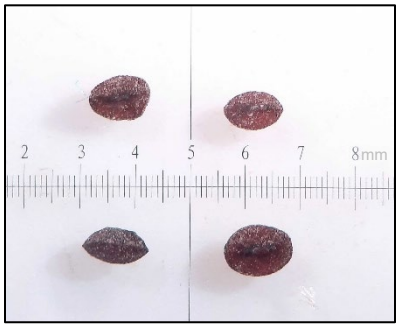


Figure 3. Left, **seed coat**. Right, a viable embryo (pink) surrounded by **endosperm**.

Primulaceae

Glaux maritima
Sea milkwort

1% TZ; 48 hours



Preparation: **Seeds** are football shaped. Shave off one of the three angles with an exacto knife (Figure 1). A thick double-sided tape helps to hold seeds in place. Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining. Be careful to only shave off a bit of the edge; the seed will still stain just fine. But, if you cut too near the middle of the seed you will damage the embryo enough to make evaluation difficult.

Evaluation: Bisect the **seed longitudinally** to evaluate. Be sure to view the entirety of the embryo. It is sometime possible to gently squeeze the seed, so the embryo squishes out and view the embryo with this method (Figure 2, far right).

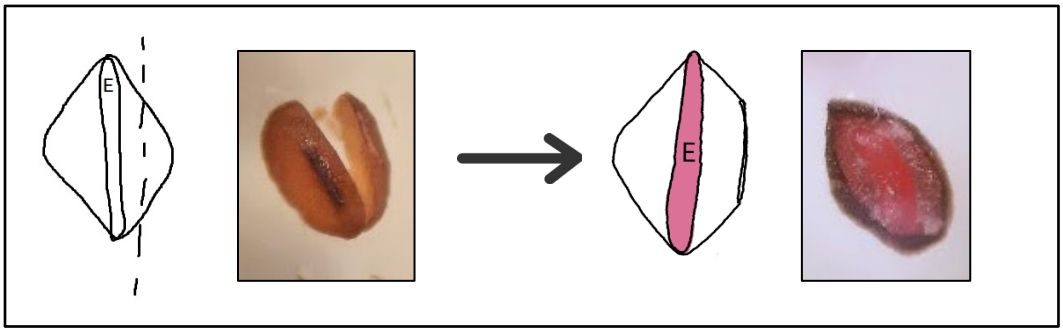


Figure 1. Images illustrating seed preparation and evaluation.

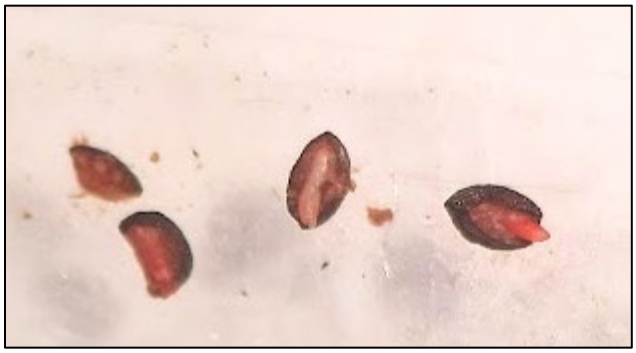
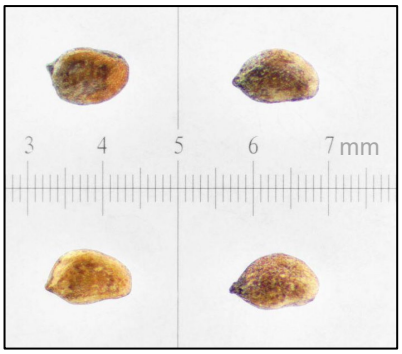


Figure 2. Two **viable** (left, right) and one **non-viable** seed (middle) with an unstained embryo.

Ranunculaceae

Ranunculus sceleratus
Cursed buttercup



1% TZ; 48 hours

Preparation: The cut recommended in the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, is hard to do without **imbibition**, as the **seeds** break. Implementing a 24-hour imbibition is helpful so that the seed stays intact. Make a small **longitudinal** slice opposite of the embryo side (Figure 1).

Evaluation: Pink **embryo** (E) and at least a lightly stained **endosperm** are necessary for counting as **viable**.

Notes: The embryo is not ready after 24 hours in 0.1% TZ.

