

Whitebark Pine Germination: Is It Really That Difficult?

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Abstract

The U.S. Department of Agriculture, Forest Service, Dorena Genetic Resource Center (DGRC) has been producing whitebark pine (*Pinus albicaulis* Engelm.) seedlings for outplanting and for testing for resistance to white pine blister rust (caused by the exotic pathogenic fungus *Cronartium ribicola*) since 2000. During the past 15 years, DGRC has designed and implemented numerous studies to improve seed use efficiency and germination percentages. In 2015, three new stratification protocols were tested against operational protocols on eight seedlots from three national forests to examine differences in speed of germination and total germination. The stratification treatments included (1) 140-day stratification in sand, (2) presoak in 1,000 parts per million gibberellic acid and 140-day stratification, (3) 140-day stratification in peat moss, and (4) control (operational method). No significant difference in speed of germination among treatments was observed, but the seeds stratified for 140 days in sand had significantly higher total germination than all other treatments. This paper was presented at the annual meeting of the Western Forest and Conservation Nursery Association (Eugene, OR, October 26–27, 2015).

Introduction

Why Do We Still Care?

Whitebark pine (*Pinus albicaulis* Engelm.) is an important ecosystem component and is considered a “keystone” species in certain high-elevation northwestern forests (Tomback et al. 2001). The seeds are a major food source for a variety of mammals, ranging from the red and Douglas squirrels (*Tamiasciurus hudsonicus* and *T. douglasii*) to black and grizzly

bears (*Ursus americanus* and *U. arctos*). The Clark’s nutcracker (*Nucifraga columbiana*) also depends on whitebark pine seeds and is one of the main sources of whitebark pine seed dissemination (Mattson et al. 2001). Whitebark pine can be one of the first tree species to colonize an area following catastrophic disturbances, including fire and landslides, and to play a vital role in soil stabilization and cover for regeneration of other tree species. As one of the few tree species found in many alpine areas, mature whitebark pine trees can be an important contributor to high-country aesthetics.

Whitebark pine populations, however, are declining due to a number of factors, including mountain pine beetle (*Dendroctonus ponderosae*), fire, and global climate change. In addition, white pine blister rust, caused by the exotic pathogenic fungus *Cronartium ribicola*, is a significant threat to the survival of the species in the Pacific Northwest and western Canada (Aubrey et al. 2008). In July 2011, the U.S. Fish and Wildlife Service issued notice that listing of whitebark pine as threatened or endangered is warranted but currently precluded by higher priority actions. Whitebark pine currently resides on the candidate species list (USFWS 2011). In addition, the Canadian Government listed whitebark pine as Schedule 1 Endangered under its Species at Risk Act (Government of Canada 2016).

Why Are Seedlings So Expensive?

Very few commercial nurseries have produced whitebark pine seedlings during the past 20 years. Because whitebark pine is a high-elevation species, seeds are often difficult to obtain. Late-spring snow and cold can disrupt or delay flower pollination, resulting in either minimal seed crops or seeds that are immature when cone harvesting occurs in the

fall. Cone collection is also expensive. To prevent competition for seeds from nutcrackers and squirrels, cone-bearing trees, often located in remote sites, must first be climbed in the spring and early summer to cage conelets (figure 1). Trees must again be climbed in the fall to collect cones.



Figure 1. Caging whitebark pine conelets to prevent competition from nutcrackers and squirrels for seed crops. (Photo by Haley Smith, USDA Forest Service, 2016)

Whitebark pine seeds are difficult to extract from the cones, often requiring special extraction equipment or hand labor. In addition, due to a high lipid content, seed viability may be significantly reduced in long-term storage compared with other pines.

Whitebark pine seedlings are also expensive and challenging to produce (Overton et al. 2016). Seeds can be difficult to germinate, requiring special stratification, scarification, and handling during germination. Even with special handling, germination is erratic, depending on seed maturity. Seedlings are often slow growing and may require extended photoperiods during the growing season. Depending on the outplanting situation, seedlings from some seed sources may require three growing seasons before reaching the target size.

Previous Trials and Tribulations of Growing Whitebark Pine Seedlings at Dorena Genetic Resource Center

The U.S. Department of Agriculture (USDA), Forest Service, Dorena Genetic Resource Center (DGRC) is primarily a disease-resistance testing center and tree-improvement seed extractory in Cottage Grove, OR. The oldest program focuses on testing for resistance of five-needle pines to blister rust. Although western white pine (*Pinus monticola* Douglas ex D. Don) and

sugar pine (*P. lambertiana* Douglas) historically have been the main focus, DGRC has begun working with all five-needle pine species native to North America and also with many European species.

