Resolving Western White Pine Seed Germination Differences Between Lab Testing Protocols and Operational Greenhouse Protocols at Webster Nursery

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Abstract

The efficient production of western white pine seedlings (*Pinus monticola* Douglas ex D. Don) has historically been a challenge for growers in the Northwestern United States. Great progress has been made in unlocking the dormancy of this species, resulting in better, more uniform seedling crops, but room for improvement remains. This article addresses the discrepancy between seed stratification protocols for lab germination tests, on which sowing calculations are based, and stratification protocols for operational sowing. The goal was to develop a repeatable lab stratification approach to mirror as closely as possible the results of improved operational stratification methods. Results showed that alternative lab stratification approaches outperformed standard lab protocols, thereby providing a better measure of operational greenhouse performance. Comparisons between this study, which was performed without bleach as a surface sterilant, and an earlier trial of the same seedlots using a bleach rinse, suggest that a surface treatment can elevate germination capacity in western white pine.

Introduction

The deep seed dormancy of western white pine (*Pinus monticola* Douglas ex D. Don; WWP) has resulted in low nursery performance expectations relative to other conifers in the northwestern United States for many years. Extensive research into stratification techniques by the British Columbia Ministry of Forests Tree Seed Centre (BCTSC); now the BC Ministry of Forests, Lands, and Natural Resource Operations) and operational greenhouses in recent years has brought the quality and consistency of nursery crops to a level comparable with other species (Kolotelo 2013). These advances are significant, because the high cost of WWP seed bred for resistance to white pine blister rust (*Cronartium ribicola* J.C. Fisch), along with the large seed size of this species and its historically inconsistent germination performance, combine for very high per-seedling costs.

Despite recent advances, some difficulties in overcoming dormancy in WWP still remain. The WWP germination testing protocols used by seed labs in northwest North America accredited by the International Seed Testing Association (ISTA) have not been modified to reflect recent research and continue to report chronically low germination capacities. On the other hand, container nurseries in northwest North America have modified operational WWP germination protocols over the years in response to ongoing research and now achieve high germination capacities and vigorous germination rates. The problem lies in where these two processes come together. Seed sowing calculations are based on lab germination capacities rather than operational greenhouse germination capacities. As a result, WWP sowing rates (based on lower germination capacities) are often higher than they need to be to achieve the desired number of seedlings, resulting in the waste of expensive seed. Seeds that would not normally be released from dormancy under current lab stratification protocols germinate successfully under operational protocols, which results in higher greenhouse thinning costs as the excess seeds germinate at higher rates than predicted by lab data (figure 1).

Figure 1. Operational greenhouse germination of Seedlot 643, showing multiple germinants per cell. (Photo by Jeff deGraan, 2013)

The discrepancy in germination protocols has a genetic component as well. Seeds subjected to stratification protocols, which result in uniform dormancy release followed by homogeneous germination, are given the opportunity to initiate growth concurrently. Differences in seed dormancy levels as well as in germination speed result in size differences among multiple-sown germinants, and greenhouse thinning to leave the largest germinants will likely favor parents that produce less dormant, faster germinating seed (Edwards and El-Kassaby 1996). For WWP, the favored characteristics at thinning in the nursery may not be aligned with those that promote blister rust resistance in the woods, so some families displaying resistant attributes could be lost.

Project Objectives

Our goal with this project was to develop a new lab stratification protocol to better mirror current operational stratification practices at the Washington Department of Natural Resources Webster Nursery Greenhouse (WA DNR Webster Nursery) and thus optimize seed use and reduce associated costs. A successful lab protocol will meet the following criteria:

- Identify the variables that can impact germination.
- Reflect operational protocols so that factors such as moisture content, stratification, and germination assessment are comparable.
- Consistently reflect germination performance of operational stratification protocols with an acceptable degree of accuracy.

Similar efforts to reconcile lab and nursery results have been made by the BCTSC and others (Danielson 1985, Kolotelo 2001, Kolotelo and others 2001). To place official lab testing protocols in perspective, seed testing is not intended to reproduce the conditions under which seed may be sown, rather, it seeks to produce conditions and procedures so that results of different labs can be compared (Edwards and Wang 1995). One of our goals is to determine if a consistently repeatable lab protocol can return results in line with operational nursery results.

