Seed Preparation Techniques to Maximize Germination of Pacific Northwest Conifers

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Abstract

Stratification, the historical name for moist chilling seeds to mimic natural processes, is the primary means of releasing seed dormancy in most Pacific Northwest conifer species. This treatment removes internal, or physiological, dormancy, the main dormancy mechanism, but can also address external, or seed coat-imposed, dormancy by leaching chemical inhibitors and weakening mechanical restraints of the seed coat. Nursery personnel intentionally move seeds through the three stages of germination: hydration, activation, and emergence. To fully release dormancy, technicians target specific moisture contents during the activation phase. Maintaining seed in a surface dry condition for part, or all, of the activation phase allows for removal of dormancy while minimizing risks such as excess respiration, mold development, and premature germination. Several "advanced" techniques to maximize germination parameters include: extended stratification, delayed dryback during stratification, mid-stratification grading, and thermal priming (seed warming). These techniques do not involve specialized equipment but require close attention to detail to realize their potential. This paper is based on a webinar presentation given September 16, 2020 as part of the 2020 North American Forest and Conservation Nursery Technology Webinar Series, which can be viewed at https://vimeo.com/458771879. An accompanying online bulletin board is at https://padlet.com/nabilkhadduri/seedgermwebinar.

Introduction

Reforestation nurseries strive to produce a uniform crop that meets target specifications (Landis et al. 2010). This effort starts with complete, fast, and uniform germination followed by adequate time to complete the rapid growth and hardening stages of seedling development. Nature takes a different approach, preferring in many cases to delay and spread out germination, even in ideal conditions, through some form of seed dormancy (Kildisheva et al. 2020). Spreading out germination over weeks, months, or even years helps ensure that some percentage of seedlings survive the gauntlet of environmental challenges to eventually reach reproductive maturity (Baskin and Baskin 2014).

In natural regeneration for most Pacific Northwest (PNW) conifers, seeds ripen, cones flare, and seeds fall to the forest floor in late summer or early fall. These seed banks are often exposed to ideal germination conditions in mid-fall, where regular rains return while temperatures are still relatively warm. Most PNW conifer seed lots have a range of dormancy, including some non-dormant seeds that will readily germinate whenever warm, moist conditions are present. The challenge for those early germinants is to establish rapidly enough to be able to endure harsh winter conditions. Due to a chilling requirement, however, most seeds hydrated by fall rains will not release dormancy for several weeks to months, generally when temperatures are too cold for germination. Only when warmer spring temperatures arrive will germination commence. To further reduce the risk of germinating into adverse environmental conditions, many seed lots include seeds that will not germinate for weeks, months, or even years (Landis et al. 1999).

Stratification

Nurseries release seed dormancy in most PNW conifers through a cool, moist chilling treatment, commonly known as stratification. This treatment has been in widespread use with many reforestation species for hundreds of years (Bewley and Black 1994). Traditionally, layers of seed were alternated between a medium such as peat moss or sand. Today, nurseries commonly use "naked" stratification where no media are layered or mixed with seed during moist chilling to allow for the

close control of moisture content, which is crucial for maximizing stratification benefits. Seeds are typically hydrated in an initial water soak or rinse, then drained and chilled for several weeks or months in plastic bags that allow air exchange (Bonner and Karrfalt 2008).

Benefits of Stratification

By fully releasing dormancy during stratification, we can improve germination capacity, defined as the total germination potential. Seed vigor associated with dormancy release means we can also increase germination speed, especially in cool germination conditions found in bareroot soils, outdoor container compounds or unheated (or insufficiently heated) greenhouses. Finally, we can increase uniformity, where a crop germinates not only quickly, but close together in time.

Successful stratification shortens the "germination window," thereby reducing time spent misting new germinants, a practice which cools the growing environment and slows germination. A shorter germination window also decreases susceptibility to pest attack, as emerging seedlings are particularly vulnerable to animal predation and disease proliferation. Heating greenhouses can be expensive and a shorter germination window decreases these costs. Ultimately, seeds that have had their dormancy fully removed and germinate quickly, uniformly, and completely result in seedlings that are easier to cultivate and less costly to handle in the nursery.

Seed Dormancy Types in PNW Conifers

The *Woody Plant Seed Manual* (Bonner and Karrfalt 2008) defines dormancy as, "…a state in which a seed disposed to germinate does not, even in the presence of favorable environmental conditions." Seed dormancy in PNW conifers falls into two main categories: seed coat-imposed dormancy (physical and chemical) and, most commonly, embryo-imposed (physiological) dormancy (figure 1).

Physical Seed Coat Dormancy

Physical seed coat dormancy is often referred to as "hardseededness"—impermeable seed coats that do

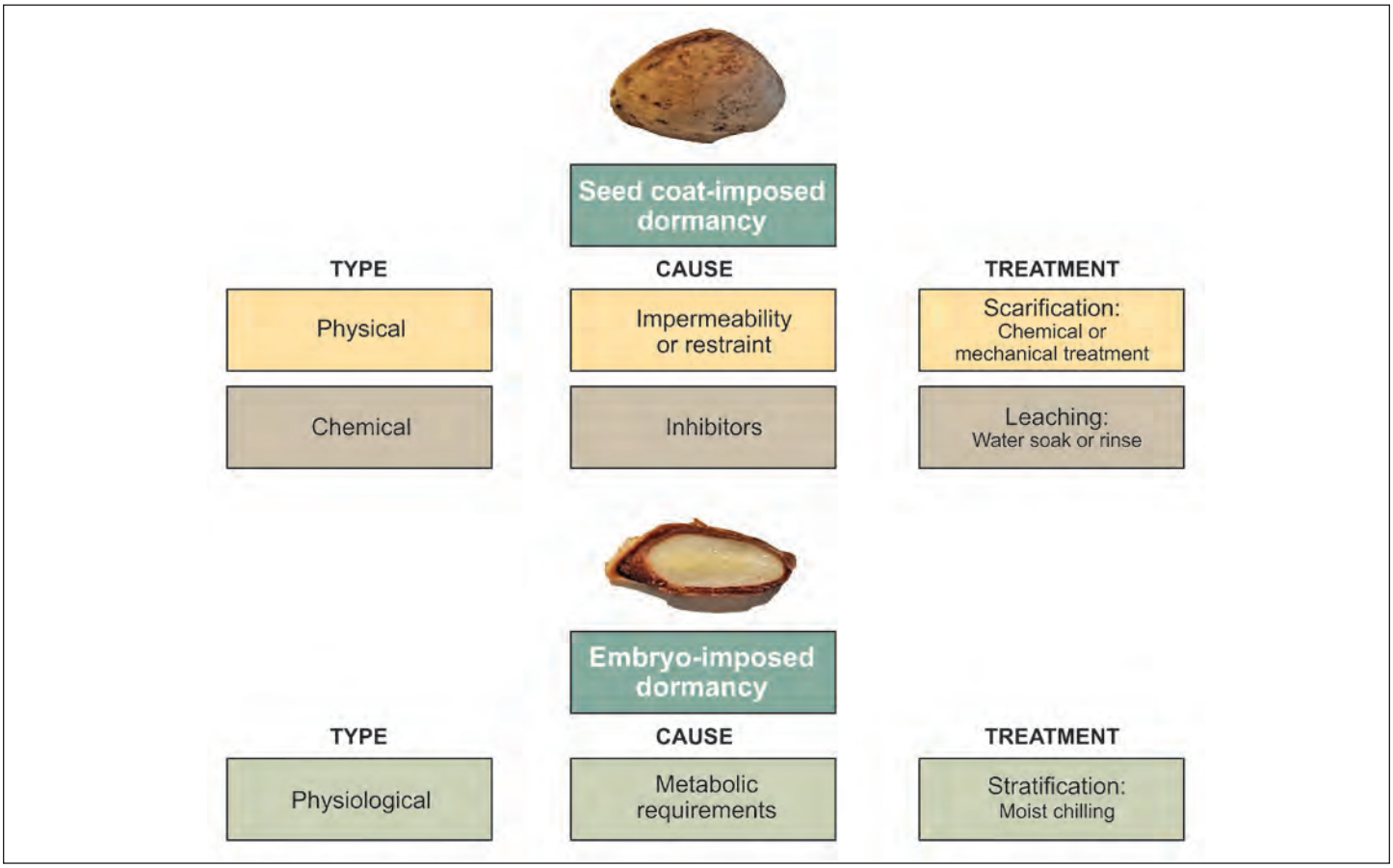

Figure 1. PNW conifer seeds can have physical or chemical seed coat imposed-dormancy along with the typical embryo-imposed dormancy. Except for impermeable seeds, the stratification process is a useful tool to break dormancy. (Photos by Nabil Khadduri 2020)

not allow gases or liquids to pass through. Many trees in the Leguminoseae family exhibit this kind of dormancy, such as locust (*Robinia* spp.). Hard seed coats can be broken down with sulfuric acid, hot water, or mechanical abrasion treatments (Khadduri et al. 2003). The only PNW conifer species thought to contain an impermeable seed coat is whitebark pine (*Pinus albicaulis* Engelm.) (Leadem 1996).

