Preparing Seeds To Minimize the Risk of Seedlings Damping Off

Robert P. Karrfalt

Director (retired), National Seed Laboratory,
U.S. Department of Agriculture, Forest Service, Dry Branch, GA

Abstract

Damping off is a disease of newly germinated seedlings prior to their development of woody tissues. High-quality seeds invigorated with moist, cold stratification will germinate rapidly under suboptimal conditions, and therefore reduce the time a seedling is vulnerable to damping off. An optimal invigoration of the seeds requires stratification times that will result in sprouted seeds, unless moisture levels are properly balanced to be high enough for stratification to be effective, yet low enough to prevent premature radicle emergence. A four-step process for adjusting seed moisture and conducting cold moist stratification can reduce damping off risks. This paper was presented at the joint annual meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers' Association (Troutdale, OR, September 14–15, 2016).

Introduction

Cram (2003) gave a thorough review of damping off in forest and conservation nurseries in which she noted that seeds are sometimes the source of the disease, but environmental and soil factors are more significant in determining whether the disease organisms are present and if the disease develops. Applying fungicides and sterilants to the seeds is sometimes helpful but must be used with caution because of potential phytotoxicity. Cram also noted that damping off is a disease of very young seedlings that have not yet developed woody tissue, and that rapid seed germination is an important defense because it minimizes the time the seedlings are vulnerable. This article focuses on using quality seeds and cold, moist stratification to speed germination and reduce the number of days a seedling is at risk for damping off. The discussion predominately relates to desiccation-tolerant seeds, although some of the principles also apply to seeds that are desiccation intolerant, such as oaks (*Quercus* sp. L.).

Seed Vigor is Key

To understand seed vigor, we must first understand seed germination. Germination is the emergence of a seedling, from a seed, with all the essential parts necessary to produce a normal plant. Germination also refers to the number or percent of individual seeds germinating out of 100 seeds. In considering an entire seed lot, the terms "germination," "germination percentage," and "percent germination" are used interchangeably. Seed vigor is a relative measure comparing the performance among seed lots, within one species of plant, of similar germination. Vigor is expressed in three ways. One of these is how long a seed remains alive in storage. A seed lot that maintains high germination for 50 years is more vigorous than one that only maintains high germination for 10 years. A second measure is how fast germination occurs. Seed lots that complete germination in 7 days are considered to be more vigorous than those taking 14 days to complete germination. The final measure of seed vigor is how much germination occurs under sub-optimal conditions. A seed lot is more vigorous when, under environmental conditions less favorable to germination, it has higher germination than other lots. From the standpoint of protecting a seedling crop from damping off, it is the speed of germination and the ability to germinate under stress conditions that are the most important expressions of vigor. Both of these types of seed vigor can be enhanced through seed management. To realize high seed vigor in the nursery, seeds of highest quality are used, and they are stratified in a way that will produce a rapid and complete germination.

Producing Seeds of Highest Quality

As a general rule, a seed has its maximum vigor when it reaches physiological maturity on the mother plant, just before it is shed to the environment. With some species, this maturation process can be completed after the seeds are harvested, but generally it is best to delay harvest

until seeds reach maturity on the mother plant. Vigor continually decreases over time, from harvest through all the seed-processing steps. Species can vary considerably in their rate of vigor loss during the extraction and cleaning periods, but all are susceptible to loss. Therefore, seeds should be processed as promptly as possible and with no or minimal mechanical injury or other stress. Keeping the seeds dry during this process is also very important because higher moistures favor higher enzyme activity, which reduces seed vigor. Seeds are very hygroscopic and will readily take up moisture from the air if they are left exposed in open seed containers or machine hoppers. Therefore, keep seeds in moisture proof containers or covered with a moisture barrier unless they are actively being worked with. For some species, it could be necessary to monitor seed moisture while seeds are exposed to ambient conditions. The equilibrium relative humidity test (Karrfalt 2014) is a good way to test seeds that have not yet been finished as well as finished seeds. Seeds should not be left at high moistures for long, as vigor loss will be the result. The definition of "long" is somewhat relative to the amount of dormancy the seeds have and exactly how moist they are. High-moisture seeds can deteriorate even within 24 hours.

