

HANDLING UNFAMILIAR SEEDS OR HOW NOT TO DESTROY YOUR SEED SAMPLE

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Abstract: Beginning work on seeds of endangered and exotic species often means starting with little or no knowledge and working toward successful handling and storage. It is often found that, in the past, exotic or poisonous plants (such as poison ivy) have been regarded as high in nuisance value and low in usefulness. Thus little research has been done on the care and handling of their seeds. Alternatively, seeds from endangered plants are often regarded as too precious to waste on needless experimentation. We now realize that information on the germination characteristics and the biochemistry of seeds of exotic and invasive species can be used to devise means of controlling their spread; and the more we learn about endangered species, the better chance we have of either establishing new colonies or storing germplasm for future generations. In this paper, we present methods for handling seeds that are an unknown entity in order to gather the greatest amount of information, often from limited seed supplies, with minimal waste.

INTRODUCTION

When beginning work on seeds from species that are unfamiliar, approach as you would any scientific problem. Consult colleagues and initiate a thorough search for information in the library or on the internet. If information about other species in the same genus or family is found, use procedures to treat and store seeds of those species as a guideline. But proceed with caution. While appropriate in some cases, in others the destruction of the entire sample may result. For instance, seeds of *Acer saccharum* Marsh., sugar maple, are orthodox, meaning they can be dried to a moisture content (MC) of less than 12% (g/g fresh wt.) and stored under refrigeration for long periods of time. However, the seeds of *Acer saccharinum* L., silver maple, although similar in shape and size to those of sugar maple, are recalcitrant. If these recalcitrant seeds are dried below a critical MC, they lose viability. This susceptibility to moisture loss makes any period of storage for some recalcitrant seeds very short, while others, still classified as recalcitrant, can survive for 3 years under proper storage conditions.

You will also need to determine optimal germination conditions, dormancy requirements, best temperature for seed storage, and the length of time seeds can be stored and still retain high viability. We provide here some standard techniques and procedures that can be adapted, with caution, to establish a protocol for handling unfamiliar seeds.

METHODOLOGY: QUICK AND DIRTY

COLLECTION AND HANDLING

Have a clear plan of action before going into the field. Your objective is to collect seeds when they are fresh and fully ripe and the best way to do this is by tracking seed development. If possible, collect more seed than you think you will need. You'll rarely have 100% viability. Color change is the most common and easily identifiable indicator of maturity, although larger seeds are often collected from the ground after dissemination. Changes in MC and biochemical makeup are also common but less easy to test in the field. Cut open a few seeds to see if the seeds are filled and if the interior color is good.

It is vital that you maintain seed MC after collection. Remember that you know little if anything about these seeds. If you allow them to dry, you may kill them. Place seeds in a plastic bag and loosely seal for transport to the lab. It is important to keep the seeds cool but refrigeration is not necessarily recommended.

THE TIME LINE

In addition to a plan of action for the field trip, everything should be in place when you arrive back at the lab. Most temperate-zone seeds can be successfully stored under refrigeration (even when fully hydrated) for short periods of time but if you can begin work immediately, all the better.

TESTS

The following steps are proposed to minimize errors in the test process: Remember, don't assume the seeds are either orthodox or recalcitrant. If you have a large supply of seeds, we suggest you treat them as recalcitrant to avoid destruction of the seed sample.

Moisture Content (MC):

MC at time of shedding may be the first clue of whether a seed is orthodox or recalcitrant. A phase called maturation drying often occurs at the end of the ripening cycle of orthodox seeds. Their MC may drop slowly as seeds mature. In orthodox seeds with pulp, MC increases but mainly in the pulp. Recalcitrant seeds do not go through a maturation drying phase and have a high MC when shed (Bonner *et al.* 1994).

Dry seeds overnight in a 103°C oven for 17 h to determine MC. Do this before undertaking any other tests. If you have some seeds to spare, weigh 21 of them in groups of 7 seeds. Then leave them on the lab bench to dry 12-24 h if small, 24-72 h if large. Put one group in the oven to determine dry weight, rehydrate the another group and put in the germination cabinet, and immerse the third group in liquid nitrogen (LN₂). Leave the seeds in the LN₂ for 1 h, then remove and allow to warm slowly at room temperature. Rehydrate the seeds before testing for germination. If seed MC has dropped below 12% (fresh weight basis) and the seeds survive immersion in LN₂ (or germinate in the cabinet), you're dealing with orthodox seeds.

