

Chapter 4

Assuring Seed Quality for Seedling Production: Cone Collection and Seed Processing, Testing, Storage, and Stratification

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Abstract

This chapter summarizes current technology concerning cone collection and seed processing, testing, storage, and stratification for the six major conifer species—Douglas-fir, ponderosa pine, lodgepole pine, noble fir, white fir, and western hemlock—produced as seedlings in Northwest bareroot nurseries. Though great advances have been made in the past 20 years, further refinements are deemed necessary to continue improving seedling-production technology, especially as use of valuable seed-orchard seed is favored over natural-stand seed. Suggested future refinements should include: (1) determining patterns of seed retrievability to capture maximum seed yield; (2) devising a method for separating nonviable and low-vigor seed from viable and high-vigor seed; (3) developing a method for improving the correlation between laboratory and field germination; (4) designing an effective long-term seed-storage method for true firs; and (5) developing a quick seed treatment for nursery sowing which shortens or eliminates stratification requirements.

4.1 Introduction

Seed quality has great impact on the quality of planting stock. For the last 20 years, the technology of producing seedlings has advanced greatly. Parallel to this advancement, seed quality also has improved dramatically. This chapter brings together information on cone collection and seed processing, testing, storage, and stratification drawn from the current literature and from questionnaires sent to 21 nurseries and eight seed-processing plants (extractories) in the Northwest (OSU Nursery Survey; see chapter 1, this volume). Discussions mainly focus on the six major coniferous species being produced by these nurseries: Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*], ponderosa pine (*Pinus ponderosa* Dougl. ex Laws. var. *ponderosa*), lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *contorta*), noble fir (*Abies procera* Rehd.), white fir [*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.], and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.]. Where knowledge is lacking on these conifers, information on others is cited to illustrate important points.

4.2 Cone Collection

Careful attention to cone collection is critical to obtaining good quality forest-tree seed. Successful collection depends on understanding seed maturation and dispersal characteristics of each species, knowing local weather trends, and evaluating crop quality, harvesting procedures, and cone-storage methods.

4.2.1 Seed maturation

Cone collection should begin only when seed is mature. Immature seed can bring about various problems including (1) slow and incomplete germination [4, 29, 97], (2) low-vigor seed, resulting in smaller seedlings [30, 107], (3) greater susceptibility to disease [20, 124], (4) reduced storage capability [63], and (5) increased incidence of abnormal seedlings [76]. In addition, extraction of immature seed is more difficult than that of mature seed [85, 116]. Various maturation indicators, reflecting visual, physical, biochemical, or climatic changes, can be used effectively to prevent harvest of immature seed.

4.2.1.1 General maturation indicators

Cone color [26, 57, 100], bract color [30], seed wing color [96], scale color [103], and color and firmness of embryo and megagametophyte [78, 96] can be **visual indicators** of seed maturity. These indicators, though indirect and subjective, have proved reasonably practical in many instances [131].

Cone moisture content [33, 86], cone specific gravity [53, 85, 96], and embryo development [30, 96, 103] can be **physical indicators** of seed maturity. Loss of cone and seed moisture is closely associated with seed ripening [46], and the decrease in cone moisture content and cone specific gravity has been used to indicate maturity. Of these two indicators, specific gravity (SG) is usually preferred because it can easily be determined in the field. This method has been successfully applied to various pines (*Pinus* spp.) and true firs (*Abies* spp.) using flotation liquids such as water (SG = 1.0) and various mixtures of kerosene (SG = 0.80), light motor oil (SG = 0.88), and linseed oil (SG = 0.93). The ratio of embryo length to embryo cavity length, which can be determined quickly in the field with a sharp knife and a 10X magnifying hand lens [41], also can be used to judge maturity [30].

Changes occurring within conifer seeds can be **biochemical indicators** of seed maturity. On the basis of observed correlation of reducing sugar content and germination, Rediske [106] recommended that Douglas-fir cone collection be initiated when reducing sugar content has fallen to 13 mg/g of seed weight. In a subsequent study, Rediske and Nicholson [108] found that, in noble fir, the increase in crude fat content is more closely related to seed maturation and recommended the threshold value of 250 mg/g of seed for beginning cone collection. Although measuring biochemical indicators is time consuming and requires special laboratory equipment, it is thought to be more reliable than methods based on visual observation.

Changes in temperature, particularly during the summer in which seeds mature, can strongly influence the rate of seed maturation and are used as **climatic indicators**. Consequently, degree-day summations should be potentially more reliable than calendar date, especially at high latitude or high altitude, where summer temperature may limit seed development. Tanaka and Cameron [135] reported that 1,310 degree-days are required for ponderosa pine seed to mature at high elevations in southeastern Oregon. Zasada [152] related cone and seed development in white spruce [*Picea glauca* (Moench) Voss] to summer heat-sum and found that 625 degree-days were required to produce cones that could be successfully after-ripened in Alaska. Heat-sums are not extensively used for cone-collection purposes, probably due to lack of sufficient information. However, together with other climatic parameters such as precipitation and radiation, heat-sums would be a useful tool for field collection of coniferous cones in the Northwest [46].

Information (as of 1974) on cone- and seed-maturation indicators for many coniferous species in the United States is available in *Seeds of Woody Plants in the United States* [138]. Edwards [46] also provides an extensive discussion on various types of maturation indicators.

4.2.1.2 Maturation indicators used in the Northwest

Maturation indicators for the six major coniferous species in the Northwest are summarized in Table 1. Those used by the one nursery and six seed-processing plants involved in cone collection (OSU Nursery Survey) are, in order of frequency: cone, wing, and scale color, firmness of embryo and megagametophyte, and embryo development. Somewhat surprisingly, seed moisture content and specific gravity are not currently used, probably indicating that visual observation of the above characteristics is preferred because it is less time consuming. One seed plant extensively relies on biochemical indicators, using crude fat for noble fir and ponderosa pine and reducing sugar for Douglas-fir; on the basis of past experience, these biochemical indicators seem highly reliable.