Although the period from harvest to optimal storage should be as short as possible, compromising care of the seeds for speed should never occur. Hasty seed cleaning leads to mechanical damage or an unfinished product. I have observed both a large western conifer and a southern pine seed cleaning facility that destroyed a large portion of a pine seed crop because the seeds were pushed too rapidly through the dewinging step. Enough time needs to also be invested in the removal of empty, insect- and fungal-damaged, and poorly developed seeds. These types of seeds are potential vectors of microorganisms, some of which can be pathogenic but can be removed with the right procedure Karrfalt (1983). A more complete discussion on seed cleaning can be found in Chapter 3 of the *Woody Plant Seed Manual* (Karrfalt 2008).

Rapid and Complete Germination

Obtaining high-quality seeds is one part to realizing a vigorous germination and lowering the susceptibility to damping off. Another part is to provide an optimal environment, including proper drainage, adequate and timely moisture, optimal temperature and light, and correct sowing depth (generally close or at the surface with a 0.25-in [6-mm] deep or less covering). The final part is to optimize seed imbibition and stratification to bring the first two parts together, especially for species that have seeds with dormancy. Dormancy is most frequently defined as the failure of a live seed to germinate when placed in environmental conditions favorable for germination. Another manifestation of dormancy is a slow and protracted germination. Therefore, optimized stratification will prepare a seed not only to germinate, but germinate promptly. Optimization usually involves extending the stratification period beyond what is needed to simply obtain germination with favorable environmental conditions.

Stratification is obligatory for dormant seeds, but can also improve the vigor of nondormant seeds. In one example of this improved vigor, Gosling and Rigg (1990) demonstrated that Sitka spruce (*Picea sitchensis* [Bong.] Carrière) seeds can be induced to germinate at suboptimal temperatures following cold stratification (figure 1). This benefit has also been observed with longleaf pine (*Pinus palustris* Mill.) and slash pine (*Pinus elliottii* Engelm.), two other species considered to be nondormant. Several nurseries now routinely stratify these two southern pines. Bonner et al. (1974) presented data that showed that loblolly pine (*Pinus taeda* L.) seeds cold-stratified for 60 days completed germination in 14 days, whereas seeds stratified for 30 days did not complete germination until approximately 21 days. Therefore, all other factors being equal, the longer stratification potentially reduced the time for damping off to develop by 7 days.

The challenge in stratifying nondormant species, or in stratifying dormant species for extended periods, is

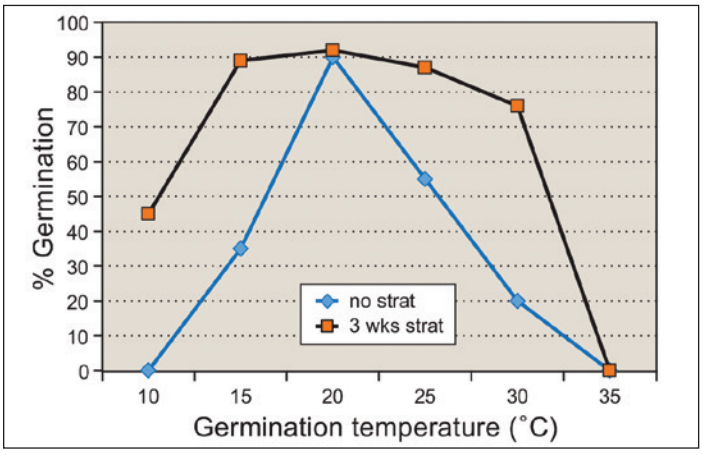

Figure 1. Cold stratification can invigorate Sitka spruce (*Picea sitchensis* [Bong.] Carrière) seeds, enabling them to germinate at suboptimal temperatures. (Adapted from Gosling and Rigg 1990)