If successful, this new lab method should better reflect the optimum germination capacity of WWP seed at WA DNR Webster Nursery, taking into consideration processes specific to that facility. Development of the optimum protocol should result in greater seed-use efficiency, reduced greenhouse labor costs, and potential expression of a fuller array of blister rust-resistant parents.

Variables Influencing Comparisons Between Lab and Operational Greenhouse Germination Practices

For this project, variations exist not only among the treatments, but also between our proposed protocol and the operational practice it is designed to try to reproduce. Therefore, results will likely be dependent on how practices at individual nurseries are conducted. The following paragraphs describe some of the potential sources of variation that influence seed germination protocols.

1. Variations in seedlots

   Seed dormancy varies among seedlots as a result of differences among trees and stands, crop years, and in response to cone collection timing (Kolotelo 1997, 2002a; Wang and D'Eon 2003). Most of these variables cannot be controlled.

2. Variations in imbibition between operational greenhouse practices and lab trial methods

   In operational stratification, seeds are often placed into 1.0 to 5.0 gal (3.8 to 18.9 L) mesh bags, keeping them in contact with their neighbors on all sides and providing fairly consistent and resilient moisture levels during stratification (Kolotelo 2001). Were this same approach downsized to accommodate an operational lab trial, one could expect smaller mesh bags to result in stratification moisture levels that were more variable and less resilient. In the lab, germination trays are used for standard germination testing, and seeds are isolated from the outside environment but no contact is made among them. As a consequence, moisture levels could vary significantly from those of the operational approach. Kolotelo (2001) found that lab germination tray samples at the BCTSC were maintained in stratification with an average moisture content 3.4 percent higher than operational greenhouse seedlots.

   Other variables related to moisture content that should be taken into account are the effect of water temperature on imbibition (Kolotelo and others 2001, Feurtado and others 2003) and the precision of the moisture meters employed in making comparisons between methods.

3. Variations in germination environments between operational greenhouse practices and lab methods

   Lab germination is conducted in closed containers in a clean, controlled environment, while greenhouse germination takes place in a less clean, more variable environment. Temperature ranges can be greater in a greenhouse setting as well (Kolotelo and others 2001) and daily photoperiod

is manipulated to promote growth. Operational greenhouse germination at Webster Nursery takes place in conditions of 16 to 18 hours of daylight and 6 to 8 hours of darkness, with a mean temperature of 70 °F (21 °C) for both day and night. The temperature in this setting can occasionally reach 85 °F (29 °C) depending on outside environmental conditions. Operational lab germination for the purposes of this study occurs in alternating cycles of 86 °F (30 °C) in daylight for 8 hours and 68 °F (20 °C) in darkness for 16 hours. One could expect these environmental variations to result in differential germination rate and capacity.

4. Variations in how germination is defined

   One difficulty cited by Kolotelo and others (2001) in making a comparison of lab germination and operational germination is the different measures of success between the two. By lab standard, a seed is germinated when its radicle length has extended to four times the seed length. In the greenhouse, a seed is characterized as germinated when its cotyledons are unfolded and have begun photosynthesis, at which time the radicle is approximately 10 times the seed length. It is conceivable that a seed defined as germinated in a lab setting may never reach the greenhouse threshold.

   The trial described in this article is a step in the process toward evaluating the impact of some of these variables, with an eventual goal of identifying the best pairing of an optimal operational protocol with a lab test protocol.

Materials and Methods

Eight WWP seedlots were chosen for the trial. Five of these seedlots were from the Inland Empire Tree Improvement Cooperative's R.T. Bingham seed orchard (Moscow, ID) and the other three were from the U.S. Department of Agriculture, Forest Service Coyote Seed Orchard (Vancouver, WA) and Dennie Ahl Seed Orchard (Shelton, WA). The lab germination trials were developed in collaboration with WA DNR Webster Nursery and were conducted at the WA DNR Seed Center (both in Olympia, WA).