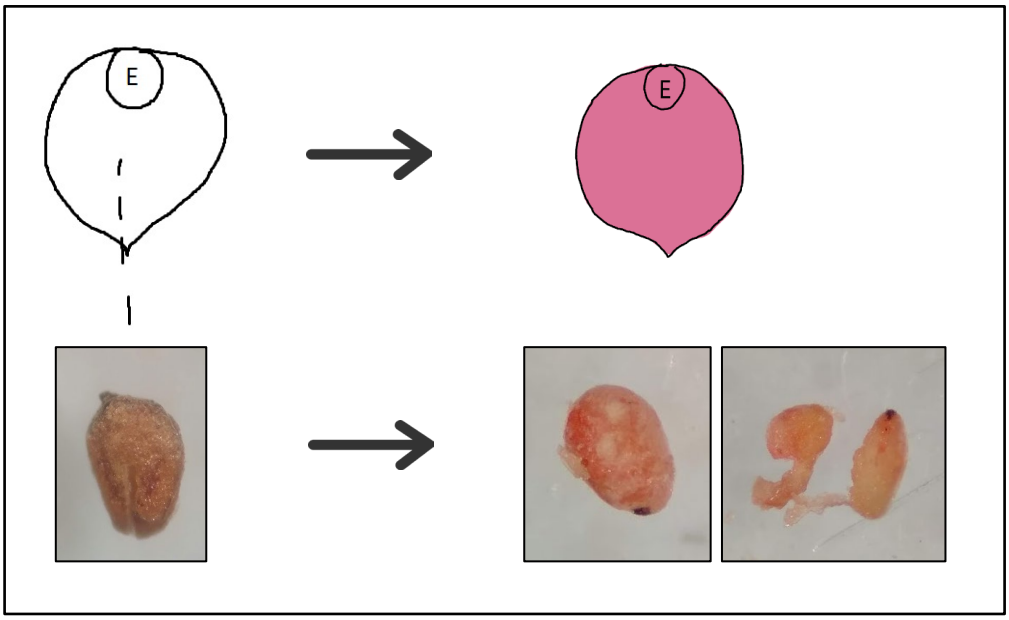
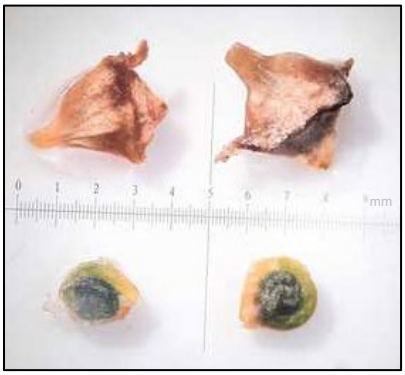


Figure 1. Images illustrating seed preparation and evaluation.

Sarcobataceae

Sarcobatus vermiculatus
Greasewood



1% TZ; 48 hours

Preparation: **Seeds** are found within the **pericarp** (P). It is often hard to tell which pericarps have seeds and which do not, so it is recommend to remove the pericarp and pierce the seed (Figure 1). It is difficult to remove the pericarp without **imbibition** so seeds should be imbibed for 12–24 hours before preparation. The TZ process is equally effective if the seed is left in the pericarp, and both the pericarp and the seed are pierced.

Evaluation: If applicable, remove the **embryo** (E) from the pericarp (P). If the pericarp was removed in preparation, view the embryo. A fully pink embryo is **viable** (Figure 2). Most **non-viable** seeds are not fully formed embryos and are unstained (Figure 3).

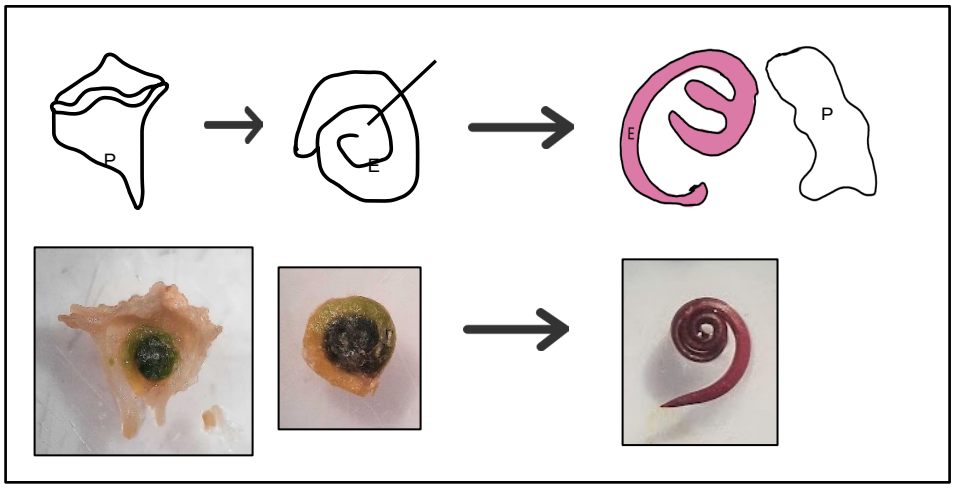


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. Two viable seeds will full dark staining.

