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Chapter 4

Eyeing Emergence: Modified Treatments for Terminating Dormancy of Conifer Seeds

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Abstract

Many seeds of coniferous species display a deep primary dormancy at maturity and require several weeks of pretreatment to produce seed populations that germinate in a vigorous and timely manner. Facilitating an efficient transition from dormancy to germination by devising improved protocols for dormancy breakage is not only important to conifer seed research, aiding in the study of the dormancy process itself, but is also of interest and applicability to commercial forest nursery operations. In the forests of British Columbia, Canada, several conifer species are well-adapted to their environment, with seeds needing to experience long durations in the moist state at cool or fluctuating temperatures. These include yellow-cedar (*Callitropsis nootkatensis*), western white pine (*Pinus monticola*), and true fir species, such as Pacific silver fir and subalpine fir (*Abies amabilis* and *A. lasiocarpa*, respectively). In this chapter, we discuss the development of new dormancy-breaking protocols for the aforementioned species that centre on the balance of several key aspects: (1) reducing the time needed to terminate dormancy in the seed population; (2) synchronicity of germination; (3) ease of use; (4) cost-effectiveness; and (5) repeatability. Where possible, any new or modified protocol should be further tested in relationship to promoting rapid seedling growth in a forest nursery greenhouse setting and after planting at natural stands. Based on the five criteria listed above, very significant improvements compared to traditional dormancy-breaking methods have been achieved for the targeted conifer species. Where tested (e.g. yellow-cedar), the modified dormancy-breaking treatments result in vigorous growth in the greenhouse and after planting at natural stands.

Key words: Seed dormancy, Conifers, Moist chilling, Traditional dormancy-breaking protocols, Modified dormancy-breaking protocols, Alcohols, Gibberellic acid, Solid matrix priming, True firs (*Abies* spp.), Yellow-cedar, Western white pine

1. Introduction

Seed dormancy, an adaptive trait that facilitates the distribution of seed through the dimensions of time and space, has been a key trait in the evolution of both angiosperm and gymnosperm species – helping plants maximize their fitness through successful

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establishment of subsequent generations (1, 2). The capacity of the imbibed seed to modulate its developmental schedule to germinate only when environment signals predict that growth and seedling establishment will be successful is a remarkable phenomenon. However, when seeds fall into human hands for use as cultivated crops, dormancy becomes an unwanted and undesirable trait since the grower can usually ensure that conditions in cultivated fields or in the greenhouse are optimal for seedling establishment and plant growth. It is not surprising, then, that domestication of a crop almost always removes or significantly reduces seed dormancy (3, 4). However, this has not been the case for tree species used in reforestation efforts. Despite the development of a more comprehensive silvicultural programme as seen in tree seed orchards and seedling nurseries, most conifer species have significant innate primary dormancy. Of course, lack of domestication in conifer species is understandable; breeding programmes are still rather juvenile, especially compared to the typical life cycles of our tree species. And few would argue that reducing dormancy to negligible levels in conifers is a much more arduous task when compared to any current domesticated crop. Fortunately, seed dormancy in species used for reforestation efforts is still a much needed trait; once the window of seed production and seedling establishment associated with the first generation in the nursery has passed, the transition from dormancy to germination of mature dispersed seeds of the next generation must then rely on signals from the "natural surroundings". Thus, retaining an inherited mechanism for seed survival is an utmost priority. As such, conifers are rather unique among crops that are cultivated – seed dormancy remains a necessary genetic trait for re-establishment of renewable natural stands. Accordingly, rather than attempting to breed out inherent dormancy mechanisms, forest nurseries require efficient dormancy-breaking treatments to ensure timely, economical production of vigorous seedlings.

Seeds of yellow-cedar (formerly known as *Chamaecyparis nootkatensis*, and now referred to as *Callitropsis nootkatensis*), true fir (*Abies*) species, and western white pine (*P. monticola*) all display deep dormancy at dispersal (5–7) and are prime examples of species that require efficient dormancy breakage in tree seed nurseries. Their deep physiological dormancy is, of course, due to the natural habitat in which each species exists.