Three stratification treatments were included in the trial:

1. Association of Official Seed Analysts (AOSA) Standard—A 24-hour soak with 90-day cold stratification in germination trays.
2. Webster Operational-based—A 14-day running-water soak in mesh bags followed by 30-day high moisture content, then 90-day low moisture content, cold stratification regime.
3. Low Moisture Operational-based—A 14-day running-water soak in mesh bags followed by a low moisture content regime.

Treatment 1—AOSA Standard

This protocol was based on AOSA "Rules for Testing Seeds" (AOSA 2007), which have been validated by the ISTA. Representative 400-seed samples for each of the 8 seedlots were collected and soaked for 24 hours in a capped plastic vial filled with tap water at room temperature 64 to 72 °F (18 to 22 °C). No seedcoat sanitation process, such as bleach or hydrogen peroxide, is described in the AOSA guidelines. The excess water was drained from the vials to bring the samples to an initial moisture content of 45 percent as measured by a Steinlite model 400G moisture meter. Seeds from each seedlot were then split into eight 50-seed samples and spread out evenly onto Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, labeled by treatment, and placed into a cooler at 35 °F (1 to 2 °C) for 90 days' stratification before placement in a germination chamber.

Treatment 2—Webster Operational-Based

The Webster Operational-based lab protocol follows WA DNR Webster Nursery's operational naked stratification for WWP, which was developed from the method described in the British Columbia Ministry of Forests *Seed Handling Guidebook* (Kolotelo and others 2001). Operational practices incorporate a Trimaco Supertuff 1.0 to 5.0 gal (3.8 to 18.9 L) paint strainer bag for imbibition and stratification of seedlots up to 2 lb (900 gr) in weight. For this treatment the representative 400-seed samples from each seedlot were placed into individual 100 percent nylon Organza 4 by 6 in (10 by 15 cm) mesh bags, tied off at the top, and subjected to a 14-day full-immersion, running-water rinse alongside operational greenhouse seedlots. The study plan for the operational-based treatment included a 10-minute soak in a 2:3 solution of 5.25 percent household bleach: water, followed by a 10-minute water rinse before the 14-day running-water rinse, to follow operational Webster Greenhouse practices. This step was inadvertently left out of all operational-based treatments in our trial, creating a significant variable between operational-based treatments and actual operational stratification. Research has shown that running-water treatments and pre-stratification bleach treatments can reduce surface-borne pathogens and increase germination capacity in some pines (*Pinus* spp.) (Axelrood and others 1995, Kolotelo and others 2001). Bleach treatments are specifically recommended for both high-value seed orchard seeds and high-risk species like WWP (Campbell and Landis 1990). Some growers

prefer a 3-percent hydrogen peroxide solution for its reduced occupational-worker risk. For both of these chemicals, a post-immersion water rinse is important to minimize damage to seeds (Kolotelo and others 2001).

Following the running-water rinse, the seeds in the mesh bags were dried to a moisture content of 45 percent as measured by a Steinlite model 400G moisture meter, and the filled mesh bags were placed into a cooler at 35 °F (1 to 2 °C) for 30 days of stratification. During this period, the mesh bags were manipulated weekly to ensure uniform moisture content and were visually monitored for the presence of pathogens (Landis and others 1998). Following the 30-day period, the seed was removed from the mesh bags, dried to 35 percent moisture content, returned to the mesh bags, then stratified at 35 °F for an additional 90 days. One strategy behind this dual moisture content approach is to maintain sufficient surface moisture during the first stratification period to promote dormancy release, then to reduce moisture content during the second stratification period to minimize the spread of seedborne pathogens (Kolotelo and others 2001).

It proved difficult to maintain the moisture content at the desired 45 percent throughout the initial 30-day stratification period for this protocol, This problem is found to a lesser degree with the greenhouse's operational stratification, and reflects one of the characteristics observed by Kolotelo (2001)—the difficulty in producing equivalent results and conditions with both small and large quantities of seed.

At the end of the 120-day stratification period, the 400-seed samples for each of the 8 seedlots were removed from 35 °F (1 to 2 °C) conditions, a moisture content measurement was taken, and each sample was split into eight 50-seed replications. Each replication was spread evenly onto Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, and labeled by treatment before placement in a germination chamber.