Table 1. Cone and seed maturation indicators for the six major conifers in the Northwest.

Species	Maturation indicators	Reference
Douglas-fir	Reducing sugar 13 mg/g or less	[106]
	Embryo:cavity length ratio greater than 90%	[30]
	Browning of cone bracts	[30]
	Cone moisture content lower than 50%	[104]
	Firm, nonmilky megagametophyte enclosing a yellowish-green embryo	[78]
	Main harvest period of squirrels	[80]
Ponderosa pine	Specific gravity 0.85 or less (central Idaho)	[85]
	Specific gravity 0.84 or less (California)	[51]
	Specific gravity 0.94 to 0.99 (South Dakota)	[139]
	Specific gravity 0.88 or less (Arizona and New Mexico)	[111]
	Heat-sum 1,000 to 1,110 degree-days	[135]
Lodgepole pine	Specific gravity 0.43 to 0.89	[77]
Noble fir	Specific gravity 0.90 or less	[53]
	Crude fat 0.25 g/g of seed	[108]
White fir	Specific gravity 0.96 or less, uniformly brown seed wing, embryos pale yellow-green, 94% of the embryos fully elongated	[96]
Western hemlock	Brown cones with red-brown tips	[56]
	Cones opening after drying	[46]

4.2.2 Seed dispersal

Although seed-maturation characteristics have been extensively studied, little is known about timing of seed dispersal in relation to cone characteristics or climatic variables. Most observations relate the timing of seed dispersal to calendar dates, but such correlations may be of little value in the field because of yearly variation in weather patterns.

We have found that ponderosa pine seed in southeastern Oregon starts disseminating when cone moisture content drops to approximately 120% on a dry-weight basis. Once the rate of moisture loss in early August has been determined, it has been possible to predict approximate dates of seed dispersal for this species. Together with knowledge of seed maturation rate,

approximate seed-dispersal dates could be of practical importance to cone collectors. The field observations made by our laboratory also indicated that the earlier seed matured, the more quickly it started disseminating, probably due to faster drying of cones. Similar observations should be of value in capturing the maximum seed yield of other conifers that have a responsive reflex of cone scales.

4.2.3 Artificial ripening

It is important that cone collection be initiated after seed has attained full maturity. However, immature seed can be artificially ripened during cone storage in certain species. Artificial ripening has been successful on noble fir [108], grand fir [*Abies grandis* (Doug.) ex D. Don Lindl.] [102], white fir [96], and Nordmann fir [*Abies nordmanniana* (Stev.) Spach] [95]. Because of this potential increase in germination during cone storage, true fir cones are usually stored longer than those of other conifers before seed extraction. Douglas-fir [125], several species of pines [13, 76], and white spruce [150, 152] have also shown increased germination during artificial ripening. However, despite the findings of various researchers and the potential benefits, artificial ripening has not been extensively used for conifers other than true firs in the Northwest—probably because there is more risk of poorer germination and reduced seed yield in other species.

4.2.4 Weather

Weather conditions significantly impact cone collection. Except for pines with serotinous cones or some cypresses (*Cupressus* spp.) or junipers (*Juniperus* spp.) for which year-round collection is possible, the optimum cone-collection period for most conifers at any given location is relatively short. This period occurs sometime between late summer and late fall but could vary by up to 2 to 3 weeks depending on weather conditions. For example, if the snow melts late at high elevations during a cool spring, flowering may be so late that seed maturation could be delayed significantly [131]. A hot, dry summer may shorten the optimum cone-collection period by causing early seed fall, whereas cool, rainy conditions may delay it. Seed generally ripens earlier at lower elevations and on south and west slopes and later at higher elevations and on north and east slopes [122].

In addition to general spring and summer weather trends that determine seed-maturation and dispersal patterns, weather conditions during the cone-collection period itself are also important for the cone-harvesting operation. High winds or rain may preclude tree climbing, disrupt access to collection areas, and reduce pickers' productivity. In many areas in the Northwest, a drying east wind during the fall collection period may cause seed to disseminate too quickly, thereby reducing seed yield. For these reasons, daily forecasts and 5-day outlooks are valuable aids to coordinating cone-collection activities [41].

4.2.5 Crop quality

Once seed maturity has been determined, the quality of cone crops to be harvested must be evaluated. This generally is done by estimating the number of good seeds present in several representative cones, sliced lengthwise with a sharp knife. Cones of Douglas-fir, western hemlock, and pines are sliced through the center; those of true firs are cut lengthwise ¼ to ½ inch to one side of center [43]. A variety of knife assemblies is available for slicing conifer cones [123, 148, 149].

Minimum acceptable seed-count requirements may vary from year to year according to supply and demand. Average good seed counts are 6 for Douglas-fir, 8 for western hemlock, 10 for ponderosa and lodgepole pine, and more than 50% of

the seed (if seed has good appearance) for noble and white fir [43]. Lodgepole pine in certain areas produces cones that are very hard and, therefore, difficult to section. To extract seeds, such cones can be dipped in boiling water for 10 seconds, then placed in an oven at 65°C for 3 to 4 hours [41]. A minimum of 20 filled seeds per cone is required before a crop can be harvested. In addition to the filled-seed count, damage by biotic agents such as insects and disease, climatic extremes, or other abnormalities also should be assessed because these affect seed yield and are important factors in selecting areas from which to collect. Dobbs et al. [41] do not recommend collection if more than 50% of seeds are damaged. Several articles may be of help in identifying and assessing insect [59, 74] and disease [25, 62] damage.