Some PNW conifers exhibit a physical dormancy where mechanical restraints keep the embryo from expanding. Water and air can pass through to the embryo, allowing it to enlarge, but growth and full imbibition are mechanically restricted by woody structures of the seed coat. This is a typical form of dormancy in southern pines (e.g., loblolly pine [*Pinus taeda* L.]) and is believed to be one form of dormancy in western white pine (*Pinus monticola* Douglas ex D. Don) (Bonner 1991).

Chemical Seed Coat Dormancy

A poorly understood mechanism of physical dormancy in PNW conifers involves the presence of chemical inhibitors in the seed coat. Some phenolic substances in the seed coat could be germination inhibitors, though their main role may be to limit growth of pathogenic organisms (Mohammed-Yaseen et al. 1994). In stratification, an initial running water rinse or simple water soak may leach chemical inhibitors (Leadem 1997).

Embryo Dormancy

Embryo-imposed dormancy is a physiological barrier to germination where metabolic blocks need to be removed and growth-promoting enzymes activated. This is the most common type of dormancy in PNW conifers and is treated with stratification. For example, one of the main internal impediments to germination of conifer seeds is high levels of abscisic acid (ABA). Feurtado et al. (2004) showed that embryo ABA levels rapidly drop during moist chilling of western white pine, greatly reducing this germination impediment.

Stratification: Nurseries Intentionally Move Seeds Through Three Stages of Germination

The three stages of germination that seeds must eventually pass through are: (1) hydration, (2) activation, and (3) emergence (figure 2a-d) (Bewley and Black 1994). In artificial regeneration, nurseries mimic natural dormancy release and stimulate germination through a series of intentional practices, with careful control of moisture content. We collect seeds either in the woods (woods-run or wild) or

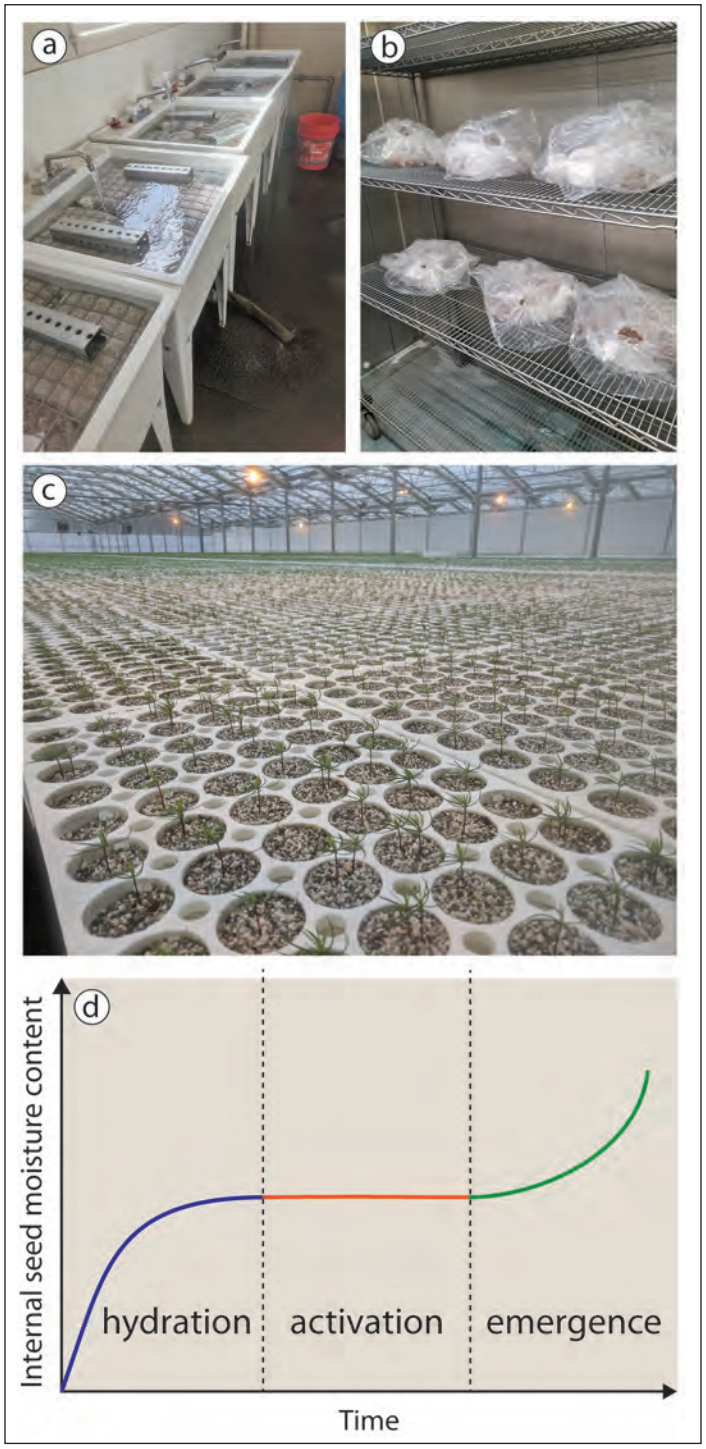

Figure 2. The three stages of seed germination are: (a) hydration, (b) activation, and (c) emergence. During hydration and emergence, (d) internal seed moisture increases. (Photos a, b, c by Nabil Khadduri 2020; d adapted from Bewley and Black 1994)

from managed seed orchards. Our seed banks are controlled storage facilities where temperatures are maintained at -18 °C (0 °F) and between 5 to 10 percent moisture content.

For most PNW conifer seeds, a stratification treatment acts as a panacea by addressing several dormancy mechanisms. Chemical inhibitors are assumed to be leached by the running water rinse or water soak during the hydration phase of the stratification treatment. The length of time spent in moist, cool conditions not only releases metabolic blocks and cues enzymatic growth processes, but may also allow mechanical restraints in the seed coat to break down. Thus, stratification may relieve chemical, physical, and physiological dormancy in one treatment (Leadem 1997).

Hydration stage

At Webster Nursery (Washington Department of Natural Resources, Olympia, WA), we initiate the hydration stage of germination with an aerated water soak or running water rinse. Seeds respire upon introduction to water and the aeration of the water soak (using a fish tank bubbler, for example) or a running water rinse introduces supplemental oxygen into the process (figure 2a). A rinse may more effectively remove chemical inhibitors (as well as pathogens on the seed coat) than a soak. After imbibition is complete, generally 24 to 48 hours, seeds are drained of excess moisture.