Treatment 3—Low Moisture Operational-Based

The Low Moisture Operational-based lab protocol was developed as a simplified version of WA DNR Webster Nursery's stratification process. This treatment explores the lower end of the recommended range of moisture contents described by Edwards (1982) for removal of physiological dormancy with a goal of creating a condition less favorable for pathogen growth during the long stratification. The 8 representative 400-seed samples for this treatment were placed into individual 100 percent nylon Organza 4 by 6 in (10 by 15 cm) mesh bags, tied off at the top, and subjected to a 14-day full-immersion running-water rinse alongside operational greenhouse seedlots. As with treatment 2, the bleach treatment was in the study plan but was inadvertently left out of treatment 3.

Following the running-water rinse, the seed was surface dried in the mesh bags to a moisture content of 30 percent, as measured by a Steinlite model 400G moisture meter, and the mesh bags containing the seed were placed into stratification at 35 °F (1 to 2 °C) for 120 days. During this period, the mesh bags were not manipulated or closely monitored for the presence of pathogens.

At the end of the 120-day stratification period, the 400-seed samples for each of the 8 seedlots were removed from 35 °F (1 to 2 °C) conditions, a moisture content measurement was taken, and each sample was split into eight 50-seed replications. Each replication was spread evenly on Anchor Paper Steel Blue Seed Germination Blotter Crocker #7 paper in 4.0 by 4.0 by 1.5 in (10.0 by 10.0 by 4.0 cm) plastic germination trays. The germination trays were then capped, sealed in 2-mil plastic bags, and labeled by treatment before placement in a germination chamber.

Germination Measurements

Germination trays for each treatment were placed into a Hoffman SG30 germination chamber, in which they were subjected to alternating cycles of 86 °F (30 °C) under lighted conditions for 8 hours and 68 °F (20 °C) under darkened conditions for 16 hours. Four germination counts were performed at 7-day intervals with the first at day 7 (figure 2). Each count included tallying and removing germinants whose radicle exceeded four times the length of their seedcoat. During these counts, abnormal germinants were recorded and discarded. At the end of the 4-week evaluation period, both the rate of germination and the cumulative germination capacity were calculated.

Assessment of Seedborne Pathogens

The long stratification required by WWP provides conditions favorable to the development and spread of several seedborne pathogens. Of these, *Fusarium* spp. and *Caloscypha fulgens* are often the most damaging. Seed moisture in excess of that needed for stratification can increase the growth of seedborne

Figure 2. Germination tray showing germinants for Seedlot 643 at day 7. From left to right: Treatments 1, 2, and 3. (Photos by Sheree Pickens, 2012)

pathogens, which is why surface drying is so important (Kolotelo and others 2001). *C. fulgens* can spread rapidly from diseased to healthy seeds during stratification, killing them before they have an opportunity to germinate. It can also infect adjacent seeds in multiple-sown cavities (Kolotelo and others 2001, Kolotelo 2013). *Fusarium* can also spread through contaminated seedlots during imbibition and stratification (Axelrood and others 1995, Kolotelo and others 2001).

Levels of pathogen infestation in the germination trays were observed and noted, but no attempt was made to identify specific fungi. The characterization of fungi was intended solely as a comparison of moisture contents and pre-stratification rinsing methods among the treatments. A useful description for identifying specific seedborne fungi can be found in Campbell and Landis (1990).

Statistical Analyses

Analysis of variance (ANOVA) was used to test the statistical significance of differences among treatments, seedlots, and the interaction between treatment and seedlot. The response variables for the ANOVAs were the number of seeds that had germinated by day 7 (initial germination) and by day 28 (final germination) (Neter and others 1996). Because most germination was expressed by day 14 for all seedlots, values for day 28 were considered to be representative of days 14 and 21 for the purposes of this analysis. Means for different treatments and seedlots were compared using linear contrasts and the error rate was controlled using the Tukey Honestly Significant Difference method (Neter and others 1996). ANOVA model terms were considered statistically significant at $\alpha < 0.05$. Analyses were conducted with the R statistical language and with the lsmeans package (Lenth 2013).