4.2.6 Collecting methods

Cones are collected from western conifers: (1) by climbing standing trees, (2) from felled trees, and (3) from squirrel caches. Collecting cones from standing trees—the surest method to harvest seed of known origin, quality, and maturity—is often time consuming, expensive, and dangerous. Cones can be picked much more easily from felled trees in logged areas, but pickers should ascertain whether seeds were sufficiently mature when the trees were felled. Cones should be picked immediately after felling so as to minimize seed loss due to cone opening or mammal, bird, and insect damage. Squirrel-cached cones are easy to collect, but their use is sometimes questioned because the source and quality of the crop tree are not known. No evidence suggests, however, that seeds collected by squirrels are inferior to those collected by other means. All three of these methods are commonly used by cone collectors in the Northwest (OSU Nursery Survey).

Other methods less frequently mentioned in the Survey were helicopter collection and mechanical seed harvester. Helicopter collection has been experimentally tested in Canada by Dobbs et al. [42]. Mechanical tree shakers, regularly used on southern pines [27, 75, 137], have been tried only experimentally for western conifers. Although not easily adaptable to Northwest terrains for natural-stand collection, mechanized cone collection should play an important future role when western seed orchards are in full production.

4.2.7 Cone storage

Cones are stored (1) because processing equipment is not usually capable of extracting seeds from all harvested cones at once [81]; (2) to decrease cone moisture content, thereby reducing kiln drying time; and (3) to artificially ripen seeds of species such as true firs and improve seed-germination potential.

In large-scale cone collection, cones are usually placed in burlap bags, which are stored either temporarily near collection sites or in storage sheds at the extractory. However, great care should be exercised to maintain seed quality during cone storage. Burlap bags should not be filled to the tops, so that cone scales can fully expand upon drying; if scales cannot open sufficiently, seed extraction may be severely impaired [131]. Burlap bags should not be stacked up in large piles; this can lead to seed losses due to overheating or to insect and disease damage. Warm, moist environments can harm seed quality [81, 109]; hence, good ventilation should be provided. At a few seed-processing plants, cones of true firs and spruces are stored on ventilated mesh screens for artificial ripening (OSU Nursery Survey).

There has been an attempt to rank the different species according to relative ability to withstand prolonged cone storage [81]. At one seed-processing plant in the Northwest, cones of western hemlock are extracted first and those of true firs last. The ranking is primarily based on intuition and experience, but such information is valuable in scheduling cone processing.

OSU Survey respondents from most seed plants indicated that they store cones from 1 to 6 months, depending on species and size of cone crops. Several studies conducted with western species have confirmed the success of current cone-storage practices and have shown that, if cones are handled properly and storage conditions are optimum, seed could be safely stored in intact cones for up to 4 to 6 months [79, 82, 106, 109]. For cone storage beyond 4 months, it may be advantageous to install frost protection because subfreezing temperatures could significantly reduce seed germinability [133].

4.3 Seed Processing

After cones are harvested and stored, seeds are extracted and prepared for either immediate sowing or storage. This series of operations, called seed processing, includes kiln drying, cone tumbling, scalping, dewinging, and cleaning and sorting. (Seed-processing equipment is also discussed in chapter 3, this volume.)

4.3.1 Kiln drying

Given good drying conditions, cones of most conifers open readily. Under natural storage conditions, however, cones may not be thoroughly and uniformly dried, especially when weather is humid and cool. Cones should therefore be kiln dried to facilitate extraction.

Kilns are of two types: rotating and progressive [131]. In rotating kilns, a batch of cones is loaded into and dried within a drum where temperature and humidity are usually controlled. Such kilns, although not suitable for drying a large quantity of cones, can provide specific drying temperatures and relative humidities for small-lot processing. In progressive kilns, loaded trays are moved at certain time intervals to expose cones to increasingly warmer air as they dry. This type of kiln is more suitable for large-batch processing.

Kilns are generally operated at temperatures between 32 and 60°C [1]. Although studies have shown that the biologically lethal temperature of most tree seed is around 66°C [12, 113], the operational maximum temperature should not exceed 43°C [32, 54]. Because cones often have a high moisture content after storage, however, drying should be started at low temperatures that are progressively elevated. Drying cones with high moisture content immediately at high temperatures should be avoided because it could lead to case hardening and result in partial cone opening and incomplete seed extraction [78]. However, the problem of case hardening can, to some extent, be overcome by moistening scales or soaking cones in water.

Air humidity is as important a factor as temperature. Low humidity is the key to more complete drying. For example, cones can be successfully dried at the relatively low temperature of 32 °C if relative humidity is below 30% [1]. Cones of most major conifers in the Northwest readily open upon drying. However, lodgepole pine cones from certain geographic areas are serotinous and require a short soak in hot water before kiln drying [112]. Additional soaking cycles with water have been reported to increase seed extraction by 20 to 84% [140, 144].

4.3.2 Cone tumbling

In rotating kilns, cones are dried and tumbled simultaneously, and seeds fall out as cones open. Generally, loose seeds drop through perforations in the drum. Cones dried in progressive kilns are subjected to shaking action by tumblers to extract seeds from cones. A tumbler is a rectangular or round wire-mesh container mounted horizontally on its long axis, which turns at a slow speed. Small quantities of cones may be tumbled in batches. In large-scale continuous operation, the

tumbler axis is inclined so that rate of cone movement through the tumbler can be regulated [131].

4.3.3 Scalping

Seeds coming from the tumbler must be separated from a mixture of cone fragments, hardened pitch, foliage, dust, and other debris. This step, called scalping, is achieved by vibration, air movement, or screens, alone or in combination. The most commonly used equipment has several layers of vibrating screens of different-sized mesh. Coarse materials such as scales and twigs are retained on the uppermost screen and slide down to be collected in one bin, while fine particles are screened to be deposited in another bin: the seed is usually collected through an intermediate screen [47].