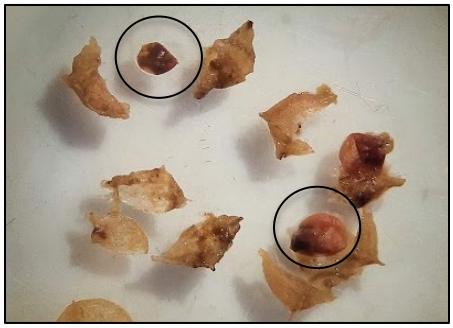
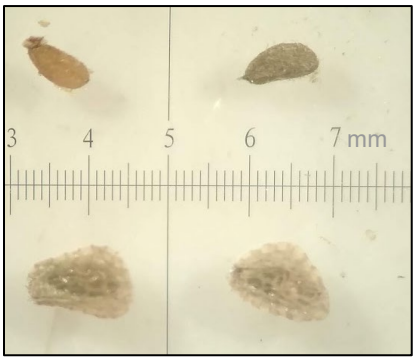


Figure 3. Two non-viable seeds (circled) surrounded by pericarp pieces.

Scrophulariaceae

Castilleja minor
Lesser Indian paintbrush

1% TZ; 48 hours



Preparation: Cut off the top, rounder (**cotyledon**) end of the **seed** (Figure 1). Discard the small portion that was cut off and keep the large part of the **embryo** (E) for staining. The seeds can remain in the airl (the fleshy coating around the embryo).

Evaluation: Bisect **longitudinally** to evaluate. A **viable** embryo is pink with a stained orange **endosperm** (Figure 2). A **non-viable** seed is white or clear with a yellow endosperm (Figures 3–4). Some endosperms look white outside but are orange inside. Disregard **artifact damage** at the cut location.

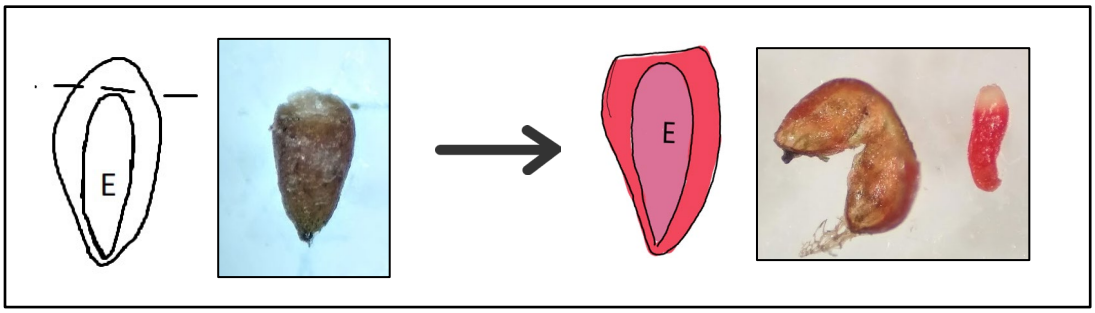


Figure 1. Images illustrating seed preparation and evaluation.

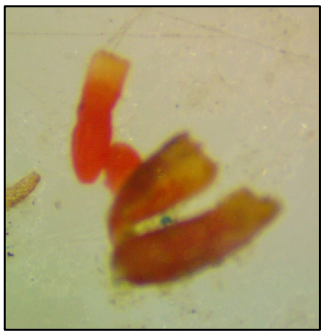


Figure 2. Viable seed; all red with an orange endosperm, some artifact damage.

Figure 3. Non-viable seed. It has completely white embryo and endosperm.