Activation Stage and the Importance of Surface Drying Seeds

For the activation stage of germination, we place seeds in plastic bags no thicker than 0.102 mm (4 ml) and filled with no more than 50 to 75 percent of the bag's volume (figure 2b). We use U-line 1 ml plastic bags (Pleasant Prairie, WI) at Webster Nursery. The thin plastic and partial filling ensure sufficient oxygen and carbon dioxide exchange (Landis et al. 1999). Partial filling also facilitates "massaging" of seed during stratification, typically carried out once or twice per week, to encourage further aeration and avoid heat build-up from excess respiration. Typical stratification temperatures are 1 to 5 °C (34 to 41 °F). At Webster Nursery, we maintain temperatures at 2 °C (35 °F). This relatively low temperature still releases dormancy, but also reduces premature germination or mold build-up during stratification that may accelerate on the higher end of the temperature range.

A major advancement in stratification protocols in recent years has been the careful monitoring and drying back of seed surface moisture content prior to, or in some cases during, the chilling period (Jones and Gosling 1994). The presence of a film of moisture on seed coats during stratification can lead to excess respiration, which depletes seed reserves (figure 3a). Also, gas exchange is reduced when seeds are in a surface wet condition, and massaging to break up clumps and increase air flow is more difficult. Observable problems include rapid mold development (figure 3b) and premature germination. To reduce these risks, chilling duration may be shortened, but this could result in failure to fully remove dormancy. The goal, then, is to safely remove dormancy by surface drying seed while maintaining internal seed moisture content.

Surface drying can be considered a form of "hydropriming" where we target specific seed moisture contents (weights) to prolong the activation phase and

Figure 3. (a) Surface dry (left) and surface wet (right) seeds. After the initial soak or rinse, seeds should be surface dried for part or all of the chilling period. Surface drying reduces (b) mold, excess respiration, and premature germination during stratification. (Photos by Nabil Khadduri 2020)

delay the start of stage 3 emergence (Kolotelo 2020b). By limiting surface moisture and prolonging the activation phase, we allow all seeds to release dormancy, even the more dormant seeds, thereby improving germination. Some species are best surface-dried immediately after hydration and draining, while others benefit from several weeks of "wet stratification" prior to surface drying (see section on Delayed Dryback).

Emergence Stage

The emergence stage ideally begins after we sow seeds in a greenhouse or field and visible germination takes place. A favorable warm and wet environment encourages germination, but only seeds whose dormancy has been completely removed will germinate with full vigor. Importantly, complete dormancy removal helps seeds cope with any suboptimal conditions they might experience during emergence.

Useful Tools to Aid in Surface Drying

Using Storage Seed Lot Moisture Content to Accurately Surface Dry Seeds

Seed plant operations use ovens or meters to accurately determine moisture content for long-term seed lot storage. The most common practice is to weigh several representative seed samples, oven dry at a set temperature for 24 hours, then re-weigh to determine current moisture content based on total weight loss. Seeds are only placed into long-term storage at 5- to 10-percent moisture content, a range that optimizes seed longevity.

In stratification, one can use this previously determined storage moisture content to monitor and manipulate current moisture content throughout the process. Jones and Gosling (1994) first described this non-destructive and rapid moisture content monitoring concept. At Webster Nursery, we have adapted the operational practices used at the BC Ministry of Forests Tree Seed Centre (Kolotelo et al. 2001).

The first step is to determine the oven dry weight (0 percent moisture content) of the seed lot intended for stratification. This information can be determined indirectly from the storage moisture content of the seed lot, a number that is generally available and can be requested from the seed vendor. If this is not available, you will need to determine the oven dry weight yourself through direct oven drying, but will only need to do this one time.

In Equation 1, we determine the oven dry weight of a seed lot withdrawal by plugging in the storage moisture content (in decimal form) along with the withdrawal weight.

Equation 1: *Oven dry weight = withdrawal weight * (1 – storage moisture content)*

For example, if we withdraw 980 g (2.16 lb) of coastal Douglas-fir seed from a seed orchard that was stored at 8.0 percent moisture content, the oven dry weight would be:

Oven dry weight = 980 g * (1 - .08) = 980 g x 0.92 = 901.6 g (1.99 lb)

Once we know the oven dry weight, we can determine the current moisture content at any point in the stratification process by weighing the fresh weight and plugging that into Equation 2.

Equation 2: *Current moisture content = (fresh weight – oven dry weight)/ fresh weight*

Using our example above, we know from Equation 1 that our oven dry weight for our withdrawn seed lot is 901.6 g (1.99 lb). If the current weight of our seed lot is 1,610 g (3.55 lb), after we have imbibed the seed but before surface drying, the current moisture content would be:

Current moisture content = (1,610 g – 901.6 g)/1,610 g = 0.44, or 44.0 percent

We can use Equation 3 to determine the target weight for a target moisture content.

Equation 3: *Target fresh weight = (oven dry weight)/ (1 – target moisture content)*

Continuing with our example, we know from experience that coastal Douglas-fir from a seed orchard surface dries to about 33 percent. So, the targeted fresh weight for this target moisture content would be:

Target fresh weight = 901.6 g / (1 – 0.33) = 901.6 g/ 0.67 = 1345.7 g (2.97 lb)

For additional examples, see Kolotelo (2018), as well as a typical spreadsheet from our nursery operations with equations plugged in at: https://padlet.com/nabilkhadduri/seedgermwebinar.

Kolotelo notes that one of the benefits of using this process to non-destructively monitor seed, as opposed to destructive tests, is that sampling error is reduced by weighing an entire bag of seed as opposed to small destructive samples to determine moisture content. Most PNW conifers surface dry to between 25 and 35 percent moisture, with relatively narrow ranges based on species. See Kolotelo et al. (2021) for a list of typical surface dry weights of PNW conifers based on their origin (woods-run vs. orchard grown).

Using a Laundry Spinner to Expedite Surface Drying

Following the hydration phase, we drain excess water from the seeds, but a film of water often remains. We generally surface dry seeds using some combination of indirect heat, forced air, and regular hand-mixing so that excess moisture is uniformly removed. Drying can take time, but there is a convenient tool to expedite the process for some species: a laundry spinner. Gosling et al. (1994) demonstrated that a laundry spinner can serve quite well as a seed spinner by quickly and consistently removing free moisture from a seed lot after the soak/rinse hydration phase.

For the past 15 years, we have used a Spin-X laundry spinner for this purpose (figure 4), the same brand used by Gosling et al. (1994). This spinner costs $495 USD, and a recent internet search (summer 2020) found several alternatives priced $200 lower than the Spin-X. We have been pleased with the durability of the Spin-X and cannot vouch for the longevity of other models. We developed a seed spinner guide for our nursery species, available at https://padlet.com/nabilkhadduri/seedgermwebinar. Seeds of some species, such as those with resin-vesicles (pitch sacks in the seed coat) are not recommended for spinning since damaged resin vesicles can release extracts that inhibit germination (Keeling et al. 2018). As with any new process, we advise trialing small lots before using at operational scale.

Figure 4. Excess water can remain following imbibition and draining. A laundry spinner repurposed as a seed spinner can be used for some species to efficiently remove excess water by placing seeds in the drum, balancing the weight of the seeds, and removing free water through centrifugal force during a preset (approximately 2-minute) spin cycle. (Photo by Nabil Khadduri 2020)

Advanced Techniques to Improve Germination

The "advanced" techniques detailed here require little additional equipment to carry out. What they do require is close attention to detail, persistence, and patience. Most utilize surface drying at some point in the process to be successful. As with all new techniques, try these on a small scale first, then gradually scale up as experience and confidence grows. A successful germination treatment for one species may harm performance in another, and even within species each seed lot can, and often will, respond differently to the same treatment. Try to develop treatments that are conservative enough to be applied to a broad range of seed lots within a species, and continue to evaluate to make sure they do not harm certain seed lots.

Extended Stratification

Why should growers consider extending stratification longer than what might be suggested in lab germination tests or the literature? Unlike field, or even greenhouse, conditions, lab tests are conducted in ideal situations with warm, controlled temperatures. By extending stratification one is more likely to completely remove dormancy from all seeds within a lot. Thus, operational tests in nursery conditions are important since extended stratification benefits may not be realized in ideal lab conditions (Edwards and El-Kassaby 1995).