4.3.4 Dewinging

Once debris has been eliminated, wings must be removed from many conifer seeds. Although wings are often loosened during tumbling and scalping, dry or wet dewinging may also be required. Dry dewinging, a technique which employs a rubbing action to remove wings from dry seed, is generally used for Douglas-fir, pines, and true firs. Small lots can be dewinged in a cloth bag; lots of up to 5 kg are better handled in a Dybvig macerator [19]; and large lots are best dewinged with a brush-type dewinger, although auger-type dewingers have also been used successfully.

Because dry dewinging is the processing step that is most likely to cause seed damage, extra caution should be exercised to use proper equipment and to minimize unnecessary friction. In one study, for example, three cycles of brush-dewinging seeds of subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.] destroyed 50% of the originally viable seeds [pers. commun., 49].

Because dry dewinging can mechanically damage seed, many seed workers prefer wet dewinging, especially for pines and spruces. The principle of wet dewinging is that wings are more hygroscopic than seed and, upon wetting, are released cleanly. The Kason Vibrator [47] and a rotating cement mixer with a soft brush [144] have been successfully used for wet dewinging. However, because seed absorbs moisture during wet dewinging, it must be redried sufficiently before storage. Germination tests verified that a 20- to 30-minute water soak, followed by wet dewinging and air drying for 16 hours at 26 to 30°C to 4 to 8% moisture content, did not adversely affect seed quality [144].

4.3.5 Cleaning and sorting

Empty seed, partially filled seed, and other foreign particles are removed from good seed in the final cleaning. Scalpers and fanning mills are often used for species that have few scales, such as Douglas-fir and pines, but vibratory gravity tables are best for true firs. Pneumatic seed cleaners have also been successfully used for various conifer species [45, 126, 151]. All this equipment, in combination, further improves sorting efficiency. Flotation sorting with water, alcohols, and other organic liquids has been used to clean red spruce (*Picea rubens* Sarg.) [8], true firs [pers. commun., 49], and several pines [10, 88, 143], although this method has only been tested experimentally with western species.

A noteworthy development in seed sorting is the IDS (incubation-drying-separation) method, developed by Simak [129], which can separate nonviable, as well as empty and partially filled, seed from viable seed. Fully imbibed seed is first incubated for a short time, then gradually dried, and finally separated by various specific-gravity methods. Because empty and nonviable seeds lose water more quickly during the drying phase, differences between nonviable and viable seeds

are magnified, making subsequent separation by standard gravity methods more effective. Scots pine (*Pinus sylvestris* L.) seed of low germinability was successfully upgraded by this method experimentally [129].

4.3.6 Seed processing in the Northwest

Most processing work is done at seed plants in the Northwest. All eight seed-processing plants responding to the OSU Nursery Survey process their own seed as well as seed harvested by other organizations. However, four of the 16 nurseries responding do at least part of the processing at their own facilities. The remaining nurseries have private or state plants process their seeds.

Seven of the eight seed-processing plants and seven of the 16 nurseries set their own standards of purity for commercial seed transactions or nursery sowing (Table 2). The seed plants and nurseries replying to the Survey had a generally higher standard of purity than the Western Forest Tree Seed Council [130] recommendations for four of the six major coniferous species; the lower accepted purity standards of the true firs (see Table 2) may indicate possible difficulties in removing nonseed components without adversely affecting seed germination. Seeds of true firs are known to be especially sensitive to handling and mechanical damage [47].

Table 2. Minimum purity standards recommended by the Western Forest Tree Seed Council [130] and established by seed-processing plants and nurseries (OSU Nursery Survey) for the six major conifers in the Northwest.

Species	Tree Seed Council ~~~~~ % ~~~~~	Seed plants and nurseries
Douglas-fir	95	95-99
Ponderosa pine	95	95-99
Lodgepole pine	90	99
Noble fir	95	90-98
White fir	95	90-98
Western hemlock	90	95

4.4 Seed Testing

Seed testing evaluates seedlot quality and is essential for both seedling production and commercial seed transactions. Most tree-seed tests are conducted with methods based on rules of the Association of Official Seed Analysts (AOSA) [7] or the International Seed Testing Association (ISTA) [66]. Testing methods pertinent to western conifers are also available from the Western Forest Tree Seed Council [130].

4.4.1 Sampling

The first step in seed testing is to draw a sample that represents the entire seedlot. A seedlot is defined as a unit of seed of reasonably uniform quality from a particular location or elevation [21]. Seedlot size varies with testing rules and among laboratories. ISTA [66], for example, has determined that a seedlot should be less than 5,000 kg for seeds the size of beech (*Fagus* spp.) seed or larger, or 1,000 kg for seeds smaller than beech. The Western Forest Tree Seed Council [130] recommends that lots in excess of 227 kg be divided into equal smaller lots for sampling.

Loose seeds in containers should be sampled with seed-sampling probes long enough to reach all areas in the containers. The sample should be composed of equal portions taken from evenly distributed volumes of the lots to be sampled, each sample proportional to the size of the container. Samples should be subdivided in the testing laboratory with a mechanical divider until a subsample of the desired weight is obtained.

4.4.2 Physical characteristics

4.4.2.1 Purity

Purity tests measure the percent by weight of four major components: (1) pure seeds of the test species, (2) seeds of other crop species, (3) weed seeds, and (4) inert matter (leaves, cone scales, etc.). The purity test is usually the first test performed for a given lot and is especially important for commercial transactions, which are based on weight.