The natural habitat of yellow-cedar occurs along the Pacific Northwest, through Oregon to British Columbia (BC), and northwards to Alaska (8, 9). Yellow-cedar has a long reproductive cycle, taking between 2 and 3 years to produce seeds (10). Problems with yellow-cedar seed production often occur because of poor reproductive success in its natural habitat. In seed orchards, which are usually established at lower elevations (11), cone production is

likewise inconsistent from year to year. Non-optimal environmental conditions associated with seed orchards (e.g. low elevation, warmer temperatures, and prevalence of insects) may further lead to problems with seed maturation, and often seeds are empty or only partially filled at maturity and the rates of seed abortion are high. We have also observed a high frequency of seeds in which there is development of the megagametophyte in the absence of the embryo. Exacerbating problems of poor seed production are problems associated with inadequate dormancy termination; as a result, this species is at a competitive disadvantage. In nature, only a low percentage of seeds germinate the first year after dispersal. The majority of seeds need longer durations (sometimes, up to another year) of moist chilling to break dormancy. Throughout this time, seed numbers can decline dramatically due to consumption by birds and small animals or deterioration caused by fungal attack (12). Currently, the common or traditional method of breaking dormancy of yellow-cedar seeds in a lab or forest nursery setting is approximately 3 months in duration, consisting of a 3-day running water soak (10–20°C), 4 weeks of warm "stratification" (in which seeds are maintained in the moist state at ~21°C), and 8 weeks of moist chilling (2–4°C) (7, 13).

Western white pine grows along the west coast in southern British Columbia and southwards through Washington, Oregon, and Northern California. In the interior of North America, western white pine can be found in pockets of Idaho and Montana (14). Western white pine was a very important commercial species in the nineteenth century; however, due to the North American introduction of white pine blister rust from infected pines in Europe and the subsequent death of a great proportion of the trees, its commercial impact is limited even today. However, rust-resistant stocks of western white pine are being produced and the impact of the disease today has mostly been mitigated (15, 16). In order for germination to be elicited, seeds must be subjected to a prolonged moist-chilling treatment for 3 months or longer (17). The standard method used in British Columbia (e.g. at the BC Ministry of Forests and Range, Tree Seed Centre, Surrey, BC) is a 14-day running water soak (10–15°C) and 98 days of moist chilling at 2°C (13). Yet, even with this lengthy moist-chilling treatment, the germination capacity of different seedlots can be vastly different. Differences in germination performance can also occur when the same seedlot is chilled at different times or when there are large numbers of seeds subjected to the treatment (e.g. in a commercial operational setting) (17, 18).

Pacific silver fir (*A. amabilis*) trees grow throughout Oregon, Washington, and north through coastal British Columbia into southern Alaska. Subalpine fir (*A. lasiocarpa*) has one of the widest distributions of any fir; its habitat occurs from New Mexico, Arizona, and Northern California into Oregon, Washington, and

north into British Columbia and Alberta, stopping in southern Alaska and the Yukon (19, 20). Seeds of true fir species, including Pacific silver fir and subalpine fir, display pronounced seed dormancy at maturity. Accordingly, breaking dormancy in these species requires a prolonged moist-chilling treatment of 3–4 months (21–23). In addition, germination of these firs can be impaired by seed pathogens and select seedlots can have high numbers of empty seeds (24). Seeds of the true firs can be prone to germination during moist chilling; thus, a re-drying of the seeds during chilling has been incorporated to prevent this (21). A typical protocol for dormancy termination of Pacific silver and subalpine fir seeds consists of soaking of seeds for 2 days, followed by moist chilling (2–4°C) at >45% moisture content for 4 weeks. Subsequently, seeds are subjected to a "dry-back" (to 30–35% moisture), which is employed to inhibit germination and control fungal growth during moist chilling. Following "dry-back", the seeds are moist-chilled for an additional 8 weeks. Thus, the total protocol is approximately 3 months in duration (13).