DGRC began growing whitebark pine seedlings for blister rust resistance-testing and outplanting trials in 2000. In early small trials, the seeds were stratified and handled in a similar manner to the traditional pine species, using extended cold-stratification and direct-sowing methods. Germination in these trials was poor to nonexistent, and the few seedlings that were produced were often damaged or lost to birds and mice.

Trials to improve germination and culturing methods were initiated in 2002, based on work done by Burr et al. (2001). During the past 14 years, studies have included work on stratification length, scarification methods, fresh versus stored seeds, long-term storage seed viability, and seed viability based on embryo size (table 1).

Operational seed-handling and germination protocols evolved at DGRC based on the results of these previous studies, eventually leading to operational protocols in use by 2014. Only seeds that had been stored for at least 1 year were sown for rust testing and outplanting. Seeds were placed in mesh bags (figure 2) and soaked for 24 hours in hydrogen peroxide (H_2O_2), rinsed, and soaked an additional 24 hours in water (H_2O). Mesh bags were placed in plastic tubs, placed in warm stratification at



Figure 2. Mesh bags used for stratifying individual seedlots in tubs. (Photo by Richard Sniezko, USDA Forest Service, 2009)

Table 1. Whitebark pine germination studies designed and implemented at Dorena Genetic Resource Center, 2002 to 2014.

Year	Objective	Treatments	Results
2002	Prestratification seed soak	24 hr H ₂ O ₂ 24 hr H ₂ O 48 hr running H ₂ O	No difference.
2002	Stratification length	30-d warm, 30-d cold, 30-d warm 60-d cold, 30-d warm, 90-d cold	Highest and most consistent germination with 30-d warm, 90-d cold.
2002	Germination temperature	17 °C day, 15 °C night 20 °C day, 18 °C night	Highest germination with higher temperature, but more moldy seeds.
2004	Seed scarification	Nicking Sanding	No difference; sanding much more consistent and safer.
2004	Photoperiod length	No extended photoperiod 18-hr photoperiod 24-hr photoperiod	No significant difference between 18- and 24-hr photoperiod; both better than no extended photoperiod.
2006	Embryo length	< 25% cavity fill 25 to 50% cavity fill 50 to 75% cavity fill	Seeds with embryos filling 50% of the cavity or greater are considered viable.
2006	Long-term storage seed viability	1-yr freezer storage 5-yr freezer storage 10-yr freezer storage	Better germination with storage lengths less than 5 years, but 10 years still exhibited good viability.
2010	Long-term storage seed viability	Repeat of 2006 trial with same seedlots	Seedlots stored for 14 years starting to lose viability.
2010	Fresh vs. stored seeds	Seeds from current year 1-yr freezer storage 5-yr freezer storage	Better germination with seeds freezer-stored for 1 to 5 years.
2013	Stratification length	Operational (30-d warm, 90-d cold) Extended (30-d warm, 110-d cold)	Better germination with extended stratification.
2014	Stratification length	Operational (30-d warm, 90-d cold) Interrupted (30-d warm, 90-d cold; 21-d warm, 30-d cold)	Slightly better germination with interrupted stratification, but very moldy seeds.

C = Celsius. d = day. H₂O = water. H₂O₂ = hydrogen peroxide. hr = hour. yr = year.

10 °C (50 °F) for 30 days, and moved to cold stratification at 1 to 2 °C (34 to 36 °F) for 110 days.

Upon completion of the stratification period, seeds were individually hand-scarified using sanding machines that were designed and built at DGRC (figure 3). Scarified seeds were placed on blotter paper in 10-x-10-x-2.5-cm (4-x-4-x-1-in) germination containers that were placed in a germinator at 19 °C day/17 °C night (66 °F day/63 °F night) with a 12-hour photoperiod (figure 4). As seeds germinated, they were sown into individually labeled containers (figure 5).

By 2014, germination of whitebark pine seeds under DGRC operational protocols ranged from 5 to 95 percent germination, depending on seed maturity, with an average germination of 71 percent. Although germination in most species depends on seed quality, maximum germination for even minimally viable whitebark pine seeds is important due to the high value of the seeds.