In our experience at Webster Nursery, most PNW conifers almost always require stratification lengths longer than standard lab recommendations. Exceptions to this rule may include seed lots that are improperly stored or otherwise deteriorated. Think of lab stratification lengths, such as those from the Association of Official Seed Analysts, as the minimum, and extend from there for greenhouse and especially field sowing. For exam-

ple, if a 4-week stratification is recommended in lab testing, compare germination capacity and speed for 4 weeks with 6 or 8 weeks. Kolotelo (2020a) and Lei (2021) note a consistent pattern of increased dormancy in orchard-grown seed compared with woods-run seed across several PNW species, regardless of seed size. This pattern concurs with common grower observations that orchard-grown PNW species seem to benefit from longer stratification than woods-run seed from a similar zone and elevation.

One of the biggest arguments for extending stratification is increasing germination capacity across a range of temperatures. Jones and Gosling (1994) stratified coastal Douglas-fir (Pseudotsuga menziesii Mirb. Franco) seeds for 0, 3, 6, or 18 weeks. They found that the lots they tested were only shallowly dormant, with 65-percent germination at ideal temperatures between 20 and 30 °C (68 and 86 °F) after 42 days for those with 0 weeks (i.e., no) stratification. For seeds with 3 weeks stratification, however, germination increased across a range of temperatures. For seeds stratified for 6 or 18 weeks, germination was further enhanced in the 10 to 20 °C range (figure 5).

Extended Stratification Trial for Douglas-fir: Lab and Greenhouse Comparison Trial

In 2010, we compared 4-, 8- and 12-week stratification durations in both lab (figure 6a) and greenhouse (figure 6c) settings on four orchard-grown coastal Douglas-fir lots. In the greenhouse, stratification lengths were 10 days longer than lab lengths due to sowing delays. Lab temperatures followed a standard 20 °C, 8-hr light and 30 °C, 16-hr dark protocol (AOSA 2007). While there was no significant difference in final total germination, 12 weeks of stratification resulted in seeds with higher germination speed compared with shorter stratification durations (figure 6b). In the greenhouse, three of the four lots showed incremental increases in germination speed and total germination with increasing stratification lengths (figure 6d). One lot, however, germinated faster at 66 vs. 94 days, illustrating that not all seed lots will benefit from extended stratification durations. It is critically important to monitor seed condition and the presence of excess moisture or seed drying when extending seed stratification.

Extended Stratification Trial for Western Hemlock: Lab and Greenhouse Comparison Trial

Seeds of western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) surface dry (seed weight of imbibed seed after external moisture is dried off) to below 30 percent on average (Kolotelo 2018, Kolotelo et al. 2021). Previously, we maintained western hemlock at higher moisture values, with occasional pre-germination and/or mold during the chilling period as a result. By surface drying to lower levels, we hypothesized that

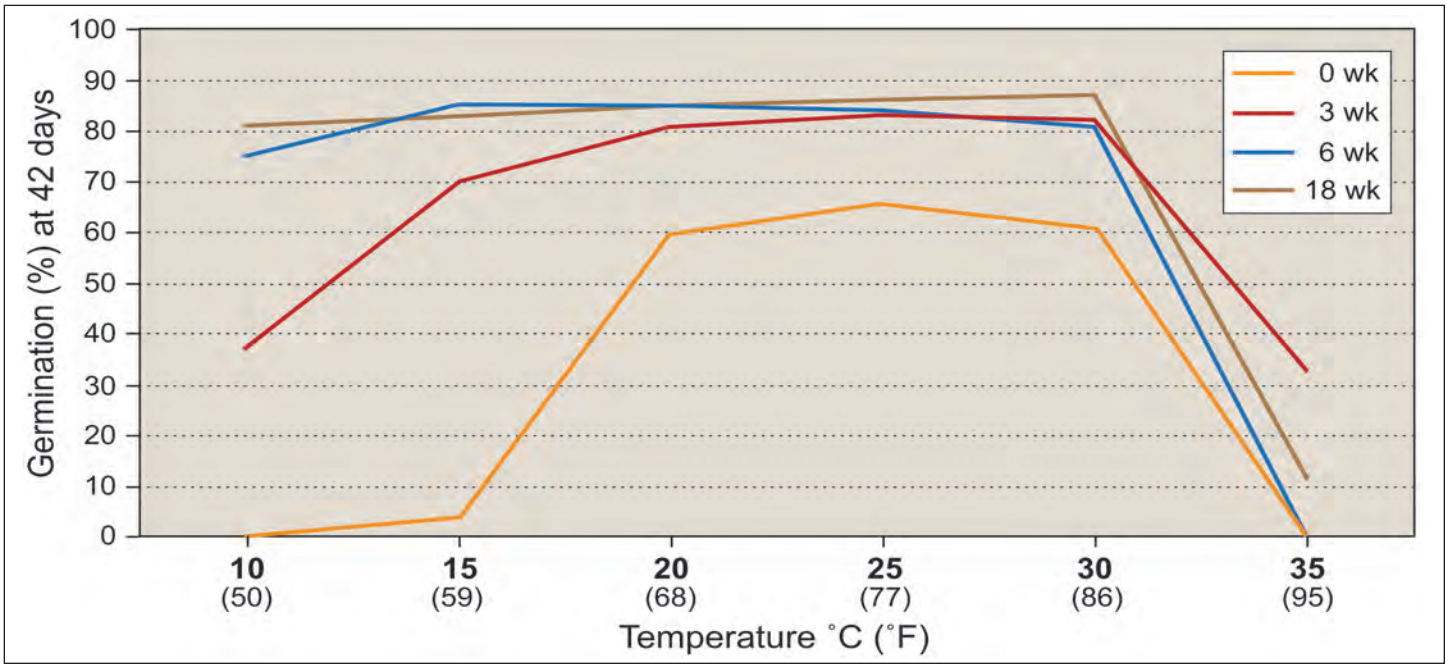

Figure 5. Extending stratification up to 18 weeks increased coastal Douglas-fir germination capacity across a range of temperatures, particularly temperatures below 20 °C (68 °F). (Adapted from Jones et al. 1994)