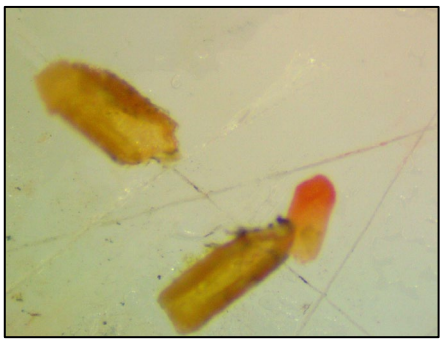
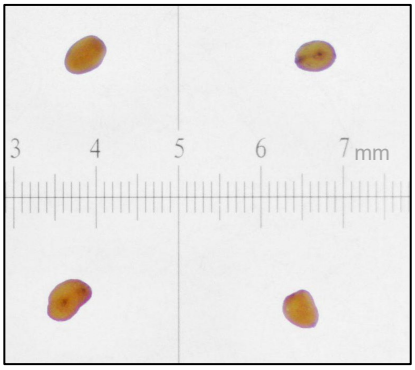


Figure 4. Non-viable seed. Half of the embryo is white with half pink.

Scrophulariaceae

Veronica anagallis-aquatica
Water speedwell

1% TZ; 24 hours



Preparation: Imbibe for 1 hour in water and then soak in 1% TZ for 24 hours at 35℃ (e.g., in a temperature regulated growth chamber; wrap in aluminum foil to prevent light from degrading TZ or triggering germination).

Evaluation: Observe if the seed (embryo (E) and endosperm) has turned pink through its transparent seed coat (Figures 1–2). Disregard any artifact damage.

Notes: If seeds become too mucilaginous once wet, mucilage can close over the preparatory cut. If you suspect this may be a problem (e.g., many of the seeds don't stain using the above protocol), you can use aluminum potassium sulfate to dissolve the mucilage. In the past, we've tried 60-minute soaks in a 10% $AlK(SO_4)_2 \cdot 12H_2O$ solution (see the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition). Reducing the time seeds are wet helps reduce mucilage, which is why the imbibition and TZ soak time is shorter than others in this protocol.

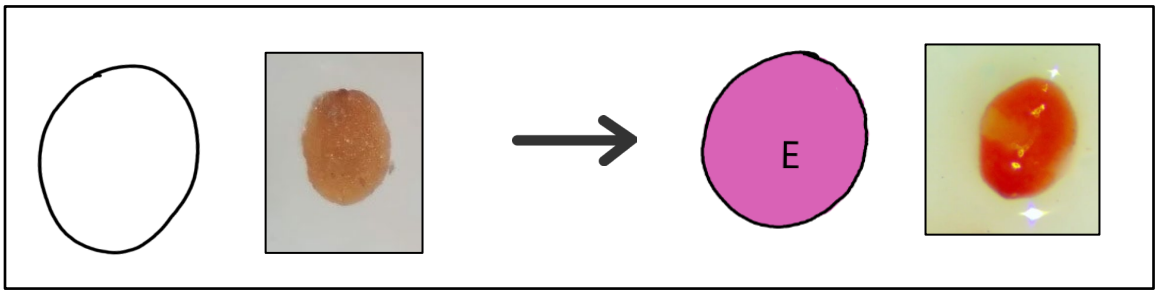


Figure 1. Images illustrating seed preparation and evaluation.

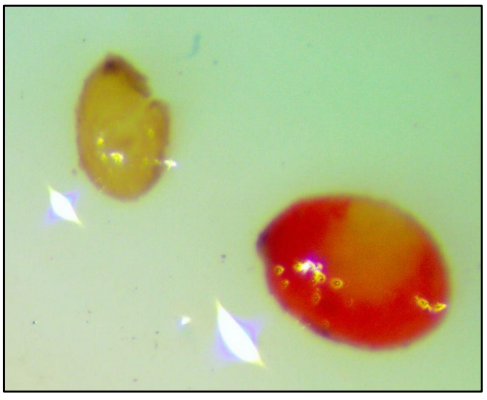


Figure 2. Non-viable, unstained seed (left) and viable seed (right); disregard artifact damage.

Verbenaceae

Verbena hastata
Swamp verbena

1% TZ; 48 hours

Preparation: A 24-hour **imbibition** makes the **seed coat** slightly softer and facilitates piercing the **seed**. Use a very sharp exacto knife to pierce the rounded part of the seed in the center (Figure 1).

Evaluation: Bisect and evaluate. When evaluating viability, there may be very slight discoloration of the **embryo** (E) at the pierce location (**artifact damage**), so disregard it if the rest of the seed is fully pink (**viable**; Figure 2).

Notes: 24 hours at 1% seems to stain the seed slightly, but there is white artifact damage. 48 hours at 1% works better.

