

Recent Work at the National Seed Laboratory on Seed Storage, Upgrading Native Plant Seed Germination, and Germination Protocols for New Species

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Abstract

Equilibrium relative humidity is an excellent emerging technology for managing seed moisture for storage purposes. This article describes how this technology is improving seed storage and some pitfalls to avoid in using this technology. Native plant seeds are often of lower quality than needed for efficient nursery production of seedlings or the application of advanced seed-sowing technologies such as seed pelletizing. Work on Wyoming big sagebrush (*Artemisia tridentata* Nutt. spp. *wyomingensis* Beetle & Young) indicates that upgrading technologies used successfully in forestry will also produce better seed lots with other native plants. This paper was presented at a joint meeting of the Western Forest and Conservation Nursery Association, the Intermountain Container Seedling Growers Association, and the Intertribal Nursery Council (Boise, ID, September 9–11, 2014).

Introduction

Most native plants are regenerated from seeds. Therefore, a reliable supply of good-quality seeds is needed for restoration and maintenance of many plant communities. Storing seeds until needed and using them efficiently are two important capabilities in achieving a reliable seed supply. The demand for seeds can vary greatly and unpredictably from year to year. Supplying such an unpredictable need can be very problematic because seeds are usually not available every year in the wild and seed production in cultivated fields requires years of advanced planning. Long-term seed storage, however, can help solve the problem by acquiring seeds when available and keeping them alive until needed. Another important factor in increasing seed supply is using available supplies efficiently, which generally requires upgrading seed qualities to high levels. This article summarizes some recent work at the U.S. Department of Agriculture (USDA), Forest Service, National Seed Laboratory (NSL) in Dry Branch, GA, on preparing seeds for storage and improving seed quality and performance.

Managing Seed Moisture for Seed Storage With Equilibrium Relative Humidity

Moisture is the most critical factor in seed storage. The most advanced methods for assessing seed moisture status is equilibrium relative humidity (ERH; Baldet et al. 2009, Karrfalt 2014; figure 1). ERH is fast (producing an answer in about 5 minutes), nondestructive, and universally applicable to all orthodox seeds at any state, from raw harvested seeds to the finished cleaned seed lot. In addition, this method is economical because it uses very little energy compared with oven methods and can be measured with a relatively low-cost hygrometer. Working with the ERH test at NSL has led to a better understanding of managing seed moisture as described in the following sections.



Figure 1. Equilibrium relative humidity of seeds being measured with a hygrometer. (Photo by R.P. Karrfalt, 2012)

Refining Seed Storage Recommendations

A longstanding view of orthodox seeds is that if they are dried to any moisture level between 6 and 9 percent (an ERH of 50 percent or less) and stored at any temperature below freezing, no viability will be lost for at least 10 years. This view was based on experience with conifer species and a handful of hardwood species in which it held true. A recent study of Wyoming big sagebrush (*Artemisia tridentata* Nutt. spp. *wyomingensis* Beetle & Young) seeds (Karrfalt and Shaw 2013), however, found that such a generalized prescription did not work. Drying the seeds to an ERH of 30 percent was found to be the best practice for freezer storage. A target ERH of 40 percent was also found to be acceptable, but this level

required using -20.0 °C (-4.0 °F) for the storage temperature, whereas, at 30 percent ERH, either -8.0 °C or -20.0 °C (17.6 °F or -4.0 °F) could be used to store seeds for at least 5 years. The prescription for seed storage needs to be more specific for sagebrush; this approach is likely the case for other native species as well.

Obtaining Accurate Equilibrium Humidity Readings

A key factor in using ERH for seed moisture testing is making sure that the seeds are at equilibrium. In working with this test at NSL, we have observed that false readings sometimes occurred with larger seeds or a rapid rate of drying. Based on these observations, trials were conducted to examine the role of the following factors in reaching equilibrium: seed size, drying rate, and seed coat thickness. Dogwood (*Cornus florida* L.) and river birch (*Betula nigra* L.) were tested because they differed greatly in their physical characteristics. Dogwood seeds are much larger than river birch seeds and have a thick, stony seed coat while river birch seeds have a thin, papery seed coat. Initial ERH for both species was approximately 60 percent. Two drying regimes were used: an aggressive rate consisting of a small seed sample placed with a large amount of a chemical desiccant and a slower rate in which the seeds were air dried at 31 to 34 percent relative humidity. Three drying periods were used: 8, 19, and 32 hr. One seed lot per species was used, and it was divided into equal portions using a riffle divider and one fraction assigned to each of the six drying treatments. ERH was measured immediately at the end of the drying period and at three subsequent times until the change in reading was only 1 or 2 percent and, therefore, the seeds were judged to be at equilibrium. The first subsequent reading was taken 1 to 2 hr after drying, the second at 22 to 26 hr, and the final at 38 to 46 hr. The interval between the initial reading and the subsequent readings varied because no readings were taken during night time hours. Samples were kept in sealed containers between ERH measurements so that there was no moisture loss or gain.