Results

Germination Rates and Capacities

The effects of stratification treatment, seedlot, and the treatment by seedlot interactions were all statistically significant for both initial and final germination (table 1, figure 3). Both treatment 2 (Webster Operational-based treatment) and treatment 3 (Low Moisture Operational-based treatment) had significantly greater initial germination rates than treatment 1 (AOSA standard treatment) for all seedlots. Initial germination was greater in treatment 3 than in treatment 2 in most cases, but differences among these treatments were only significant for Seedlot 1215. Treatments 2 and 3 also tended to have greater final germination than the AOSA standard treatment; however, the differences were not statistically significant for all of the seedlots (figure 3).

Pathogen Levels

As mentioned previously, an intended bleach treatment was not applied, so pathogen levels were potentially higher than they might have been. Observations of fungal infestations were made at a treatment level, rather than at a seedlot

Table 1. Summary of analysis-of-variance results testing the effects of stratification treatment, seedlot, and treatment by seedlot interactions on the number of initial germinants and final germinants of eight seedlots of western white pine.

Effect	DF	Initial germination (day 7)		Final germination (day 28)	
		F-Value	P-Value	F-Value	P-Value
Treatment	7	50.6	< 0.001	47.7	< 0.001
Seedlot	2	426.8	< 0.001	95.2	< 0.001
Treatment by seedlot	14	6.3	< 0.001	9.4	< 0.001
Error	168				

DF = degrees of freedom

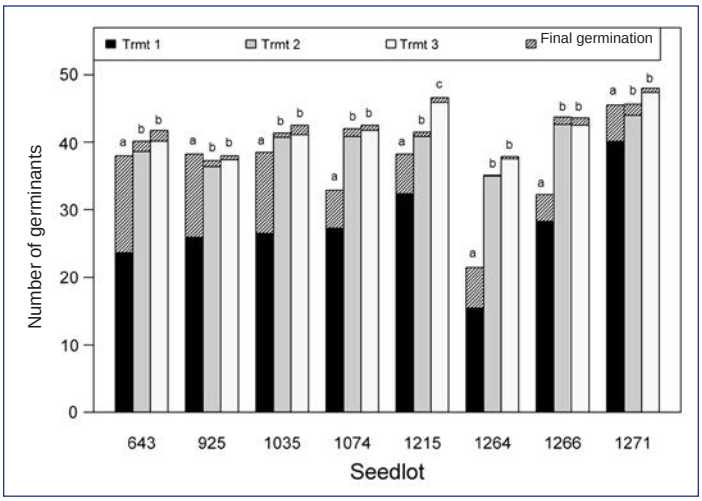

Figure 3. Germination capacity of eight seedlots for each of the three stratification treatments. Solid filled bars indicate germination capacity at day 7 (initial germination) for each treatment and diagonal patterned bars show germination capacity for each treatment at day 28 (final germination). Bars with different letters had significantly different germination capacity at day 7. (Peter Gould, 2013)

level, and were not quantified, so no patterns within seedlots could be established. For the AOSA Standard treatment, considerable fungi were present in the germination trays at the end of the 90-day stratification period. This condition was probably largely because of the absence of a running-water rinse in this treatment to cleanse the seedcoat (Axelrood and others 1995) and the lack of air exchange in the germination trays during the cold stratification and subsequent warm germination period. The Webster Operational-based treatment had a level of fungus in the germination trays less than that of the AOSA Standard treatment but still considerable, possibly because of the initial higher moisture stratification period, which provided favorable conditions for the spread of pathogens (Sutherland 1981, Cram and Fraedrich 2009). No fungi were present on seeds stratified with the Low Moisture Operational-based treatment, which was somewhat surprising given the largely unmonitored 120-day stratification period.

Discussion

Treatments 2 and 3, which included a 14-day rinse rather than a 24-hour soak, had better germination rates, better germination capacities, and lower pathogen levels. This supports research that such extended stratification treatments promoting thorough seed imbibition, the flushing of growth inhibitors from the seed coat and other structures, and the breaking of physical dormancy significantly improve germination (Kolotelo 1993; Bewley and Black 1994; Landis and others 1998; Bower and others 2011).