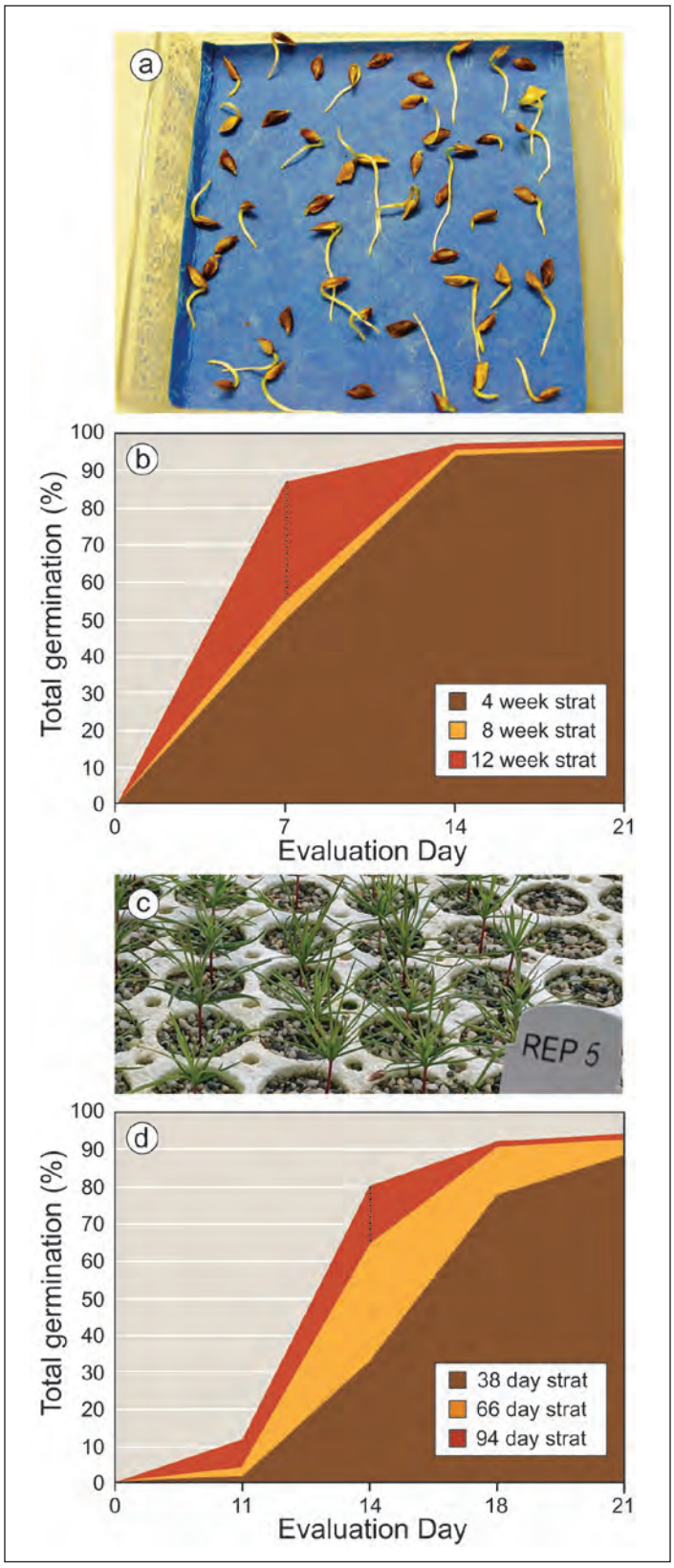

Figure 6. In a lab test under (a) controlled conditions, (b) 12 weeks of stratification increased germination by day 7 of four coastal orchard Douglas-fir lots but with no significant increase in total germination by the end of the test. In a (c) greenhouse test with the same four lots, (d) three of the lots showed incremental increases in germination speed from increasing stratification lengths. For one seed lot in the greenhouse (not shown), germination speed was fastest with the 66-day stratification but did not differ in total germination from other stratification durations. (Photos by Nabil Khadduri 2010)

extended stratification times beyond our 30-day operational treatment would expedite notoriously slow western hemlock germination.

In 2020, we ran western hemlock lab (figure 7a) and greenhouse (figure 7c) stratification trials. In the lab trial, we found that longer stratification times for two woods-run lots incrementally improved germination speed, even under warm lab conditions (23.3 °C [73.9 °F] average), though there were no differences in final germination (figure 7b). Normally, we stagger stratification dates so that all seed in a trial is sown on the same day. For logistical reasons, however, we started all stratification treatments for the greenhouse trial on the same day, so that 30-, 45-, and 60-day treatments were sown 15 days apart. While not an issue in standardized lab conditions, average greenhouse temperatures slowly increased through the trial from 19.8 °C to 20.5 °C. For this reason, we plotted greenhouse germination against growing degree days for each treatment to account for those temperature variations (figure 7d). Results paralleled the lab trial in terms of germination speed, with longer stratification treatments emerging faster. Unlike the lab trial, longer stratification durations also significantly improved final germination over the 30-day treatment.

Seed Sanitation During Extended Stratification

Occasionally seed lots, even with surface dryback precautions, will build up some level of mold during extended stratification. In addition to a presoak bleach treatment with some species, we may also use a 3.0 percent active ingredient hydrogen peroxide soak for 2 to 4 hours either in the middle or at the end of stratification, followed by a rinse of 2 to 4 hours. Some nurseries do not rinse after the hydrogen peroxide treatment, while others simply do a clear water rinse during stratification in lieu of a chemical treatment.

We continue to assess and update our seed sanitation program. For example, the use of sodium hypochlorite is both supported (Wenny and Dumroese 1987; Dumroese et al. 1988) and discouraged (Trotter 1990) in the literature. We apply sodium hypochlorite to certain species at varying concentrations, with an emphasis on seeds of species that are slow to take up water. In general, a post-imbibition hydrogen peroxide treatment should be safer to seed than an initial sodium hypochlorite treatment (Neumann et al. 1997). In the case of species with

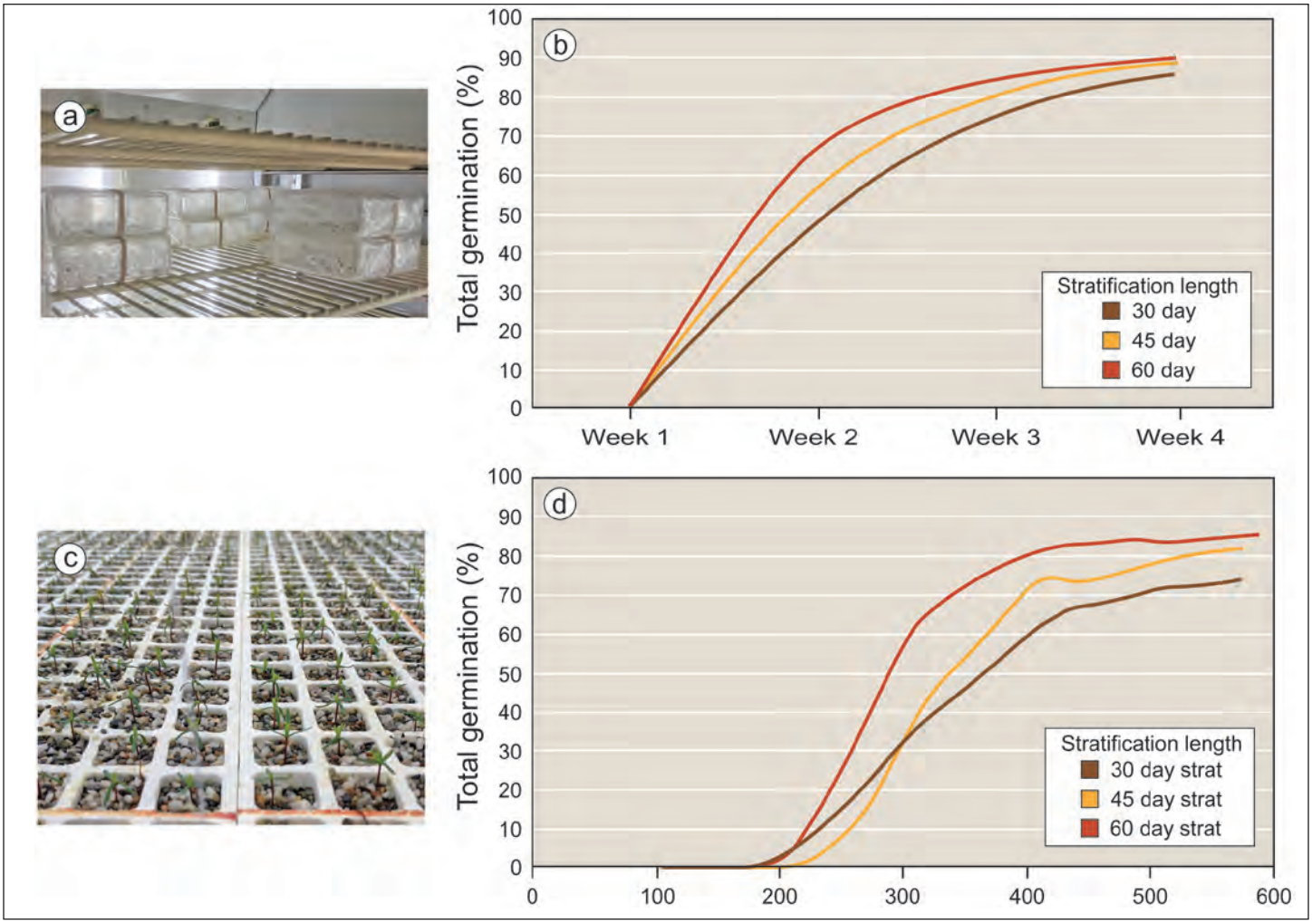

Figure 7. A western hemlock (a) lab trial demonstrated that (b) longer stratification times for two unimproved seed lots significantly improved germination speed over a 30-day treatment, even under warm lab conditions. In a corresponding (c) greenhouse trial, (d) germination speed significantly increased with increased stratification length and improved final germination over the 30-day treatment. (Photos by Nabil Khadduri 2015)

resin vesicles, neither chemical treatment is recommended, especially if resin vesicles have been damaged. In the PNW, these include true fir species as well as western hemlock. See https://padlet.com/nabilkhadduri/seedgermwebinar for an overview of our current nursery seed sanitation guidelines.