Below, we review and summarize modified dormancy-breaking protocols for yellow-cedar, western white pine, true firs, Pacific silver fir, and subalpine fir, previously published as separate entities (Fig. 1) (5, 6, 25). In brief, after testing various methods to

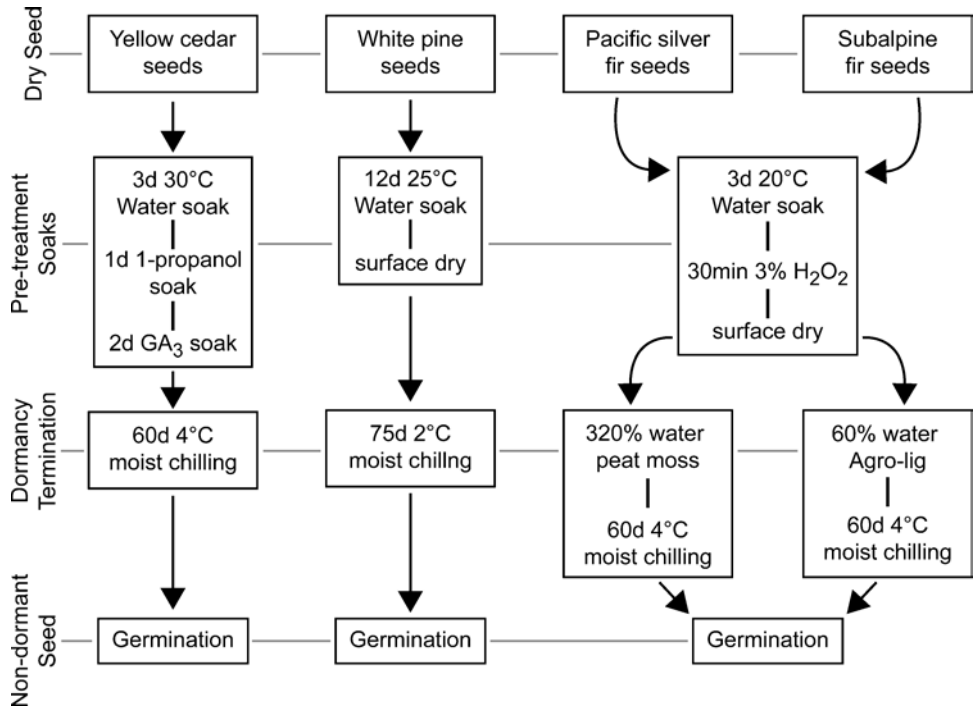

Fig. 1. Flow chart summarizing the modified dormancy-breaking methods for yellow-cedar, western white pine, and Pacific silver and subalpine firs.