Higher germination rates and fewer seeds-per-cell sown as a result of higher germination capacities reduce initial greenhouse crop variation and accelerate germinant emergence. Benefits include higher overall crop quality, more complete genetic expression, and better seed use efficiency (Kolotelo and others 2001).

Seedlot 1264 is a specific example of the potential benefits of these alternate stratification approaches. Morphological observations made when the cones for this seedlot were initially received suggested that the collection was made before the seeds were mature. The initial lab test following collection returned a germination capacity of 31 percent, and a retest 1 year later showed that the germination capacity had increased to 45 percent—still a very poor result. The AOSA Standard protocol used in this study resulted in a germination capacity of 43 percent, whereas the Webster Operational-based and the Low Moisture Operational-based protocols resulted in germination capacities of 70 and 76 percent, respectively (figure 4). In this case, a seedlot that might have been designated for disposal was shown to be suitable for operational sowing when using an alternative stratification protocol.

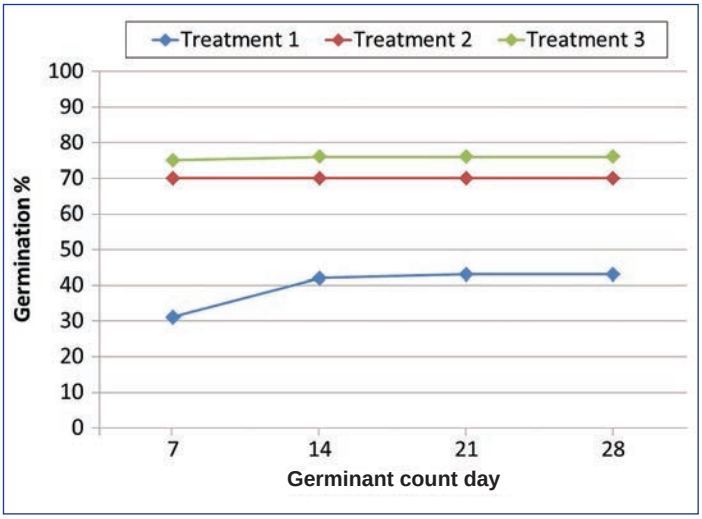

Figure 4. Cumulative germination percentage over time for Seedlot 1264, showing improved performance of alternative stratification treatments for this seedlot collected early in the season. (Sheree Pickens, 2012)

Potential seed savings as a result of the Webster and Low Moisture Operational-based alternatives are also illustrated in Seedlot 1074. Kolotelo and others (2001) describe the use of a "125 Percent Green Tree Count" to ensure that container seedling orders are fulfilled and that the probability of a viable seedling in each cavity is close to 100 percent. As part of this approach, the publication includes a table, which describes the number of seeds per cavity that must be sown to attain this goal. We used this table to determine values for Seedlot 1074 (table 2). At the AOSA Standard protocol, the germination capacity of 66 percent for Seedlot 1074 requires a sowing rate of 5.22 seeds per cavity to meet the 125 percent green tree count. Germination capacities of this same seedlot for the Webster Operational-based and Low Moisture Operational-based protocols were 84 and 85 percent, respectively, which would require sowing rates of 3.43 and 3.30 seeds, respectively, per cavity to meet the 125 percent green tree count—a 35-percent reduction in seed needed compared with the AOSA Standard protocol.

Table 2. Cumulative germination over time for Seedlot 1074, demonstrating improved seed use efficiency of alternative stratification treatments. The "125 Percent Green Tree Count" refers to a sowing approach that produces 25 percent extra seedlings beyond the requested amount from which to select shippable trees (Kolotelo and others, 2001).

Seedlot	Treatment	Germination capacity (%)	Seeds to sow per cavity to achieve 125% Green Tree Count
1074	1	66	5.22
1074	2	84	3.43
1074	3	85	3.30

Lower WWP sowing rates as a result of optimized lab germination tests not only result in seed savings, but they lead to better expression of blister rust-resistant families, as the amount of thinning is reduced (Landis and others 1998; El-Kassaby 2000). Single seed or individual family sowing would be ideal to ensure full expression of desirable traits (El-Kassaby and Thomson 1996), but consistent WWP germination capacities to make this approach economically feasible have not yet been realized.