4.4.2.2 Moisture content

Seed moisture content is most often determined with the air-oven method [66]. Seed samples are heated in ovens; the weight loss that occurs during drying is considered to be seed moisture. ISTA rules prescribe oven drying at 105°C for 16 hours for all tree seeds except those of the genera *Abies*, *Cedrus*, *Fagus*, *Picea*, *Pinus*, and *Tsuga*. Seeds of those genera contain a significant amount of volatile oils and resins which may be lost at the above temperature. Therefore, their moisture content must be determined by toluene distillation [66]. Electronic moisture meters, though not as accurate as the above methods, are frequently used by various seed workers; they give rapid measurements desirable, for example, when checking moisture in a large number of seedlots being dried before storage.

Seed moisture content can be expressed as a percentage of water loss of either total **fresh** weight or corresponding **oven-dry** weight. Seed moisture content has been expressed on a dry-weight basis in some research [99], but international usage is exclusively on the fresh-weight basis. To avoid misunderstandings, the base should always be clearly specified.

4.4.2.3 Weight

Seed weight, required for calculating sowing rates in nursery sowing and direct seeding, is a function of seed size, moisture content, and proportion of full seed in a given lot. The commonly used unit is the weight of 1,000 pure seeds (1,000 seed weight). ISTA [66] specifies weighing eight random samples of 100 seeds each from the pure-seed component; however, some laboratories use two or more samples of 500 seeds each. When means of replicates vary more than 10%, additional samples should be weighed. All weights should be accurate to three significant digits.

4.4.3 Biological characteristics

4.4.3.1 Tests to estimate seed viability

Germination potential, perhaps the most important quality measurement in seed testing, is used to determine sowing rates as well as whether seed must be sown immediately or can be stored. Seeds of different species have different requirements for optimum germination. This potential can be (1) evaluated directly by germinating seeds under predetermined conditions or (2) estimated indirectly with biochemical staining, embryo excision, cutting tests, x-ray radiography, or hydrogen peroxide tests.

The most reliable method is germination in a controlled environment. At least 400 seeds, usually divided into four replicates of 100 seeds each, from the pure-seed component of the purity test [7] are normally prechilled for up to 28 days and germinated on suitable substrates (Table 3). Substrates should (1) be nontoxic, (2) be free of molds or other microorganisms, and (3) provide adequate aeration and moisture [71]; those recommended by AOSA [7] are blotter papers, paper towels, washed sand, vermiculite, perlite, and peat moss. Most (over 70%) of the coniferous species listed in the AOSA rules are germinated under alternating temperatures (30°C for

8 hours in the light, 20°C for 16 hours in the dark). An intensity of 750 to 1,500 (\pm 250) lux [75 to 150 (\pm 25) foot-candles] is recommended [71]. Seed is counted as germinated when all essential structures appear normal. Retests are necessary when an extremely high proportion of full, ungerminated seed is left at the end of the test, or when variation among test replicates exceeds the accepted tolerances [7].

Although controlled-environment germination tests are reliable, they are often time consuming, especially for dormant species requiring prechilling. Several rapid methods of estimating viability have been proposed, two of which—tetrazolium staining and embryo excision—are now recognized as official testing procedures.

The tetrazolium test is the most commonly practiced biochemical staining method [66, 94]. Seeds are immersed in 2,3,5-triphenyl tetrazolium chloride. Living cells stain red as tetrazolium is reduced by dehydrogenase enzymes to form a stable red triphenyl formazan, which is insoluble in water. The method is fast but lacks uniformity in staining [83]; therefore, results can be difficult to interpret. Other biochemical staining methods applied to seed testing with varying degrees of success include those using salts of selenium and tellurium [9] and Indigo Carmine [73, 93].

The excised embryo test is recommended for several species of pines including Coulter pine (*Pinus coulteri* D. Don), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), and sugar pine (*Pinus lambertiana* Dougl.) [66]. Excised embryos are cultured on moist filter or blotter paper in covered dishes under light for 10 to 14 days at 18 to 20°C. Viable embryos remain firm and white and turn green, indicating growth, whereas dead ones turn dark or are covered with mold. This method is fast but requires skilled analysts.

Other quick methods include the cut, x-ray, and hydrogen peroxide tests [82]. In the cut test, seed is bisected and then rated visually; this is the simplest but most unreliable method because distinguishing seeds damaged during handling and storage is very difficult. The x-ray test is fast, especially when Polaroid film is used [44], and development of contrast techniques has greatly expanded x-ray test capabilities [127]. Disadvantages are difficulty in interpretation and relatively high equipment costs. The hydrogen peroxide (H₂O₂) test allows assessment of root growth in 1% H₂O₂ [32]. It is simpler to perform than the excised embryo test and is more objective and easier to interpret than x-ray. However, as with other quick tests, it tends to overestimate viability, compared with germination tests.

4.4.3.2 Seed vigor

Nursery bed germination is usually slower and less complete than laboratory germination. Therefore, various laboratories have attempted to define and determine seed vigor to improve prediction of nursery germination. Three major groups of expressions have been proposed: (1) mathematical values based on standard laboratory test results, (2) germination under stressful conditions, and (3) biochemical testing.

Mathematical expressions have been most widely tested. They include the number of days required to attain a certain proportion of total germination [8, 28], germination value [36], modified germination value [40], and the Weibull function [22, 114]. Germination under stressful conditions has been developed mainly for seeds of agricultural species; most widely used are the cold test for corn [31, 68] and the accelerated aging test for soybean [87, 136]. However, application of these tests or development of new procedures for tree seed has been rather limited. Biochemical tests have been tried to a limited extent; the few reported include tetrazolium staining [94] and the GADA (glutamic acid decarboxylase activity) test [21].