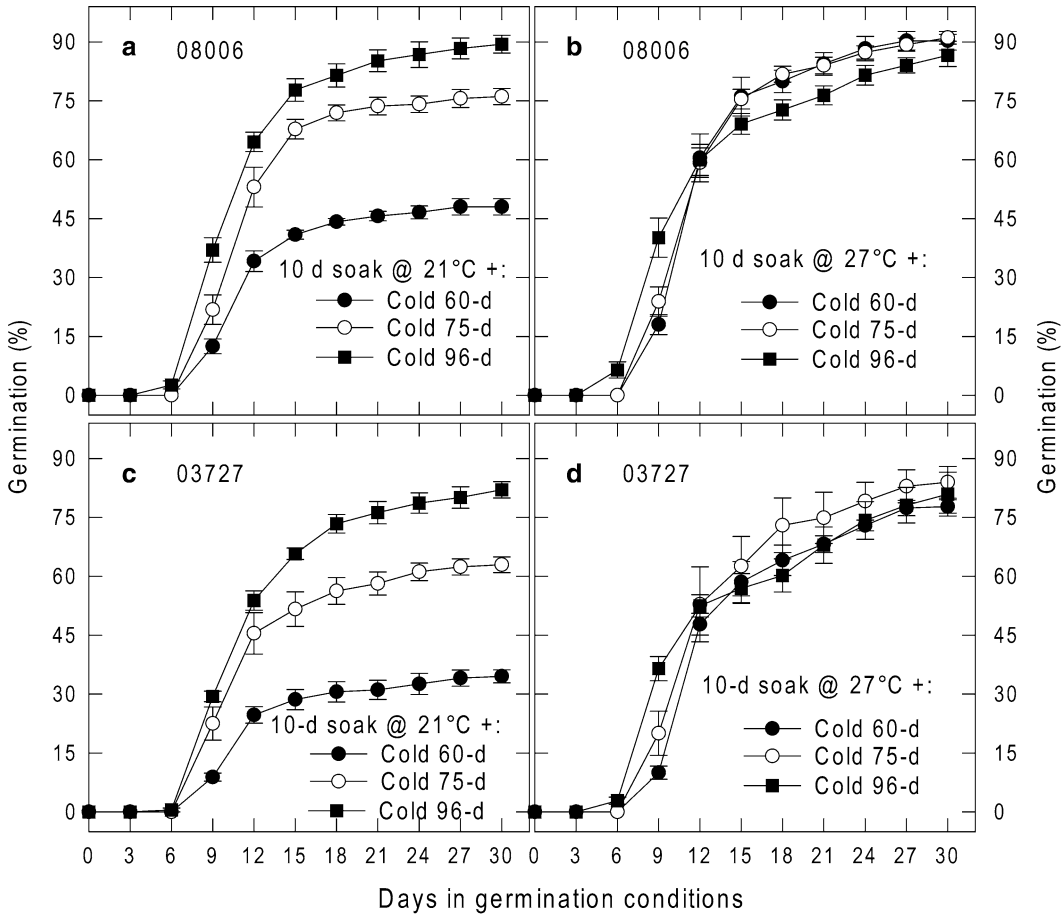

Fig. 2. Germination of western white pine seedlots 08006 (**a**, **b**) and 03727 (**c**, **d**) following a water soak and moist chilling. Seeds were either soaked at 21°C (**a**, **c**) or 27°C (**b**, **d**) for 10 days, followed by 60, 75, or 96 days of moist chilling. Reprinted from (6) with kind permission from the International Seed Testing Association.

effectively break the dormancy of yellow-cedar seeds (25–29), the most effective uses the anaesthetic 1-propanol, combined with a 3-day warm water soak, a 2-day treatment with the hormone gibberellic acid, followed by 30–60 days of moist chilling. For western white pine, a prolonged warm water soak followed by a 75-day moist-chilling period is most proficient (Fig. 2) (6). Finally, moist chilling within a solid matrix of Agro-Lig (a commercial formulation of humic acids) or peat moss proves very effective for breaking dormancy of Pacific silver and subalpine fir seeds, respectively (Table 1) (5). Compared to traditional dormancy-breaking methods, the modified protocols significantly reduce the time needed to terminate dormancy. The methods are effective for various seedlots within each species, and they further lead to synchronous germination within the seed population. For yellow-cedar, the modified protocols proved to be further effective in

Table 1
Germination percentages of fir seeds after different SMP-chilling treatments. Modified from (5) with kind permission from Springer Science and Business Media

		Moist chilling (weeks)		
Fir species	**Treatment**	**4**	**8**	**12**
Pacific silver fir	Control: moist chilling	39	44	45
	Agro-Lig Greens Grade	47	64	44
	Sand	61	65	57
	Peat moss	62	**79**	56
	Soaking only	3	3	3
Subalpine fir	Control: moist chilling	41	58	63
	Agro-Lig Greens Grade	63	**83**	70
	Sand	51	71	62
	Peat moss	55	71	68
	Soaking only	12	12	12

Bold numbers represent the most efficient dormancy-terminating protocol.