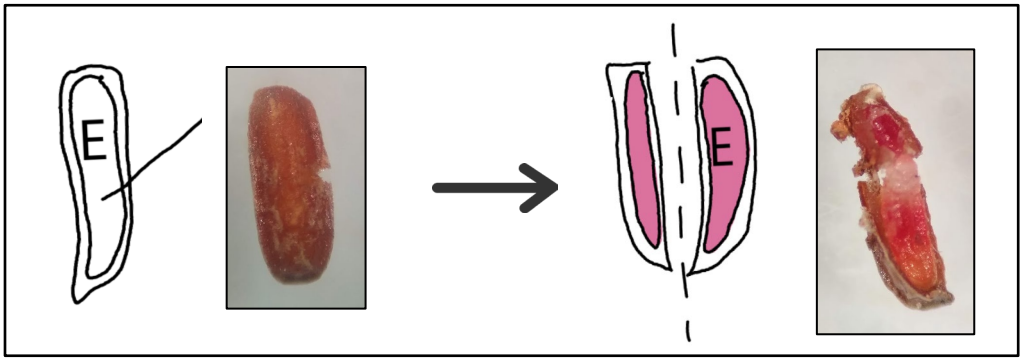


Figure 1. Images illustrating seed preparation and evaluation.

Figure 2. One viable, bisected seed.

Glossary

Note: the AOSA/SCST Tetrazolium Testing Handbook, 2010 Edition, has a great glossary. Some terms from that handbook are included below.[1] Other definitions are taken from Harris and Harris (2001).[2]

Achene: a dry, hard, one-seeded fruit.[1]
Aril: an appendage growing at or near the point of **seed** attachment; fleshy thickening of the **seed coat**.[2]
Artifact damage: damage to the **seed** from the preparation technique.[1]
Chaff: seed coverings and other debris that is separated from the seed and discarded.
Cotyledon: **seed** leaves of the **embryo**; embryonic leaves.[1]
Dicotyledon: plants with **seeds** that produce two **cotyledons**. Abbreviation: dicot.[1]
Embryo: a rudimentary plant contained within a **seed** or genetic individual.[1]
Endosperm: the part of a **seed** which acts as a food store for the developing plant **embryo**.[1]
Germination: the development of a plant from a **seed** after a period of dormancy.[1]
Imbibe/Imbibition: **seed** absorbing water into ultramicroscopic spaces or pores.[1]
Lateral: spanning the width, perpendicular to the **longitudinal** axis.
Lemma: the lower of the two bracts jutting out from a **seed**; surrounding the **palea**.[2]
Longitudinal: spanning the long axis.
Mericarp: a single unit of a **schizocarp**.
Monocotyledon: plants that produce **seeds** with only one **cotyledon**. Abbreviation: monocot.[1]
Mucilaginous: having a viscous or gelatinous consistency.
Palea: the upper of the two bracts jutting out of the **seed**; surrounded by the **lemma**.[2]
Pericarp: the fruit wall; derived from the ovary wall. It may be thin and fused with the **seed coat** as in corn, fleshy as in berries, or hard and dry as in pods of legumes.[1]
Perigynium: a fleshy cup or tube that surrounds the pistil (the female reproductive organ of a flower; i.e., the **seed**).[2]
Radicle: the rudimentary root of the **embryo** that forms the primary root of the young plant or embryonic root.[1]
Schizocarp: dry fruit that splits into one-seeded units when ripe.
Scoring: to break down the **seed**'s outer coating.
Seed: a mature ovule consisting of an embryonic plant together with a store of food, all surrounded by a protective **seed coat**.[1]
Seed coat: the protective covering of a **seed** usually composed of the inner and outer integuments. Also called the testa.[1]
Seed lot: in this context, the group of **seeds** that are being tested.
Tuber: a thickened, solid, short underground stem with nodes bearing buds.
Tubercle: a small **tuber**-like prominence or nodule.
Utricle: a thin-walled, one-seeded fruit where the **seed** is loosely attached to the **pericarp**.
Viable: a **seed** that is alive with the potential to grow into a seedling. Conversely: **non-viable** with no potential for growing into a seedling.

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Appendix: Example Viability Evaluation Data Sheet

Initials are for the personnel involved in that step. Conc = concentration, Hrs = hours, Prep = preparation, and Eval = evaluation.

Species/source: TZ conc: Hrs in TZ: Prep initials: Prep date: Prep notes: Prep time:	Eval initials: Eval date: Eval notes: Eval time:
Total TZ viable: Total TZ non-viable: Total not evaluated:	

Group	Viable	Non-viable	Notes & evidence for non-viable seeds
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
extra			