The small river birch seeds with their thin seed coats were at equilibrium at the initial reading and completely dried with all drying periods. For the rapidly dried, larger seeded dogwood, the difference between initial reading and the reading taken 1 to 2 hr later was 8 to 10 percent. The difference between the second and third readings was 3 to 4 percent, and between the third and the fourth readings was 0 to 1 percent. This difference indicates that an accurate ERH reading for a sample of a larger seeded species that had been dried rapidly required holding the seeds for about 24 hr past the cessation of drying.

The same pattern was observed in the slow drying regime but the differences among successive readings were never more than 3 percent, meaning that initial readings are close to true equilibrium and a usable reading might be taken sooner than 24 hr after cessation of drying. Length of drying did not appear to create any bias in the readings on dogwood seeds although the longer drying period removed more moisture from the seeds than the shorter periods.

In conclusion, the larger seeds, when dried aggressively with the calcium sulfate, gave highly biased ERH readings immediately following the cessation of drying. Drying with air at 10- or 15-percent relative humidity would very likely produce the same effect as using the calcium sulfate drying method. The most likely explanation for this bias is that moisture was rapidly pulled from surface layers of the seed faster than moisture closer to the center of the seed could diffuse to the surface layers. The ERH reading was of the surface layers of the seed and not the whole seed. By holding the seeds for 24 hr, the moisture content in the inner and surface layers of the seed equilibrated and ERH readings were then representative of the entire seed, not just the surface layers. In slower drying regimes, the inner seed moisture was able to diffuse to the surface layers at close to the same rate that moisture was removed from the surface; therefore, the bias was much smaller. This internal seed moisture gradient did not develop in small seeds, resulting in accurate readings immediately at the cessation of drying. The safest approach, especially with an unfamiliar species, would be to check the ERH 24 hr after the initial reading to be sure the ERH readings are not biased and the moisture level is low enough for safe storage.

Upgrading Native Seed Quality

High-quality seeds are needed in any native plant restoration work, and the larger the project, the more important this attribute becomes. Germination especially has a major effect on restoration costs and effectiveness. In container seedling nurseries, better germination means fewer seeds must be sown per cavity, and ideally it would be just one seed per cavity for the most cost-effective and genetically sound nursery program. Multiple seeds per cavities incur thinning costs, higher seed costs, and wasted seeds because of discarded seedlings. Even if seedlings are not discarded, transplanting adds cost. Direct seeding is often problematic because of the small seed size of many native plants. Pelletizing small seeds would be a great benefit to both direct seeding and nursery sowing because a pellet can be made larger and uniform in size for easy handling (Khadduri 2007). Only seeds of high purity and germination are economically suitable for pelletizing,

however, because pelletizing is an expensive process. Sizing seeds with screens followed by weight separation has worked to upgrade conifer seed lots and can also be applied to other smaller seeded native plant seeds. Wyoming big sagebrush is one example.

Upgrading Wyoming Big Sagebrush Seed

Five seed lots of Wyoming big sagebrush were acquired from the Bureau of Land Management seed warehouse (Boise, ID). These seed lots were approximately 9 months from harvest at the time of this experiment. Initial cleaning with an aspirator removed the lightest trash and reduced the volume of material by 50 percent. The seeds were then scalped with 22 by 22 woven wire screen. The numbers of a woven wire screen indicate how many wires there are per inch (2.54 cm). A 22 by 22 screen has 22 parallel evenly spaced wires per inch (2.54 cm) going from one side to the other and 22 parallel evenly spaced wires per inch (2.54 cm) that are perpendicular to the first set of wires. Therefore, a smaller number represents a larger screen hole. The seeds were further divided into nine sizes using eight screens ranging in size from 24 by 24 to 38 by 38 using only even-numbered screens. Seed size was labeled in this manner. For example, a number 24 seed passed through the 22 by 22 screen but did not pass through the 24 by 24 screen. In like manner, a 26 seed passed through the 24 by 24 screen but not the 26 by 26 screen, and so forth for all seed sizes.