Delayed Dryback

As mentioned previously, surface drying (dryback) of seeds immediately following imbibition and prior to stratification reduces mold, excess respiration, and premature germination. Some species, however, benefit from a period of "wet stratification" for at least the first few weeks of the chilling process. True firs (*Abies* spp.) in particular (Edwards 1996), as well as western white pine (*Pinus monticola* Douglas ex D. Don) (deGraan et al. 2013), should not be surface dried for 4 weeks after imbibition (figure 8). It is not clear why this works. Surface moisture may be desirable for the first few weeks if additional imbibition is needed or if seed coats need to be additionally degraded in the presence of excess moisture. Some true firs such as noble fir (*Abies procera* Rehder) benefit from a chilling period of 84 days total with surface drying after the first 28 days (Edwards 1996).

Delayed Dryback Case Study: Western White Pine

Western white pine can benefit from an extremely long imbibition period, specifically a running water rinse of up to 2 weeks (figure 9). The USDA Forest Service Coeur d'Alene Nursery tested several lots of western white pine and found that some did not fully imbibe until 6 days of soak (Rhoades 2020). At Webster Nursery, we have found the 2-week hydration phase prescribed by the BC Ministry of Forests (Kolotelo 1993a, Kolotelo et al. 2001) to be successful. It is not clear why this lengthy running

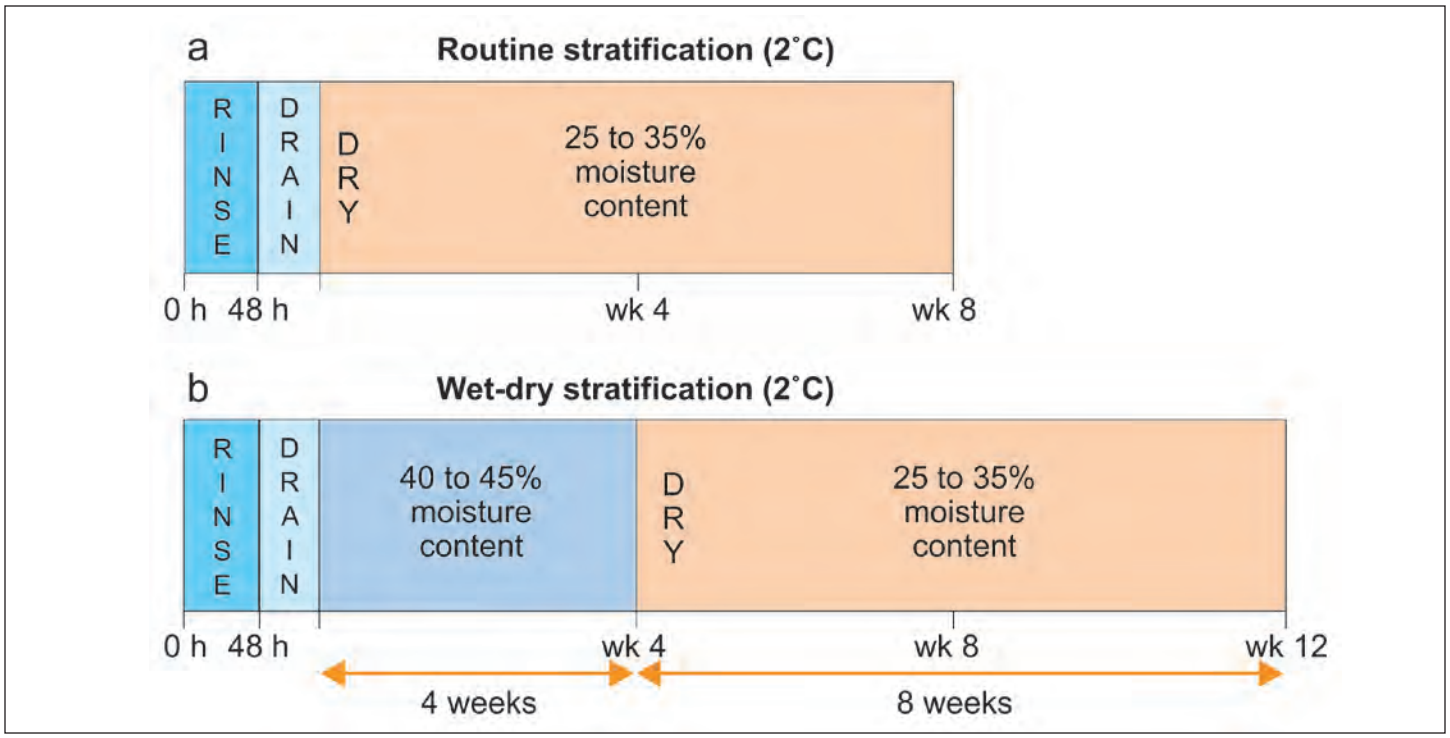

Figure 8. For (a) routine stratification, seeds are surface dried immediately following water uptake and draining. For (b) delayed dryback stratification, seeds are allowed to remain in a surface wet condition for about 4 weeks, then surface dried for the remainder of the chilling period. Delayed dryback generally allows for a longer overall chilling period than routine stratification.

water rinse works, but perhaps stubborn chemical germination inhibitors take time to leach out. Another possible explanation is that physical structures that impede embryo expansion continue to break down during the extended water treatment.

Modifying the BC protocol, we found that western white pine benefits from 4 weeks of surface wet stratification in the 42- to 44-percent range, followed by surface drying to 34 to 36 percent for an additional 14 to 15 weeks.

Western white pine is at risk for *Fusarium* fungal disease on the seed coat (Cram and Fraedrich 2009), and an aggressive seed sanitation protocol may be warranted, particularly due to the extreme length of time in chilling. We treat western white pine seed with an initial soak in 2.1 percent active ingredient bleach (sodium hypochlorite) for 10 minutes, followed by a 2-minute clear water flush. The intent is to kill fungal spores on the seed coat before active water uptake commences. The 14-day rinse presumably also helps remove bulk pathogens on the seed coat, though some *Fusarium* spp. can remain after running water rinse and/or a bleach or hydrogen peroxide treatment (Littke 1996).

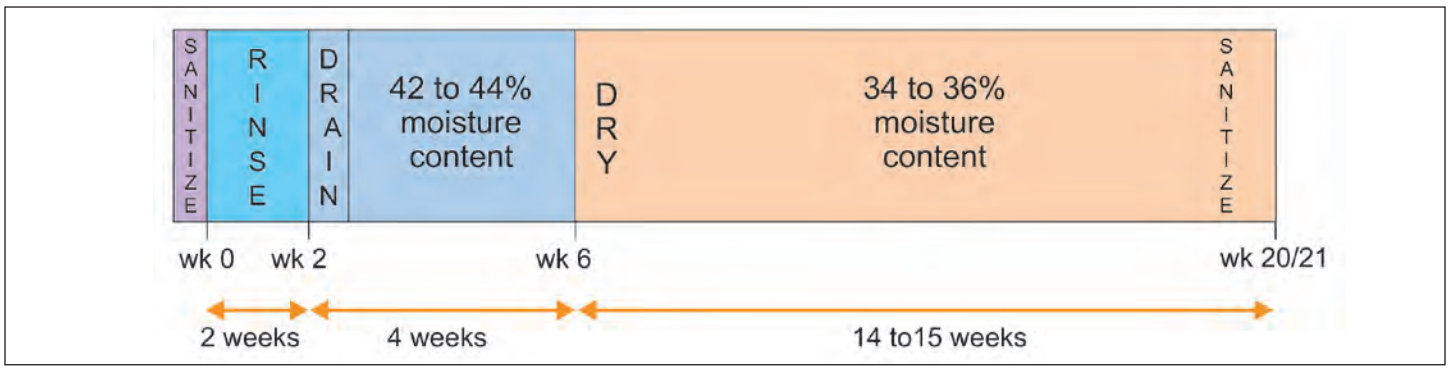

Figure 9. A series of stratification steps, including delayed dryback, can maximize germination parameters in western white pine. These steps include a pre-imbibition bleach dip (aggressively flushed), a 14-day running water rinse, a 4-week "wet" stratification, followed by an additional 14 to 15 weeks of surface dry stratification. While surface drying the seed helps prevent mold build-up, fungal development can be addressed with hydrogen peroxide and/or a short running water rinse late in the stratification period.