4.4.4 Seed testing in the Northwest

Over 70% of the nurseries and seed-processing plants conduct some type of seed-quality test at their own facilities (OSU Nursery Survey); the remaining organizations send all their samples to outside commercial laboratories. The most commonly used outside laboratory is the Oregon State University Seed Laboratory (Corvallis, Oregon). Samples are also sent to private laboratories, other state laboratories, and the National Tree Seed Laboratory (Macon, Georgia). Of the 17 organizations that conduct their own tests, three conduct all of their tests; the rest have certain types of tests done by outside laboratories—including checking their own test results. According to the OSU Survey, the tests most commonly conducted, in order of frequency, are seed moisture content (see 4.4.2.2), 1,000 seed-weight determination (see 4.4.2.3), purity test (see 4.4.2.1), and germination test (see 4.4.3.1). Cut, x-ray, and H₂O₂ tests are used less frequently. No organization indicated use of seed-vigor expressions, although a few have tried Czabator's [36] germination value.

4.5 Seed Storage

Irregular and often infrequent seed production by many of the major tree species necessitates seed storage—sometimes

Table 3. AOSA seed-testing procedures [7] for the six major conifers in the Northwest.¹

Species	Temperature, ² °C	Test duration, days	Additional directions
Douglas-fir	20-30	21	Light ³ ; prechill 21 days at 3 to 5°C. Vermiculite recommended if top of blotter not used.
Ponderosa pine	20-30	21	Light; prechill 28 days at 3 to 5°C.
Lodgepole pine	20-30	28	Light; prechill 28 days at 3 to 5°C.
Noble fir	20-30	28	Light; prechill 14 days at 3 to 5°C. Vermiculite recommended if top of blotter not used.
White fir	20-30	28	Dark; prechill 21 days at 3 to 5°C.
Western hemlock	20	28	Light; many lots complete in 14 to 21 days; few sources from the coastal region may need prechill for 21 days at 3 to 5°C.

¹ Substrates for all species were the tops of blotters and covered petri dishes with (a) two layers of blotters, or (b) one layer of absorbent cotton, or (c) five layers of paper toweling, or (d) three thicknesses of filter paper, or (e) top of sand or soil.

² Single numeral indicates constant temperature. Two numerals separated by a dash indicate an alteration of temperature, the test to be held at the first temperature for approximately 16 hours and at the second temperature for approximately 8 hours per day.

³ Where prescribed, light should be provided by a cool-white fluorescent source. Illuminance for dormant seed should be 750 to 1,250 lux (75 to 125 foot-candles). Seeds should be illuminated for at least 8 hours of every 24 and, where temperatures alternate (see footnote 2), during the high-temperature period only.

for several years—to maintain supplies through years of poor seed production. Because of this, considerable research has been carried out on seed storage. Storage is one area of forest-tree seed technology for which sufficient information is available for most species of interest.

Successful seed storage requires knowledge of the seed characteristics of different trees as well as of the factors influencing storage capacity, such as seed quality before storage, seed moisture content, and storage temperature and method. These aspects have been reviewed by Baldwin [9], Barton [17], Holmes and Buszewicz [63], Jones [70], Magini [84], Wakeley [143], and Wang [145].

4.5.1 Seed longevity

The life span of seeds varies with species. Seeds are classified into three biological categories according to their life span under natural conditions: (1) microbotic seeds (life span not exceeding 3 years), (2) mesobiotic seeds (life span from 3 to 15 years), and (3) macrobotic seeds (life span from 15 to more than 100 years) [34]. Seeds of most conifers and hardwoods are microbotic. Under regulated storage conditions, however, longevity of many tree seeds can be extended more than tenfold. For example, the viability of naturally dispersed seed of spruce and many pines extends only into the first growing season and, occasionally, into the second growing season. Under subfreezing storage, seed viability of these same species can easily be maintained at high levels for 10 years or longer [17]. Storage over 10 years is not usually required for seedling-production purposes but may become vital to future tree-breeding programs. Under optimum storage conditions, seed viability of certain trees might be maintained indefinitely, but the maximum potential for maintaining original seed viability has not yet been determined for most species [145].

4.5.2 Seed quality

Seed quality has a significant impact on storage capability. Factors affecting quality are seed maturity, cone handling, and seed extraction and processing. Immature seeds are not only poor in germinability and liable to be further damaged by seed processing but also are difficult to store successfully [2, 3, 30, 65]. Overheating during extraction [3] and damage caused by dewinging [9, 50, 72] also have been found to adversely affect seed quality. Injured seeds are not suitable even for short-term storage because they have a high rate of respiration, undergo spontaneous heating, and deteriorate rather quickly [63, 153].

4.5.3 Seed moisture content

Of all the factors influencing seed storage, moisture content may be the single most important one in maintaining germinability. Various researchers [17, 63, 70] have demonstrated the detrimental effect of high seed moisture on tree-seed viability; increased rates of respiration and changes in carbohydrates and fats presumably cause seeds to use their food reserves [84, 153]. Excessively low seed-moisture content also may reduce storage capability. Some species, including Douglas-fir, can tolerate drying to 0% moisture content [119]; however, overdrying can destroy the monomolecular layers that protect against oxidation [55]. Recommended seed-moisture content for storing Douglas-fir, ponderosa pine, lodgepole pine, and western hemlock is 6 to 9% (wet-weight basis); that for true firs is 9 to 12% [130]. However, Danielson and Grabe [38] showed that optimum moisture content for noble fir is also 6 to 9%.

4.5.4 Storage temperature

The effect of storage temperature on the retention of tree-seed viability has been thoroughly investigated [3, 17, 60, 63, 64, 70, 91, 120, 121]. The general relationship between stor-

age temperature and moisture content was described by Barton [17] as follows: at a given moisture content, the higher the storage temperature, the faster the deterioration of seed viability: the lower the storage temperature, the greater the tolerance to high moisture content and the better the retention of viability. Some studies have shown that temperature slightly above or below freezing may be sufficient to prevent deterioration for short-term storage [14,120], but the retention of viability has generally been better when seeds were stored at -18°C, particularly for longer periods [3, 13, 122]. Of the 24 organizations (nurseries and seed-processing plants) replying to the OSU Nursery Survey, 16 store seeds at their own facilities. Of these, 11 store the material -15 to -18°C (5 to 0°F) and four at -5 to -12°C (23 to 10°F); one stores them at 0.5°C (33°F), but only for short periods. For a seed moisture content of 6 to 9% (wet-weight basis), these storage temperatures generally seem within the safe range (Table 4) for storage up to 7 years. This length of storage time should maintain supplies through years of poor western-conifer seed production.