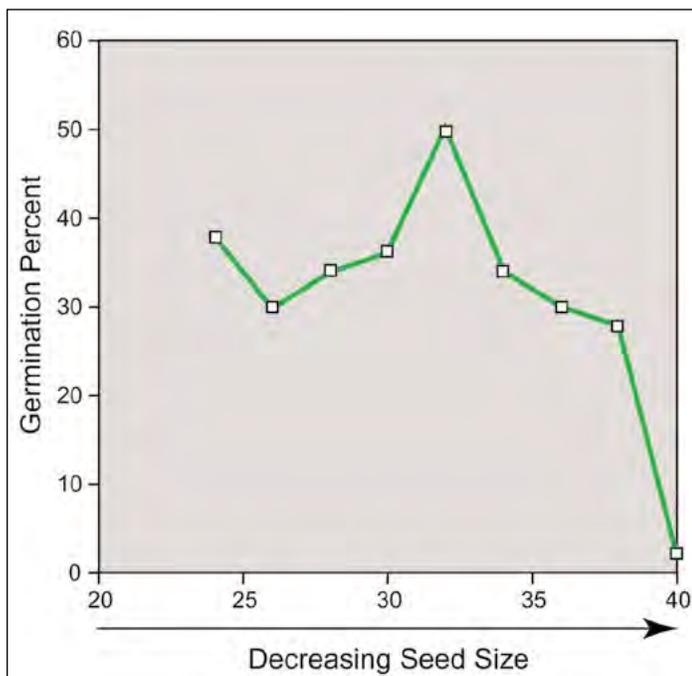


Figure 2. Effect of seed sizing on germination of *Artemisia tridentata* var. *wyomingensis*.

Fifty seeds from each size fraction were germinated at 15 °C (59 °F), and germination was counted at 7 days (figure 2). In this trial, most seeds had similar germination, with the exception of the smallest seeds (passing through the 38 by 38 screen), which did not germinate and should be discarded; these small seeds are likely immature or sterile. Among the remaining sizes, the larger the seed, the larger the 7-day-old seedling tended to be (figure 3). Seedling size differences such as these can have implications on the genetic diversity of the crop, seedling-to-seed ratios, and required cultural practices. These results indicate that native plant seed quality and performance can be improved by discarding smaller seeds to raise nursery efficiency and prepare seeds for advanced sowing technologies. Further trials on seed sizing of sagebrush seeds and seeds of other species, therefore, are warranted.

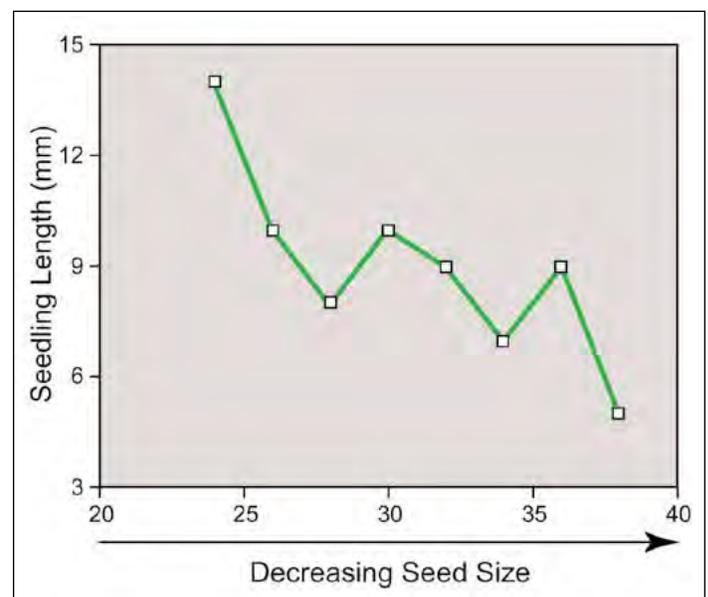


Figure 3. Effect of seed sizing on 7-day-old seedlings of *Artemisia tridentata* var. *wyomingensis*.

Equipment for Upgrading Seeds

The aspirator used to upgrade the sagebrush seeds was constructed from PVC (polyvinyl chloride) drainpipe for approximately \$100. If a vibratory feeder, such as shown in figure 4, is used, the cost increases to about \$600. Despite its low cost, the aspirator is very precise at cleaning seeds of a wide range of sizes from pine (*Pinus* spp.) to sagebrush. The aspirator is one of several inexpensive devices being developed at the NSL to meet the needs of small seeds and restoration operations that generally cannot afford an expensive set of commercially available machines but still need to produce high-quality seeds.



Figure 4. Seed aspirator constructed from PVC (polyvinyl chloride) drainpipe. (Photo by R.P. Karrfalt, 2014)

Developing Germination Protocols

NSL is developing germination protocols for several native species. The process begins by placing seeds in germination chambers programmed to provide four controlled constant temperatures: 10, 15, 20, and 25 °C (50, 59, 68, and 77 °F). Native plant seeds (either not stratified or stratified at 3 °C [37 °F] for 30 days) are tested for germination in each temperature. Longer stratification periods might be used in subsequent tests when dormancy is found. This process gives a total of eight germination tests in the initial screening for determining an optimum germination temperature and the presence of dormancy. Followup tests are conducted using the

best temperature(s) and those alternating temperatures close to the best temperature(s). Requests for the development of germination protocols can be submitted to the NSL. Seeds must be supplied to the laboratory for this research.

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