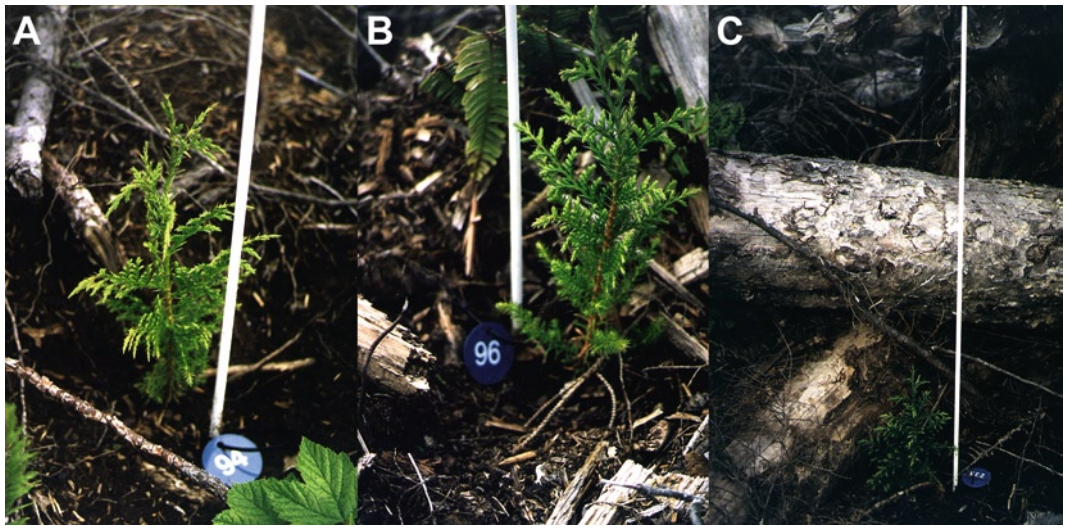

Fig. 3. Growth of yellow-cedar seedlings 2 months after out-planting into natural stands near Port McNeill, BC, Canada. These seedlings were subjected to the dormancy-breaking protocol described for yellow-cedar (Subheading 3.1). Reprinted from (29) with kind permission from Springer Science and Business Media.

promoting vigorous growth in a forest nursery greenhouse setting and after planting at natural stands (Fig. 3). These protocols described below in detail are the collaboration of several years of work in the laboratory of A. Kermode.

2. Materials

2.1. General Items

1. Polystyrene square container 156C, 10.95 mm×10.95 cm× 3.5 cm (Hoffman Manufacturing, Jefferson, OR, USA).
2. Seedburo K-22 seed germination paper (Kimpak), No. 87, 25.4×35.6 cm (Seedburo Equipment, IL, USA), cut to fit inside the container described in item 1 above.
3. Whatman 3MM Chromatography Paper, 35×45 cm, cut to fit inside the container described in item 1 above.
4. Forceps (Fine Science Tools, North Vancouver, BC, Canada).
5. Analytical scale (for milligram measurements).
6. Laminar flow hood for sterile work and air drying of seed (ENVIRCO, Sanford, NC, USA).
7. Spoonula or Spatula.
8. Mesh bag made from vinyl-coated fibreglass screen material available from most building supply stores.
9. Stainless steel tea ball available from most grocery stores.
10. Double deionized sterile water (dH_2O) (double deionized H_2O, ddH_2O, is sterilized by autoclaving).
11. Water bath (approximately 10–12 L) (e.g. Thermo Scientific, Newington, NH, USA).
12. Refrigerator capable of maintaining 2–4°C.
13. Controlled temperature growth chamber (e.g. Conviron TC16, Controlled Environments Ltd, Winnipeg, MB, Canada).

2.2. Dormancy Termination of Yellow-Cedar Seeds

1. 1-Propanol (Anachemia Science, Montreal, Canada).
2. Gibberellic acid A_3 (GA_3) (Sigma-Aldrich, Oakville, ON, Canada).
3. Petri dishes (10×1.5 cm).
4. Parafilm.
5. Mature seeds of yellow-cedar. In our case, we obtained all seedlots from the BC Ministry of Forests and Range, Tree Seed Centre (Surrey, BC, Canada). Seeds should be maintained at –20°C before use.

2.3. Dormancy Termination of Western White Pine Seeds

1. Petri dishes 10×2.5 cm.
2. Optional: 70% ethanol, 30% sterile water.
3. Optional: 10% commercial bleach, 90% sterile water.
4. Optional: Corning 150-mL Tube Top Vacuum Filter System (Fisher Scientific, Ottawa, Canada).

5. Mature seeds of western white pine obtained from the BC Ministry of Forests and Range, Tree Seed Centre (Surrey, BC, Canada). Seeds should be maintained at –20°C before use.