to prevent sprouting, which precludes mechanical sowing. Seed moisture control is key in meeting this challenge. Seeds must have sufficient water to stratify, yet insufficient water to germinate. Traditional stratification procedures focused on providing sufficient moisture levels for stratification. Bonner et al. (1974) gave the instruction that, "Full imbibition is essential for stratification in plastic bags without moisture-holding media." Therefore, they recommended soaking the seeds in water overnight or even for 3 to 4 days. Although the water was drained off the seeds at the end of the soak period, some liquid water usually remained in the stratification bag. This extra moisture kept the seeds moist for breaking dormancy and provided the water needed to initiate radicle emergence, an undesirable condition that interfered with mechanical sowing. Keeping temperatures as low as possible without freezing helped restrain radicle emergence but not sufficiently for extended stratification periods. Therefore, the practice at nurseries has mostly been to shorten the stratification period to avoid radicle emergence and forfeit the advantage of invigorating the seeds for a faster germination. In more recent studies, however, lengthening stratification periods and mixing warm and cold periods have been found possible without radicle emergence if moisture contents were properly regulated (Edwards 1981, Gosling and Rigg 1990, Suszka et al. 1996).

Controlled Moisture During Stratification

To better understand the role of restricting moisture during stratification, we need to understand the three phases of water uptake by a seed, as it moves from the resting desiccated and dormant condition to active germination (figure 2). The first phase is a relatively rapid imbibition. During the second phase, very little water is absorbed, and the seed goes through preparatory metabolic steps to germinate. The third phase is again rapid, and a large uptake of water occurs as the embryo emerges from the seed. Therefore, to effectively stratify and avoid sprouting, water uptake must be arrested in the second phase. The following four-step process gives specific directions on how keep seeds at this second phase of water uptake.

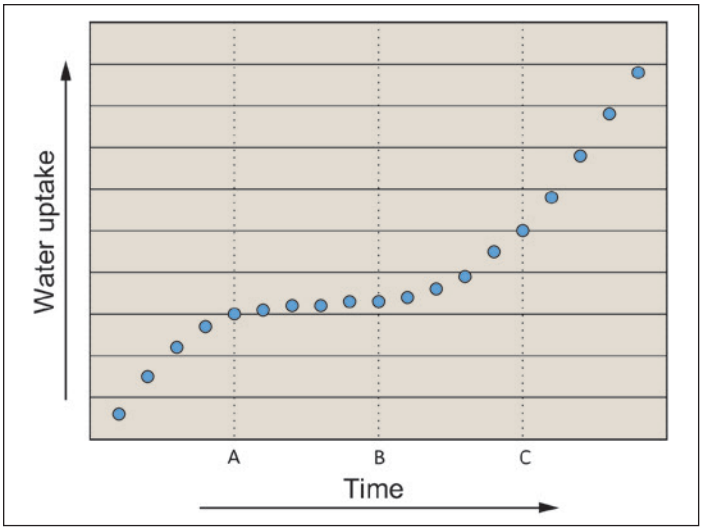

Figure 2. The three phases of water uptake as a seed germinates. Water uptake begins rapidly, then plateaus at point A when the seed is fully imbibed. Between point A and B, the water uptake is minimal as the seed prepares to germinate. From point B to point C and beyond, water uptake is again rapid as the seedling emerges from the seed.

Step 1—Imbibition

The first step in the process is to soak the seeds in water until they are fully imbibed. To determine how long this water soak must be, a series of weighing-soaking-reweighing cycles is followed. First, weigh a sample of seeds. Next, place the seeds in a water soak for approximately 24 hours, then drain off the water, surface dry the seeds, and weigh them again. Repeat this cycle until the weight stops increasing. Because very small increases can continue to occur for a long number of cycles, it is often difficult to decide when to stop the process. To give some perspective on when to end the process, I find it helpful to express the weight gain as a percentage of the original dry weight. Then full imbibition is considered the point when the weight increase is close to zero. For example, imbibition for whitebark pine (*Pinus albicaulis* Engelm.) is completed in 4 days, because the percent increase in seed weight is nearly zero by day 4 (figure 3). If possible, this process needs to be conducted on five separate samples, one from each of five seed lots. As a general guide, each individual sample should contain at least 300 to 500 seeds but not less than 1 oz (28 g). A two-place balance is sufficient for weighing. Determining the length of the water soak can be done at the nursery, or assistance can be obtained from the National Seed Laboratory (NSL; Dry Branch, GA) or other qualified laboratory.