4.5.5 Storage method

Tree seeds can be stored either wet or dry. Large seeds of hardwood species require moist conditions and are usually kept wet for short-term storage, whereas small seeds of conifer species, including all major conifers in the Northwest, are stored dry. Seed moisture content is controlled by storing properly dried seed in tightly closed containers or by regulating humidity in the storage area (as for many agricultural seeds); in the Northwest, dried seed is generally placed in closed containers, although some facilities do use humidity-regulated storage rooms. These facilities, although costly, are effective in minimizing reabsorption of moisture by dried seed, especially in areas with humid climates.

The most frequently used storage containers are plastic bottles with screw tops, polyethylene bags, and fiberboard drums [145]. More than 80% of the organizations surveyed store their seeds in rigid drums lined with polyethylene bags (OSU Nursery Survey). This method is common in many areas because it is relatively inexpensive and effectively prevents uptake or loss of moisture by seed from the atmosphere. Of 16 organizations, nine use plastic bags as containers; five of those use 1- to 6-mil plastic, and the remaining four use 7- to 8-mil plastic. Thin bags are subject to ripping but easier to handle when cold. However, plastic containers are not completely impermeable to moisture [145]. Use of thicker materials may be desirable for seed requiring low seed-moisture content if external humidity is high and seeds are to be stored for a long period.

4.5.6 Retesting

Retesting is often recommended for seeds stored for a relatively long period (5 years or more). Even under ideal storage conditions, certain poor-quality seedlots rapidly lose their viability. Fourteen of 16 organizations conduct viability tests at 2- to 6-year intervals (OSU Nursery Survey). Seed moisture content is retested by only three of the 16, however, probably indicating that moisture content does not fluctuate significantly under current storage conditions.

Even if seed moisture changes only minimally in storage, additional moisture could be introduced when seeds are withdrawn. Therefore, it is particularly important that sealed containers removed from cold storage be permitted to reach ambient temperatures before being opened to avoid condensation of water within the container [145]. Sahlen and Bergsten [118] found that temperature was completely equalized in the center of a 28-liter container, with walls 2.5 mm thick, 36 hours after the container was moved from a -16°C storage room to 22°C ambient temperature. To minimize repeated opening

and resealing and to reduce storage space, the use of small-sized containers (10- to 25-kg capacity) has been recommended [63].

4.6 Seed Stratification

Tree seeds, unlike agricultural seeds, are in many cases characterized by deep dormancy. This is true for most North-west conifers. Seeds of different species or different geographical origins often require different pretreatments and conditions for optimum germination. The most commonly used pretreatment to break dormancy is stratification—which usually is moist cold treatment for up to several months. Stratification is generally known to bring about changes in anatomy or physiology,

including embryo growth [105], and in metabolism [106, 117]. Physiologically, breaking of dormancy has often been explained in terms of a shift in the inhibitor-stimulator balance. Presumably, although it may not directly affect the level of inhibitors [147], stratification could increase growth-stimulator levels, which would then counteract the effects of inhibitors in breaking dormancy [141, 142, 146].

Successful cold stratification requires: (1) proper moisture content, (2) low temperature, (3) adequate aeration, and (4) proper length of time. In practice, seed originally was stratified by placing it between moisture-holding media such as peat moss or sand in boxes, tanks, trays, and other suitable containers and maintaining it there under cold, moist conditions [21].

Table 4. Effect of storage conditions and periods on seed viability of the six major conifers In the Northwest.