Grading Seed During Stratification Using Float Separation

Seed processing facilities use gravity tables, aspirators, and other tools to remove unfilled, partially filled, damaged, or insect-ridden seeds based on seed density. Some species, for example true firs, can present challenges to optimal cleaning due to thickened seed coats of hollow seeds, resin-filled seeds, or insect-damaged seeds. In all these instances, dead seed may have similar densities to live, filled seed that allows them to make it through the typical cleaning process.

The stratification process can facilitate seed grading because filled, live seed are more likely to bind to water and to increase in density due to biological growth in the activation stage of germination. While there are more involved forms of water-based seed separation prior to stratification such as pressure/vacuum treatment (PREVAC) or incubation drydown separation (IDS) (Karrfalt 2013, Karrfalt 1996, Simak 1984), one relatively straightforward technique is to simply float-separate seeds at some point during the stratification period. The greatest success with this technique is with lower-germination lots that have a relatively high proportion of dead, unfilled seeds (Kolotelo 1993b).

In float separation, surface dry seeds are placed in a tank (figure 10a), stirred to break surface tension, and allowed to separate based on density for several minutes or even hours. Ideally, unfilled or damaged seeds rise to the surface (figure 10b) and filled seeds sink to the bottom (figure 10c), though some seeds stubbornly remain in a suspended intermediate state. Cut tests should be used to determine proportions of viable seeds from each fraction (see Kolotelo 1997 for an excellent visual guide on cut tests of PNW conifers). Sylvan Vale Nursery (Black Creek, BC) reports that aeration may speed up the separation process. Also, float separation near the end of the stratification process can sometimes be successful when separation just after wet stratification fails to distinguish seed quality (Paquet 2020).

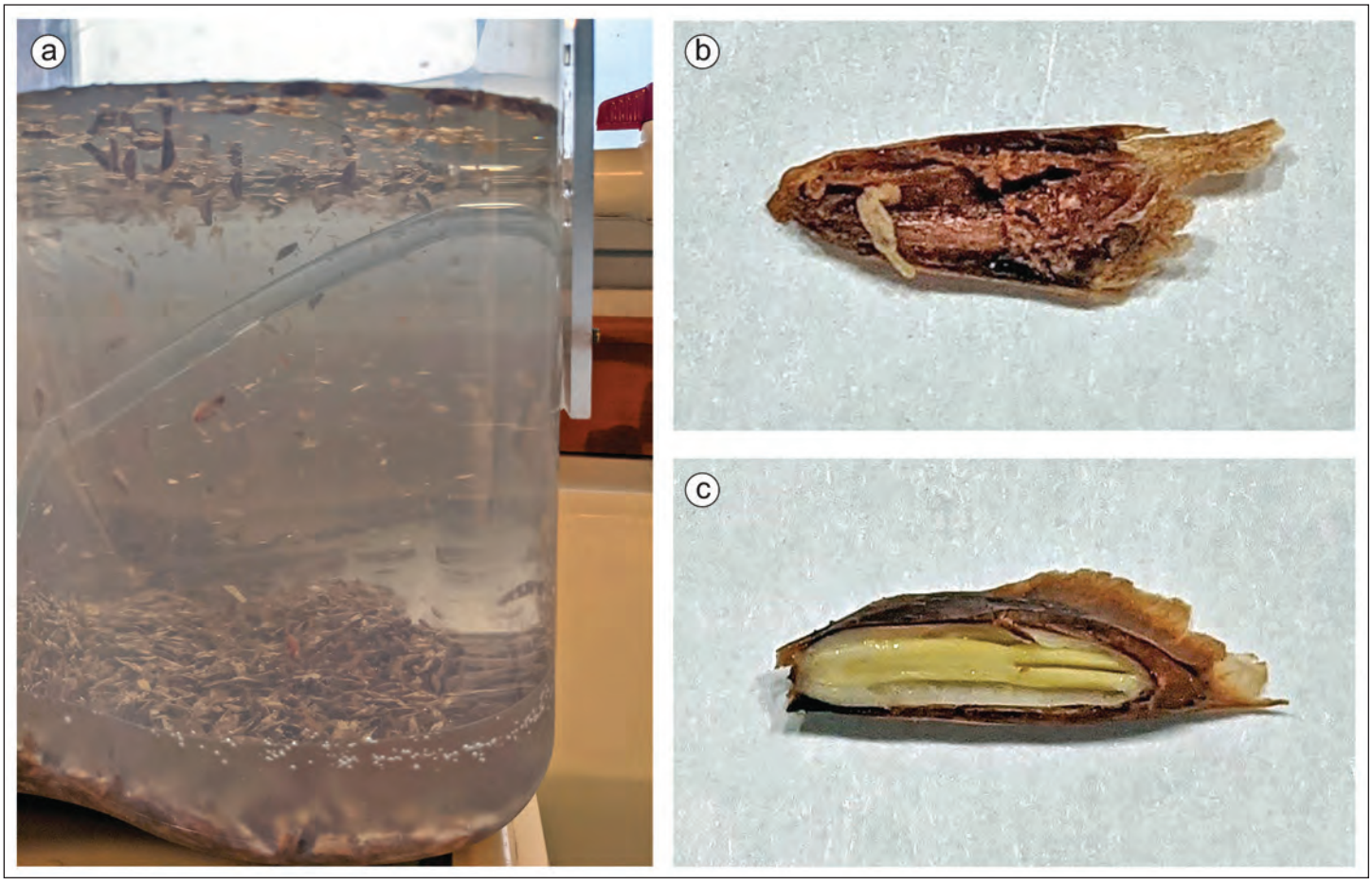

Figure 10. In float separation, surface dry seeds are placed in a (a) tank, stirred to break surface tension, and allowed to separate based on density for several minutes or hours. Cut tests can determine percentage of (b) empty or damaged seed and (c) filled seed in the floater and sinker fractions. This process is most successful for grading low germination lots with a high proportion of unfilled seeds. (Photos by Nabil Khadduri 2020).