The Thermogradient Table:

Test seeds on a thermogradient table to determine optimal germination temperatures. A two-way thermogradient table can be used to determine optimum temperature regimes for laboratory germination of the unfamiliar seeds. The thermogradient table can supply simultaneously a very large number of alternating temperature regimes which simulate natural seedbed conditions; circulating water baths pump hot and cold water through channels on opposite sides of the plate to create a very stable thermal gradient. At pre-selected times, solenoid valves automatically switch the flow to the other two sides, thus creating alternating temperature regimes at all spots on the plate except for the diagonal, which has constant temperatures. The grid size selected often depends on seed size. For larger seeds, such as those of *Lindera melissifolia* Walt. (pondberry, Fig. 1), we used an 8 x 8 cm grid - 100 different spots, four seeds per spot. If seeds are recalcitrant, they should germinate very quickly. If seeds are slower to germinate or do not germinate at all on the thermogradient table, odds are they are orthodox, and there may a dormancy requirement for these seeds.

Germination Tests:

The number of seeds used in the seed tests for germination may be limited by the number of seeds available. If you have an ample supply, 4 replications of 100 seeds is a good starting point. If seed supplies are limited, drop to 4 replications of 25 seeds. Use the temperature combination showing best results from the thermogradient table study to set the temperature program in the germination cabinet.

If you are limited by time and number of seeds, we suggest you eliminate the thermogradient study and take your best guess at setting the temperatures on the germination cabinets. For instance, when testing for germination of *Toxicodendron radicans* (L.) Kunst. (poison ivy, Fig. 2) seeds, which mature in the fall, we set the germinators on a 25/30 cycle. This means the germinator was set for 16 h of dark at 25°C and for 8 h of light at 30°C. If the seeds mature in spring, try setting the germinator for 8 h of dark at 20°C and 16 h of light at 30°C. The combination of 20/30°C is prevalent in the literature for use on fall-maturing seeds from the temperate zone.

Storage Tests

If you determine that the seeds are orthodox before beginning the storage study, dry them to a MC between 5-12% and store at the temperature of your choice. If the seeds are recalcitrant, DO NOT under any circumstances dry before storage. Store the seeds fully hydrated at either 4°C or -2°C. Test at intervals for viability. If the seeds are very recalcitrant, such as those of *Quercus alba* L., they will germinate while in storage in as little as 2 months. If they are less recalcitrant, like *Quercus nigra* L., or sub-orthodox, they can be stored, with care, for a few years. If you are uncertain if the seeds are orthodox or recalcitrant, treat them as recalcitrant. Their storage lifespan will be shorter since they are stored fully hydrated, but you will not destroy your seed sample.

Overcoming Dormancy

This may be the most difficult problem you face. Dormancy is not an issue with recalcitrant seeds. Orthodox seeds, however, can have many types of dormancy requirements. If they are not overcome, your germination tests may give poor results despite the fact that the seeds are viable.

The best bet would be to check for information on other species or genera in the same family. For instance, while we found little or no information for poison ivy, we did find various treatments for *Rhus spp.* We set up a series of

treatments and stratified the seeds for 0-90 days. The results, reported in Table 1, were encouraging (Schiff *et al.* 2004); we had success with very little first hand information.

SUMMARY

The following is a suggested protocol for handling unfamiliar seeds:

- Have a plan of action in mind before going to the field.
- Harvest fresh, fully ripe seeds. Perform a cutting test.
- Handle seeds as if they were recalcitrant.
- Determine MC of collected seeds using the oven-dry method.
- Use a thermogradient table to determine optimal germination temperatures. This step can be eliminated if seeds are in short supply.
- Germinate seeds to determine if they are viable. If you eliminate step 5, try a combination of 20/30 on temperate zone seeds.
- When in doubt, store seeds at 4°C.
- Dormancy may be a serious problem for orthodox seeds. Check other plants in the same genus or family for information.

LITERATURE CITED

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Table 1. Mean percent germination of poison ivy seeds after 4 weeks and 12 weeks for 0, 30, 60, and 90 days of stratification.

Days Stratified Treatment ^a	0	30	60	90
After 4 weeks				
1 hr soak in water	0	32*	22*	40*
Picked in February		1	40*	42*
H ₂ SO ₄ 30 min.	63	72	66	70
H ₂ SO ₄ 1 hr		34	62	50
H ₂ SO ₄ 3 hr		16	2	0
H ₂ SO ₄ 6 hr		3	0	0
After 12 weeks				
1 hr soak in water	0	82	60	70
Picked in February		1	92	96

^aTreatments marked with an asterisk are those that had viable seeds left after the 4 week test period and were tested for an additional 8 (12 total) weeks.