2.4. Dormancy Termination of Pacific Silver Fir and Subalpine Fir Seeds

1. 30% hydrogen peroxide (H_2O_2).
2. Agro-Lig Greens Grade (humic acids with particle sizes between 0.212 and 1.29 mm) (America Colloid Company, Reeder, ND, USA).
3. Peat moss (Lakeland Peat Moss Ltd., Edmonton, AB, Canada) sieved with 1.4-mm testing sieve (see item 6 below).
4. 150-mL sterile sample bags (Fisher Scientific).
5. Testing sieve #14, 1.4-mm screen opening (VWR Canlab).
6. Mature seeds of Pacific silver and subalpine firs obtained from the BC Ministry of Forests and Range, Tree Seed Centre (Surrey, BC, Canada). Seeds should be maintained at –20°C before use.

3. Methods

3.1. Dormancy Termination of Yellow-Cedar Seeds

1. Following removal of the seeds from storage at –20°C, seeds, in batches of 150, are soaked within tea strainers or mesh bags in a 30°C water bath for 3 days.
2. Seeds are then transferred to Petri dishes containing 30 mL of 50 mM 1-propanol. The Petri dishes are sealed with parafilm and gently agitated at 70 rpm on a platform shaker for 24 h.
3. Seeds are transferred to a Petri dish containing 30 mL of 200 mg/L of GA_3. Plates are sealed and agitated as in step 2 for 48 h.
4. The seeds are then placed directly on a moist substratum within a polystyrene container for 60 days at 4°C in the dark. The moist substratum consists of one layer of Whatman 3MM paper and one layer of Kimpak in a square polystyrene container ($10.95 \times 10.95 \times 3.5$ cm) moistened with 30 mL of sterile ddH_2O.
5. Following moist chilling, seeds are divided into replicates of 50 seeds each and placed on a moist substratum in polystyrene containers for germination. This moist substratum is identical to that described in step 4, except 50 mL of ddH_2O is added.
6. Seed containers are placed in germination conditions: 30°C days, 20°C nights with an 8-h photoperiod; light intensity at 25 μmoles/m^2/s, PAR 400–700 nm.
7. Germination counts can be monitored daily for the first 15 days followed by once every 3 days for 15 days (30 total days).

3.2. Dormancy Termination of Western White Pine Seeds

1. Following storage of seeds at –20°C, western white pine seeds are allowed 1–2 h to equilibrate to room temperature. During this time, seeds can be counted into needed replicates for subsequent experiments. Alternatively, if dealing with large numbers of seeds, one can calculate the number of seeds per gram and weigh the seeds.
2. Optional: Depending upon the seedlot and the presence of seed-borne pathogens, seeds may need to be sterilized. Seeds can be sterilized by soaking in 70% ethanol for 5 min, followed by 10% commercial bleach for 3 min. This is followed by rinsing with sterile water for two 5-min periods. We often use bottle-top filters to ease in the sterilization of seeds (e.g. Corning 150-mL Tube Top Filter).
3. Seeds are placed into water-penetrable containers, such as stainless steel tea balls or, if using larger quantities of seeds, into screen or mesh bags.
4. Seeds are soaked for 10–14 days in running tap water (approximately 10–15°C) or in a 25°C water bath with water exchanged daily (see Note 1). If you are using aseptic technique, the seeds can be soaked in 50-mL conical tubes or Erlenmeyer flasks with gentle shaking.
5. Following the water soak, water is drained and the seeds are placed on Whatman 3MM paper in a flow hood to dry the surface moisture off of the seeds (see Note 2).
6. Seeds (<250 per container) are placed on Whatman 3MM paper supported by K-22 germination paper in a square polystyrene container to which 25 mL of ddH_2O is added. If you are using aseptic technique or have small sample sizes (e.g. 25–30 seeds per replicate), deep dish Petri plates (10×2.5 cm) are used and 12.5 mL of ddH_2O is added.
7. Seeds are placed to moist chill at 2–4°C for 98 days in the dark (see Note 3).
8. Following moist chilling, the seeds can be transferred to germination conditions. The seeds are transferred to new square polystyrene containers (50 seeds per dish) or deep dish Petri plates (25 seeds per plate), set up as mentioned in step 6, except with 50 and 23 mL of ddH_2O, respectively. Germination conditions of 23°C and a 16-h photoperiod or 25°C days, 15°C nights, and an 8-h photoperiod have been used successfully (see Note 4). Light intensity for both conditions is kept at approximately 60–80 μmol/m²/s, PAR 400–700 nm.
9. Germination counts can be monitored daily for the first 15 days followed by once every 3 days for 15 days (30 days total).