Figure 3. Sanding machine designed and constructed at DGRC for scarifying whitebark pine seeds before germination. (Photo by Judith Danielson, USDA Forest Service, 2009)

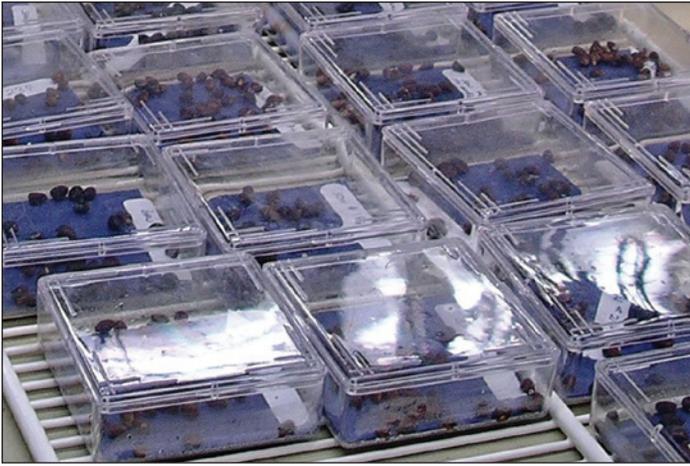


Figure 4. Whitebark pine seeds placed on blotter paper in 10-x-10-x-2.5-cm (4-x-4-x-1-in) germination containers to germinate before sowing. (Photo by Richard Sniezko, USDA Forest Service, 2010)



Figure 5. As whitebark pine seeds germinate, they are sown into individually labeled containers for emergence. (Photo by Judith Danielson, USDA Forest Service, 2009)

2015 Germination Study— Materials and Methods

In 2015, a small trial was designed and implemented to determine if three different seed pretreatment and stratification methods would result in improved germination over the standard DGRC protocols. Six hundred seeds from each of eight whitebark pine seedlots (table 2) were divided into three treatments plus a control, with three replications included in each treatment.

Table 2. Eight whitebark pine seedlots from three national forests spanning 3 collection years were included in the germination study.

Collection year	Seed origin (National Forest)	Seedlot ID	Percent filled
2009	Fremont-Winema	005030	90
2009	Fremont-Winema	005043	80
2009	Deschutes	011116	80
2010	Deschutes	011182	93
2010	Gifford Pinchot	350718	96
2010	Gifford Pinchott	350714	81
2011	Deschutes	011221	72
2011	Fremont-Winema	005164	80

Treatment 1 was a combination of DGRC protocols and protocols developed by the Alberta Tree Improvement and Seed Centre (Smoky Lake, AB, Canada) (Robb 2015). Seeds were placed in mesh bags and soaked for 48 hours in aerated water using an aquarium aerator. Bags were then layered in tubs of fine sand, and the tubs were placed in warm stratification at 10 °C (50 °F) for 30 days followed by cold stratification at 1 to 2 °C (34 to 36 °F) for 110 days. Seeds were not scarified at the end of the stratification period.

Treatment 2 was based on protocols DGRC has used to overcome internal dormancy in some native shrub species seeds. Seeds were placed in mesh bags and soaked for 24 hours in 1,000 parts per million (ppm) gibberellic acid (GA₃), rinsed, and soaked an additional 24 hours in H₂O. Bags were then placed in warm stratification (in plastic tubs) at 10 °C (50 °F) for 30 days followed by cold stratification at 1 to 2 °C (34 to 36 °F) for 110 days. At the end of the stratification period, seeds were scarified using the DGRC sander.

Treatment 3 was based on protocols that DGRC has used to soften hard seedcoats in some native shrub species seeds. Seeds were placed in mesh bags and soaked for 24 hours in H₂O₂, rinsed, and soaked an additional 24 hours in H₂O. Mesh bags were layered in plastic tubs containing peat moss and placed in cold stratification at 1 to 2 °C (34 to 36 °F) for 140 days. Seeds were not scarified at the end of the stratification period.

Treatment 4 was the standard DGRC protocol and was considered the control. Seeds were placed in mesh bags and soaked for 24 hours in H₂O₂, rinsed, and soaked an additional 24 hours in H₂O. Mesh bags were placed in plastic tubs, placed in warm stratification at 10 °C (50 °F) for 30 days followed by cold stratification at 1 to 2 °C (34 to 36 °F) for 110 days. At the end of the stratification period, seeds were scarified using the DGRC sander.

After seed pretreatments, seeds from all treatments were subjected to standard germination testing. Seeds were placed on blotter paper in 10-x-10-x-2.5-cm (4-x-4-x-1-in) germination containers in a germinator at 19 °C day/17 °C night (66 °F day/63 °F night) with a 12-hour photoperiod. Germination on all treatments was tracked every day for 3 weeks beginning 4 days following placement of seeds into the germinator. Seeds were considered germinated when the radical protruded at least 1 mm (0.04 in) and was curved.