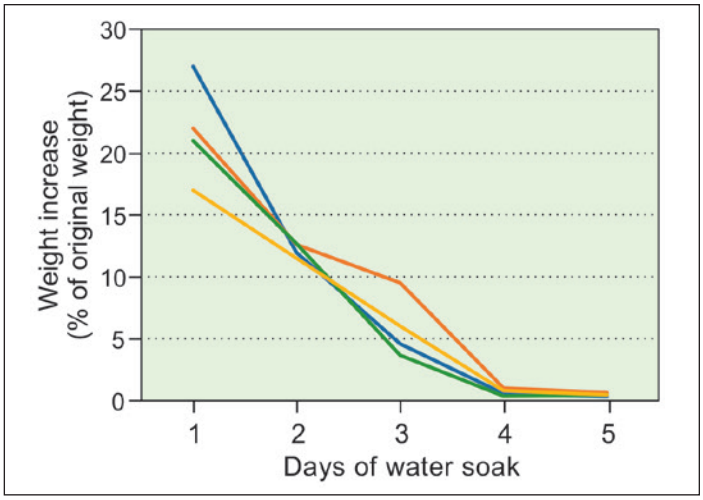

Figure 3. Whitebark pine becomes fully imbibed after 4 days of water soak.

Step 2—Water Rinse

Rinsing seeds with water is frequently practiced as an effective method to remove fungal spores from the seed coats. The imbibition phase is an ideal time to conduct this rinse. Soaking the seeds loose enables a daily stirring of the seeds to rinse their surfaces (figure 4). The water should be changed daily by pouring the seeds into a paint strainer, such as the E-Z Strainer™ (U.S. Plastic Corp., Lima, OH) (figure 5), from which they can easily be poured and rinsed back into a bucket for another day of soaking.

Step 3—Removal of Excess Water

At the end of the water soak, the excess water is removed in a two-stage process. The majority of water is first drained off in the strainer (figure 5). Then, the strainer is placed on a bucket connected to a wet-dry vacuum (figure 6). This connection can be made by simply inserting the vacuum hose into a hole cut into the side of the bucket or by attaching an electrical

Figure 4. Seeds are soaked in water until they are fully imbibed. (Photo by Robert Karrfalt)

Figure 5. The water is drained from the seeds using a paint strainer. (Photo by Robert Karrfalt)

connector to the bucket and an elbow (figure 7). The elbow can deflect the vacuumed water to the bottom of the bucket. The open strainer enables easy monitoring of the water removal process. Usually about 15 seconds of vacuuming is sufficient to remove the capillary water. Next, the surface film of water must be removed because, as demonstrated with Sitka spruce (Gosling and Rigg 1990), this surface film of water can lead to sprouting in stratification. Removing this film can be done by putting the seeds into a tumbler and passing a flow of dry air over the seeds as the tumbler rotates. A small concrete mixer is an easily acquired and low-cost tumbler. A pedestal fan is a good way to deliver the airflow because the height of the fan is usually adjustable to the height of the tumbler and can be positioned far enough from the tumbler so that no seeds are blown away. Removal of the paddles in the concrete mixer makes removing the seeds easier. When adequately dry, the seeds will look damp but no longer shiny (figure 8). The more humid the ambient conditions, the longer it will take to dry the seeds.