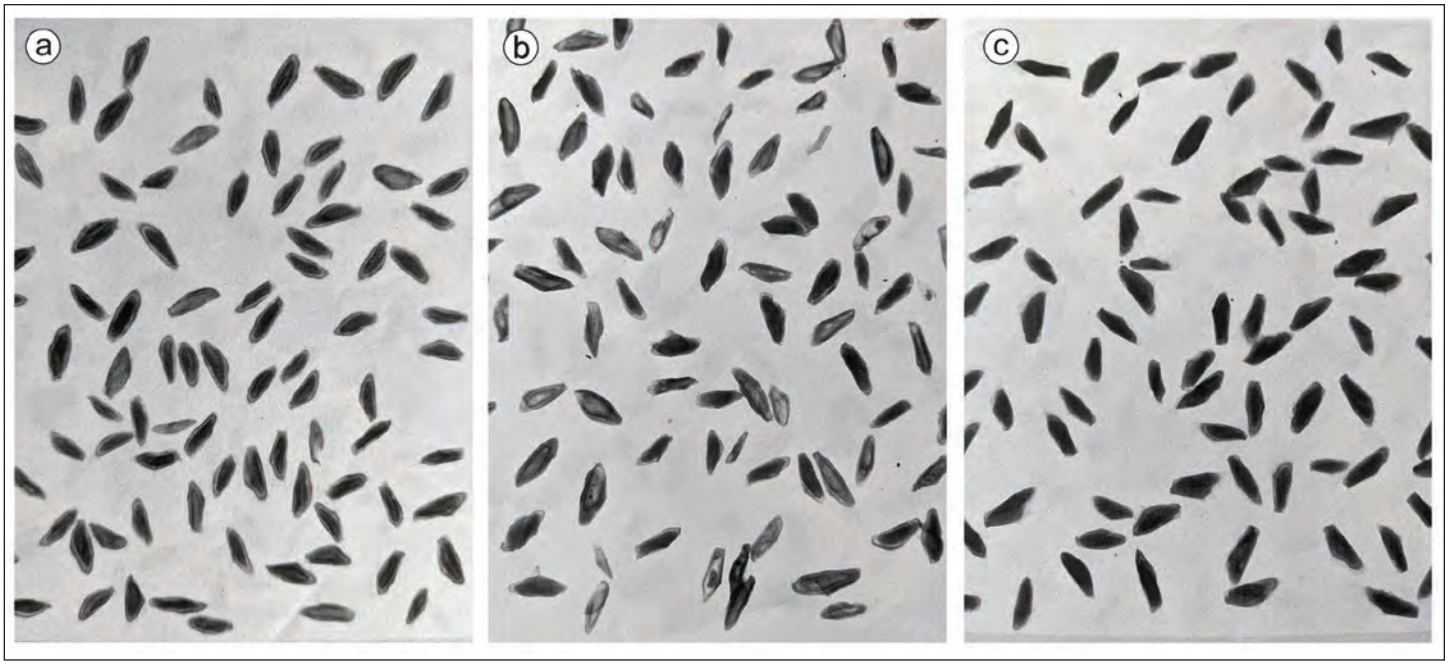

Figure 11. Seeds of Pacific silver fir seed lot 1386 were x-rayed (a) pre-stratification. Seeds were surface dried after "wet" stratification, then water separated into (b) floaters and (c) sinkers. Cut tests can also quickly help determine filled seed percentages. (Photos by Nabil Khadduri 2020)

In 2015, we separated several seed lots in a one-step separation process immediately following dryback after initial wet stratification. Figure 11 shows a Pacific silver fir seed lot (a) pre-stratification, (b) floater fraction with empty and insect-damaged seeds, and (c) sinker fraction with filled seeds. Results varied by species and by seed lot, with some floater fractions still containing significant quantities of viable seed. Nevertheless, greenhouse germination of graded seeds improved to 76 percent over a 69 percent lab test baseline (not shown) when averaged across all lots in this trial (figure 12). An important step when grading is to weigh the separations to make new sowing calculations of high-graded seed.

Thermal Priming (aka Seed Warming) to Jump Start Germination

Thermal priming refers to pre-warming seeds at the end of stratification prior to mechanical sowing. Because heat units needed for germination are unpredictable in a bareroot setting and expensive to supply in a greenhouse setting, intentionally heating seeds in a small, controlled environment prior to sowing can speed germination.

Careful attention and precautions must be followed in the seed warming process. K&C Nursery (Oliver, BC) recommends splitting lots into smaller bags and regularly rotating bags to facilitate even warming (Yang 2020). Dividing lots also allows extra air space for gas exchange during increased biological activity. Along with visual inspection, Kolotelo (2020) recommends weighing bags to make sure moisture loss is not taking place. Smaller-seeded species may benefit

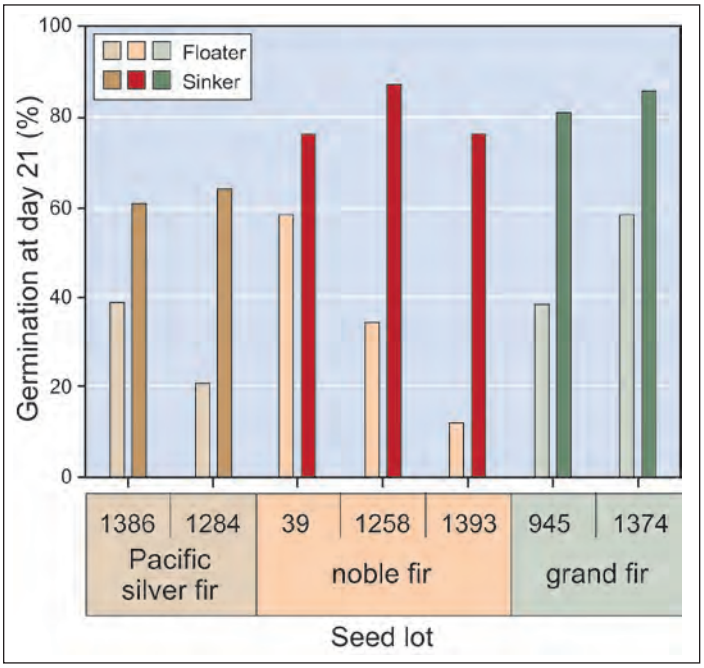

Figure 12. Seed lots of three *Abies species* (Pacific silver fir, noble fir and grand fir) were graded into floaters and sinkers mid-stratification. Greenhouse germination varied by species and seed lot and some floater fractions still contained significant quantities of viable seed. Still, overall greenhouse germination of graded seed averaged 76 percent compared with 69 percent for lab germination (lab results not shown).

from supplemental moisture. Another risk during thermal priming is stimulation of fungal growth. Fungi usually grow faster than seeds during the warming period (Dawes 2008). A third, and perhaps greatest, risk is premature radicle emergence such that most forms of mechanical sowing cannot be used.

To avoid the above risks, Yang (2020) recommends against thermal priming if abnormal fungal growth is observed during stratification, or the seed lot has a fermented odor (suggesting seed degradation), or if any radicles are observed to be emerging during stratification.

Provided stratification has proceeded normally with no complications, Kolotelo (2020b) suggests some useful guidelines for determining how much heat to supply in pre-warming without inducing germination before sowing. Radicles first emerge in lab germination tests between Day 2 and Day 5 for many species. In standard lab temperatures, this comes out to (25 °C [77 °F) x 8 hr) + (15 °C [59 °F) x 16 hr) = 440 growing degree-hours per day (based on a 5 °C [41 °F] baseline for accumulating heat). Thus, 880 degree-hours can be used as an estimate for seeds that may germinate as early as 2 days in test conditions, with 800 degree-hours a conservative starting point. Operationally, one can probably add additional heat units without risk of germination, but it is worthwhile to build up experience and comfort level. For example, K&C nursery pre-warms seeds up to 72 hours at 25 to 30 °C (77 to 86 °F), but this varies by species.

Kolotelo (2020b) points out that, for PNW conifers, how one accumulates heat units (steady or alternating diurnally) and the rate at which units are accumulated may not matter. It is just the total energy received that determines germination. In a study on white spruce (Picea glauca [Moench] Voss), Liu et al. (2013) found excellent germination characteristics using a combination of moist chilling with 72 hours of thermal priming at 15 °C (59 °F), and especially 20 °C (68 °F).

We plan to test thermal priming on a small scale at an average room temperature of 20 °C (68 °F). At a suggested conservative total of 800 degree-hours, that comes out to 2.2 days. As an added layer of insurance, we plan to evaluate our seed lots beforehand by calculating how many degree-hours are required to first see radicle emergence on fully stratified seeds. See Kolotelo (2020b) for additional information and references on the topic.

Conclusions

Several stratification strategies exist to increase germination uniformity, speed, and percentage. A dedicated, passionate, and experienced seed technician is key to successfully implementing the techniques described in this article. Through trial and error and attention to detail, a technician can develop techniques that are tailored to species and seed lots in operational sowing conditions. The goal in an operational program is to develop strategies that are aggressive enough to enhance germination performance, but conservative enough to be applied as a standard practice with minimal risk. Always remember to refine techniques by revisiting "tried and true" practices as experience dictates and time allows.

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