Pros and Cons of the Webster Operational-Based Treatment Versus the Low Moisture Operational-Based Protocol

Although the Low Moisture Operational-based protocol (treatment 3) returned slightly higher germination capacity and lower pathogen levels, drawbacks to using this protocol could exist. The moisture level used in this protocol was at the low end of the range recommended for dormancy release, so it is possible that families within particular seedlots may not have full-dormancy release, resulting in overall germination performance poorer than that of the Webster Operational-based treatment (treatment 2). This possibility should be weighed against the absence of pathogens seen in the Low Moisture Operational-based treatment.

Implications for Future Practices at Webster Nursery

The most obvious drawback of this trial was the fact that no sanitation rinse was used in the treatments. Based on past research, it can be assumed that the addition of a bleach rinse to the stratification process would result in lower pathogen levels and possibly even higher germination capacities as well. A similar earlier version of the trial supports this assumption. In the earlier trial, a bleach rinse applied to five of the same seedlots resulted in average germination counts 13 and 17 percent higher for the AOSA Standard treatment and the Webster Operational-based treatment, respectively, at day 7 compared with the later bleach-free trial described here. For all of the seedlots tested in the previous trial with the bleach rinse, the Webster Operational-based treatment resulted in germination capacities in excess of 94 percent, which approaches an efficient one-seed-per-cavity sowing.

In addition to ensuring that a bleach rinse is incorporated, subsequent modifications will likely include monitoring of water temperatures, further efforts to address the difference between a successful lab germinant and a successful greenhouse germinant, more detailed fungal assays, and closer tracking of moisture contents throughout the stratification process. As recommended by Kolotelo and others (2001), quantifying and evaluating heat sums of different stratification protocols as they relate to germination speed may also be useful. In addition, we plan to compare the results described in this article with future trials with seeds from the same seedlots sown into an operational greenhouse setting for a real-world comparison.

We encourage other greenhouse nurseries that work closely with seed testing facilities to conduct similar trials of their own to evaluate and compare WWP stratification practices. The sharing and refinement of these results over time could eventually lead to modifications in official seed testing rules to more closely mirror operational practices.

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References

Association of Official Seed Analysts (AOSA). 2007. Rules for testing seeds. Stillwater, OK: Association of Official Seed Analysts. 125 p.

Axelrood, P.E.; Neumann, M.; Trotter, D.; Radley, R.; Shrimpton, G.; Dennis J. 1995. Seedborne *Fusarium* on Douglas-fir: pathogenicity and seed stratification method to decrease *Fusarium* contamination. New Forests. 9(1): 35–51.

Bewley, J.D.; Black, M. 1994. Dormancy and the control of germination. In Seeds: physiology of development and germination. 2nd ed. New York: Plenum Press: 199–267. Chap. 5.

Bower, A.D.; Kolotelo, D.; Aitken, S.N. 2011. Effects of length of storage and stratification on germination of whitebark pine seeds. Western Journal of Applied Forestry. 26(1): 24–29.

Campbell, S.J.; Landis, T.D. 1990. Managing seedborne diseases in western forest nurseries. Tree Planters' Notes. 41(4): 3–7.

Cram, M.; Fraedrich, S.W. 2009. Seed diseases and seedborne pathogens of North America. Tree Planters' Notes. 53(2): 35–41.

Danielson, R. 1985. Stratification and germination of western white pine seeds. In Landis, T.D.; Fischer, J.W., eds. Intermountain Nurseryman's Association Meeting, 13–15 August 1985, Fort Collins, CO. Gen. Tech. Rep. RM-125. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 43–44.

Edwards, D.G.W. 1982. Collection, processing, testing and storage of true fir seeds: a review. In Oliver, C.D.; Kenady, R.M., eds. Proceedings, biology and management of true fir in the Pacific Northwest symposium, 24–26 February 1981, Seattle, WA. Institute of Forest Resources Contribution No. 45. Seattle, WA: University of Washington, College of Forest Resources; U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station: 113–137.