3.3. Dormancy Termination of Pacific Silver and Subalpine Fir Seeds

1. Following removal of the seeds from storage at −20°C, seeds are weighed to estimate the seed number needed for a typical experiment.
2. Seeds are soaked in a mesh bag submersed in an aerated-dH_2O running water bath for 3 days at 20–22°C (see Note 5).
3. Seeds are sterilized for 30 min in 3% H_2O_2 and rinsed several times with sterilized ddH_2O (see Note 6).
4. Seeds are dried for approximately 5 min in a laminar flow hood until the seed-surface moisture disappears. During this time, seeds are divided into the replicates for the experiment (e.g. 4 replicates of 50 seeds each).
5. During the final hours of the seed soak, the matrices for solid matrix priming (SMP) chilling are prepared and placed in 150-mL sample bags. For Pacific silver fir seeds, pre-sieved peat moss is combined with 320% sterile ddH_2O on a weight-to-weight (w/w) basis (see Note 7) and approximately 30 mL of wetted matrix is placed into a 150-mL sample bag together with 50 seeds from step 4. For subalpine fir seeds, Agro-Lig Greens grade is mixed with 60% sterile ddH_2O on a w/w basis and approximately 30 mL of wetted matrix is placed into a 150-mL sample bag together with 50 seeds from step 4.
6. Seeds within the various matrices are then placed at 4°C for 8 weeks of subsequent moist chilling (see Note 8).
7. Following 8 weeks of SMP chilling, fir seeds are removed from the matrices by rinsing in a sieve (1.4-mm screen) with dH_2O water.
8. Approximately 50 seeds are then transferred to a square polystyrene container with Whatman 3MM paper supported by K-22 germination paper and 50 mL of dH_2O water is added.
9. Germination is then tested using 21°C days, 15°C nights with an 8-h photoperiod; light intensity is at 40 μmoles/m^2/s, PAR 400–700 nm.
10. Germination can be monitored daily or every 3 days for a total period of 30 days. To estimate the "realistic germinability" of a particular seedlot, the ungerminated seeds are then cut open to determine the proportion of unfilled seeds (e.g. seeds missing a viable embryo due to abortion or poor seed developmental conditions, or attached by a parasite or a microorganism, such as fungus).

4. Notes

1. Using a higher temperature soak (i.e. 25°C) decreases the subsequent moist-chilling duration (6).
2. This step helps minimize fungal contamination and also aids in dormancy breakage possibly due to increased air exchange (5, 6).

3. The moist-chilling time for western white pine seeds can be shortened to 75 days if a higher temperature soak is used (Subheading 3.2, step 4).
4. Germination conditions at 23°C constant temperature elicit more effective germination (i.e. germination occurs over a shorter time frame), but 25°C days, 15°C nights may more accurately reflect conditions in a forest seed nursery.
5. In our experience, a 3-day soak is necessary to obtain a stable water content (e.g. 45% for Pacific silver fir seeds and 50% for subalpine fir seeds).
6. Sterilized water may not be needed at this point and for subsequent moist chilling since most of the fungal contamination, if present, comes from the seeds themselves.
7. Moisture content of the matrices is calculated on a weight-to-weight basis and is calculated based on: weight of water/dry weight of solid matrix × 100.
8. To determine the optimal period of moist chilling, we also considered the potential for germination during the chilling period and tried to minimize this value. Thus, longer periods of SMP moist chilling are beneficial to dormancy breakage and subsequent germination, but unwanted "pre-" germination was always increased – sometimes as much as 20%.

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