Step 4—Stratification

The final step in the process is to pour the seeds out of the tumbler and back into the strainer. Place the strainer into a polyethylene bag to keep the seeds moist and then into the stratification cooler, maintained at 2 to 3 °C for a time appropriate for the species or seed lot being grown. Appropriate stratification times for many species can be found in the *Woody Plant Seed Manual* (Bonner and Karrfalt 2008) or a laboratory seed test report on the specific seed lot. The former resource would give a general place to start, and the latter source would be more precise for the specific seed lot. Assistance in determining an appropriate time can also

Figure 6. A wet-dry vacuum is used to pull capillary water from the seeds. (Photo by Robert Karrfalt)

Figure 7. The disassembled base used to hold the paint strainer of seeds during the vacuuming of capillary water. The elbow should point to the bottom of the bucket. (Photo by Robert Karrfalt)

Figure 8. Seeds on left have a surface film of water. Seeds in center are properly surface-dried for stratification. Seeds at right are totally dry. (Photo by Robert Karrfalt)

be had by contacting the NSL. The bag should be 4 ml or less in thickness to facilitate gas exchange. Keeping the seeds in the strainer facilitates easy inspection for mold growth. If mold is detected, the rinsing and surface drying process should immediately be repeated. With the mold removed, the cleaned and re-dried seeds are returned to stratification. As a quality control measure, some strainers of seeds should be weighed upon entry into stratification and periodically reweighed to be sure the seeds are not drying any further. If much weight loss has occurred, return to the soak step for a period appropriate for the weight loss. The final weight of the surface-dried seeds following this second soak should be close to the weight at which the seeds were when originally placed into stratification.

Conclusion

The use of high-quality seeds that are adequately invigorated is one tool to prevent damping off in the nursery. The four-step process for adjusting seed moisture and conducting cold moist stratification allows for maximized invigoration while preventing premature radicle emergence that would make sowing the seeds difficult.

Address correspondence to—

Robert P. Karrfalt (retired); email: karrfalt@purdue.edu

REFERENCES

Bonner F.T.; McLemore B.F.; Barnett J.P. 1974. Presowing treatment of seed to speed germination. In: Schopmeyer C.S. ed. Seeds of woody plants in the United States. Agriculture Handbook 450. Washington, DC: U.S. Department of Agriculture, Forest Service: 126–135.

Bonner, F.T.; Karrfalt, R.P., eds. 2008. The woody plant seed manual. Agriculture Handbook 727. Washington, DC: U.S. Department of Agriculture, Forest Service. 1,223 p.

Cram, M.M. 2003. Damping-off. Tree Planters' Notes. 50(1): 9–13.

Edwards, D.G.W. 1981. A new pre-chilling method for true fir seeds. Proceedings, Joint Meeting of the Intermountain Nurseryman's Association and Western Forest Nursery Association. Gen. Tech. Rep. INT-109. Boise, ID: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station: 58–66.

Gosling, P.G.; Rigg, P. 1990. The effect of moisture content and prechill duration on the efficiency of dormancy breakage in Sitka spruce (*Picea sitchensis*). Seed Science and Technology. 18: 337–343.

Karrfalt, R.P. 1983. Fungus-damaged seeds can be removed from slash pine seed lots. Tree Planters Notes. 34(2): 38–39

Karrfalt, R.P. 2008. Seed harvesting and conditioning. In: Bonner, F.T.; Karrfalt, R.P., eds. 2008. The woody plant seed manual. Agriculture Handbook 727. Washington, DC: U.S. Department of Agriculture, Forest Service: 57–84.

Karrfalt, R.P. 2014. Assembling seed moisture testers, seed dryers, and cone dryers from repurposed components. Tree Planters' Notes. 57(2): 11–17.

Suszka, B.; Muller, C.; Bonnet-Masimbert, M. 1996. In: Gordon, A. translator. Seeds of forest broadleaves: from harvest to sowing. Paris: INRA: 1–294.University Extension. Ent. 178–15. Oct. 2015. http://extension.usu.edu/files/publications/publication/ENT-178–15.pdf. (January 2017).