Edwards, D.G.W.; El-Kassaby, Y.A. 1996. The biology and management of coniferous forest seeds: genetic perspectives. The Forestry Chronicle. 72(5): 481–484.

Edwards, D.G.W.; Wang, B.S.P. 1995. A training guide for laboratory analysis of forest seeds. Inf. Rep. BC-X-356. Victoria, British Columbia, Canada; Pacific Forestry Centre, Canadian Forest Service, Pacific and Yukon Region. 63 p.

El-Kassaby, Y.A. 2000. Representation of Douglas-fir and western hemlock families in seedling crops as affected by seed biology and nursery crop management practices. Forest Genetics. 7(4): 305–315.

El-Kassaby, Y.A.; Thomson, A.J. 1996. Parental rank changes associated with seed biology and nursery practices in Douglas-fir. Forest Science. 42(2): 228–235.

Feurtado, J.A.; Xia, J.-H.; Ma, Y.; Kermode, A.R. 2003. Increasing the temperature of the water soak preceding moist chilling promotes dormancy-termination in seeds of western white pine (*Pinus monticola* Dougl.). Seed Science and Technology. 31(2): 275–288.

Kolotelo, D. 1993. Stratification of western white pine. Seed and Seedling Extension Topics. 6(2): 12–15.

Kolotelo, D. 1997. Anatomy and morphology of conifer seed. Forest Nursery Technical Series 1.1. Victoria, British Columbia, Canada: British Columbia Ministry of Forests, Nursery and Seed Operations Branch. 60 p.

Kolotelo, D. 2001. White pine quality assurance monitoring 2001. Seed and Seedling Extension Topics. 13: 1–5.

Kolotelo, D. 2002a. New retesting frequencies and deterioration rate estimates from the BCMoF Tree Seed Centre. Tree Seed Working Group Newsbulletin #36. Surrey, British Columbia, Canada: British Columbia Ministry of Forests, Tree Improvement Branch, Tree Seed Centre, Cone and Seed Improvement Program: 3–7.

Kolotelo, D. 2002b. Germination tests: How precise are they? Tree Seed Working Group Newsbulletin #36. Surrey, British Columbia, Canada: British Columbia Ministry of Forests, Tree Improvement Branch, Tree Seed Centre, Cone and Seed Improvement Program: 14–15.

Kolotelo, D. 2013. Quantifiable germination influences. Tree Seed Working Group Newsbulletin #56. Surrey, British Columbia, Canada: Canadian Forest Genetics Association: 3–13.

Kolotelo, D.; Van Steenis, E.; Peterson, M.; Bennett, R.; Trotter, D.; Dennis, J. 2001. Seed handling guidebook. Victoria, British Columbia, Canada: British Columbia Ministry of Forests, Tree Improvement Branch. 106 p.

Landis, T.D.; Tinus, R.W.; Barnett, J.P. 1998. The container tree nursery manual. Vol. 6: Seedling propagation. Agricultural Handbook 674. Washington, DC: U.S. Department of Agriculture, Forest Service. 166 p.

Lenth, R.V. 2013. lsmeans: least-squares means. R Development Core Team 2006, R package version 1.06-05. http://cran.r-project.org/web/packages/lsmeans/index.html (February 2013).

Neter, J.; Kutner, M.H.; Nachtsheim, C.J.; Wasserman, W. 1996. Applied linear statistical models. 4th ed. Boston: WCB McGraw-Hill. 1,408 p.

Sutherland, J.R. 1981. Time, temperature, and moisture effects on incidence of seed infected by *Caloscypha fulgens* in sitka spruce cones. Canadian Journal of Forest Research. 11(3): 727–730.

Wang, B.; D'Eon, S. 2003. A brief review of germination testing requirements for eastern white pine (*Pinus strobus* L.) seeds. Tree Seed Working Group Newsbulletin #38. Surrey, British Columbia, Canada: British Columbia Ministry of Forests, Tree Improvement Branch, Tree Seed Centre, Cone and Seed Improvement Program: 11–13.

Additional Reading

Owens, J.N. 2004. The reproductive biology of western white pine. Forest Genetics Council Extension Note 04. Victoria, British Columbia, Canada. 40 p.