2015 Germination Study—Results

No significant difference was found among treatments in speed of germination; however, significant differences were found among treatments in total germination (figure 6). Seeds that were stratified in peat without

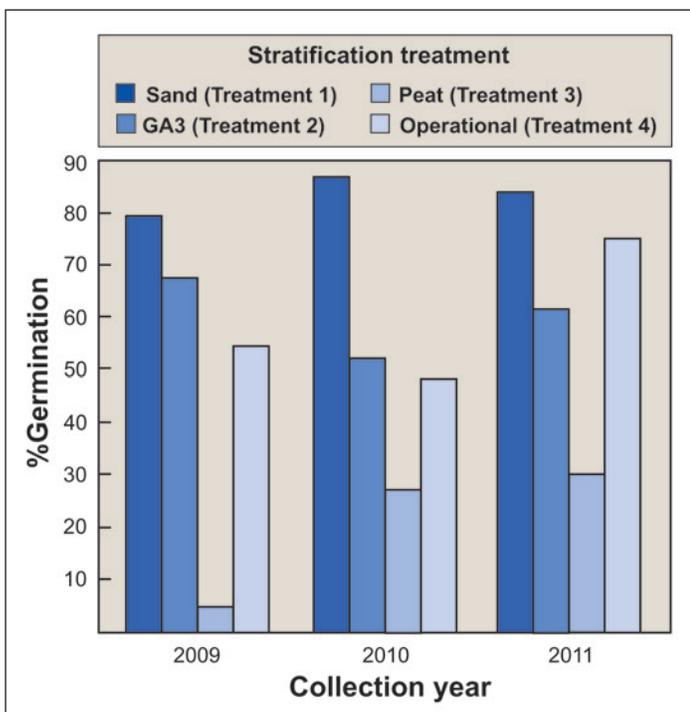


Figure 6. Significant differences were found among four stratification treatments for eight lots of whitebark pine from 3 collection years. Stratification in sand was significantly better than stratification in peat, presoak in GA₃, or the operational method used at Dorena Genetic Resource Center.

scarification (treatment 3) had significantly lower germination than all other treatments. Seeds that were soaked in GA₃ before stratification and scarified before germination (treatment 2) were significantly lower than the standard DGRC method (treatment 4) or the Alberta protocol (treatment 1). Seeds from treatment 2, however, were far less moldy than all other treatments, and the GA₃ had turned the seedcoats in all lots black. Seeds that were soaked in aerated water, stratified in sand, and not scarified (treatment 1) had significantly higher germination than those receiving the standard treatment used at DGRC (treatment 4). Seeds in treatment 1 also developed much less mold throughout the germination period than those from the control treatment.

Discussion and Conclusions

Three pretreatment and stratification methods for whitebark pine seeds were tested against standard protocols used at DGRC in an attempt to increase germination and reduce labor costs. These methods were based on protocols used to overcome both internal and external dormancy in conifers and other native species.

Presoaking of seeds in GA₃ is a common method used to overcome internal seed dormancy in a variety of native and commercial species. Depending on the species, GA₃ concentrations for presoak can range from 250 to 2,000 ppm. Several studies have found, however, that higher concentrations of GA₃ can actually inhibit germination (Machado de Mello et al. 2009; Rojas-Arechiga et al. 2011). The presoak for whitebark pine seeds used in this study was based on that used with other native species at DGRC. It is possible the concentration used in this study was higher than needed for this species and could have inhibited germination.

Layering seeds in peat has been used as a substitute for the warm stratification period sometimes required to soften seedcoats in several conifers and native species; for example, western white pine, rose species (*Rosa* spp.), and dogwood species (*Cornus* spp.). Seeds stratified in this medium, however, are often moldy at the end of the cold-stratification period, and germination may be affected by this surface mold. The whitebark pine seeds in peat in this study were quite moldy at the end of the 140-day stratification period, and germination may have been reduced as a result.

Stratifying seeds in sand without scarification may be one method to streamline the germination process. In similar studies, Robb (2015) found that seeds layered in sand are subject to less changes in moisture content than seeds stratified in bags in tubs. Therefore, seeds remain fully imbibed throughout the stratification period, and seedcoats are softened without scarification. Seed scarification by hand can be very erratic, can injure the seed, and depends on the experience of personnel. Stratification in sand removes those variables.

Although only eight lots from three national forests were used in this study, the resulting germination percentages were encouraging. Further testing is needed before any decisions can be made to switch standard protocols.

In the fall of 2015, all the whitebark pine seedlots for both operational blister rust resistance testing and outplanting (222 seedlots from Washington, Oregon, and British Columbia) were equally divided into the standard DGRC protocols and the protocols used for stratifying in sand without scarification. Germination tracking will take place in the spring of 2016. If the new protocols prove effective for seeds across this large geographic range, further testing with these methods will include direct seeding versus pregermination, reducing stratification length, and testing this method on other hard-to-germinate species.

As demand for whitebark pine seedlings for outplanting increases, the number of nurseries interested in growing this high-value species will also increase. Unless germination and growing protocols become more efficient and less labor intensive, however, it may not be cost effective for production nurseries.

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