Species	Storage condition	Storage period, years	Effect on viability	References	
Douglas-fir	Sealed, 5°C, 13.6% mc ¹	3	Reduced by 60%	[16]	
	Sealed, 5°C, 5.8% mc	3	Maintained		
	Sealed, -18°C, 5.8% mc	3	Maintained		
	Sealed, -18°C, 13.6% mc	3	Maintained		
	Sealed: room temperature, 0 and -18°C: 6.5-9.5% mc	5-7	-18°C better than 0°C; substantial loss at room temperature after 2-3 years		
	Sealed, -18°C, 6-9% mc	10-20	Maintained	[101]	
Ponderosa pine	Canvas bags, -4°C, 15% mc	3	Maintained	[15]	
	Canvas bags, -1 1 °C, 17% mc	3	Reduced by 15 %		
	Canvas bags, -18°C, 10% mc	3	Reduced by 9%		
	Sealed, 5 °C, 5.1 % mc	3	Reduced by 10%		
	Sealed, 0°C, 5.1 % mc	3	Reduced by 8%		
	Sealed, - 5 °C, 5.1 % mc	3	Reduced by 1 %		
	Sealed, -18°C, 5.1 % mc	3	Reduced by 10%		
	Sealed, room temperature, 8.1 % mc	7	Reduced by 31 %		
	Sealed, 0°C, 8.1 % mc	7	Maintained		
	Sealed, - 18°C, 8.1 % mc	7	Reduced by 9%		
		Airtight, 4.5°C	10		Maintained
	Airtight, 0 and -18°C	14	Maintained	[35]	
	Airtight, cellar	14	Substantial loss		
Lodgepole pine	Airtight, 4.5°C	9+	Maintained	[91]	
	Airtight, 4.5°C	11-20	Substantial loss in some lots	[120]	
	Sealed, 0°C, 8.8% mc	7	Maintained	[3]	
	Sealed, 0°C	2	Maintained	[6]	
Noble fir	Sealed; room temperature, 0 and -18°C; 9.0% mc	7	Reduced by 4 1 , 11, and 10%, respectively	[3]	
	Sealed; 8°C for 9 years and -4°C for an additional 7 years; 7, 8, 11, and 13% mc	16	Reduced by 6-16% after 9 years and 30-50% after 16 years		
	Sealed, 5°C	5	25%		[120]
	Sealed, -10°C	3-5	Maintained		[67]
	Sealed, room temperature	1	Total loss		[38]
	Sealed; 20, 5, and -18°C; 4% mc	2	Maintained		
	Sealed; 5 and -18°C; 6, 8, and 9% mc	2	Maintained		
	Sealed, - 18°C, 12 % mc	2	Reduced by 5-8%		
	Sealed; 20 and - 18°C; 16 and 17% mc	2	Greatly reduced		
White fir	Sealed, room temperature, 6.3% mc	7	Complete loss	[3]	
	Sealed, 0°C, 6.3% mc	7	Reduced by 17%		
	Sealed, -18°C, 6.3% mc	7	Reduced by 10%		
	Sealed, 5°C	5	4-53%		[120]
	Sealed, 5°C	10	6%		
	Sealed, 5°C	20	8%		
Western hemlock	Airtight, 5°C	20	1 and 13%	[120]	
	Sealed; room temperature, 0 and -18°C	5-7	-18°C usually superior to 0°C; complete loss at room temperature		
	Sealed, 5°C, 7.7% mc	2	Maintained		[16]
	Sealed, 5°C, 11.0% mc	2	Substantial loss		
	Sealed, -18°C, 7.7% mc	2	Maintained		
	Sealed, -18°C, 11.0% mc	2	Maintained		
	Canvas bags, -4°C, 8% mc	3	Complete loss		[15]
	Canvas bags, - 11 °C, 12 % mc	3	Complete loss		
	Canvas bags, -18°C, 8% mc	3	Maintained		

mc - moisture content.

Some nurseries use outdoor soil pits. More recently, stratification in polyethylene bags has become common at many nurseries and seed-processing plants. This method, called "naked stratification," requires no moisture-holding medium and less effort in preparing seed for subsequent sowing [6]. Seed is soaked in water in containers lined with plastic or mesh bags, drained of excess water, and kept at low temperatures for a predetermined period of time; bags are often loosely fastened to allow aeration. All nurseries and seed-processing plants responding to the OSU Survey use some type of naked stratification.

4.6.1 Water soaking

The rate of water absorption varies among species. Most conifers require 1 to 2 days of soaking to achieve full imbibition. It has been suggested that warm water can speed up water absorption by seed, and that running water and aeration can improve oxygen availability; however, this has not yet been substantiated experimentally for Northwest conifers. One study showed that running water was of no benefit to noble fir [unpubl. data, 134]. Twelve nurseries and five seed-processing plants responding to the OSU Survey stratify seed; nine of these soak seed for 24 hours, the other eight for 36 to 48 hours. During soaking, four organizations aerate water, whereas three use running water. These practices are probably beneficial, although the effectiveness should be determined for each species.

4.6.2 Temperature

After draining, seeds are stored in the fully imbibed state. A few species, such as yew (*Taxus* spp.) [61, 92] and yellow-cedar [*Chamaecyparis nootkatensis* (D. Don) Spach] [58], require storage at warm temperatures before cold storage: however, most coniferous species require low temperatures throughout. For loblolly pine (*Pinus taeda* L.), McLemore [89] found optimum stratification temperature to be 10°C, but Robinson et al. [115] reported that, for this same species, gradually increasing temperature over a 4-week period gave the best stratification results. Temperatures above 5°C are not desirable because they increase the risk of overheating and subsequent deterioration, although freezing temperatures also can damage seeds at high moisture content. Consequently, low temperatures of 2 to 5°C have been adopted as accepted operational practice in most cases. All except one organization use stratification temperatures between 1 and 5°C (OSU Nursery Survey); the exception uses 0°C. Even in this case, however, embryos would not experience freezing due to their osmotic potential, which is lower than that of water. Premature germination during prolonged stratification can be minimized if seeds are held at 2°C, rather than 5°C, for both Douglas-fir and ponderosa pine [39].

4.6.3 Aeration

Aeration during stratification is necessary to supply oxygen for seed respiration and to allow carbon dioxide and heat to escape [23]. Lack of aeration could therefore lead to deterioration of seed quality through buildup of toxic substances. The most commonly used technique is to leave a small air space at the neck of each bag in which seed is stratified and to massage the whole bag periodically. A few Northwest nurseries also use fine-meshed bags hung so that air may circulate (OSU Nursery Survey).

4.6.4 Duration

Optimum stratification length varies among species and seedlots [5]. In general, the longer the stratification period, the greater the rate of germination, especially under suboptimal

germination temperatures [5, 52, 132]. For this reason, seed destined for colder environments must be stratified long enough for quick and complete germination. However, prolonged stratification can cause seeds of some species to germinate prematurely [5, 98, 121]; furthermore, vigor and total germination may be reduced if seeds are stratified for excessively long periods [5, 98].

In the Northwest, stratification periods vary from 28 to 90 days for noble fir and Douglas-fir and 28 to 45 days for ponderosa and lodgepole pine (OSU Nursery Survey). These variations probably reflect the nursery environment under which seed is to be sown and germinated.

Premature germination sometimes occurs during prolonged stratification. Although premature germination is a serious concern in nurseries because the fragile seeds can be damaged in handling or during mechanical sowing, redrying and storing of stratified seed are possible. Danielson and Tanaka [39] reported that ponderosa pine seed air-dried to 26% and Douglas-fir seed air-dried to 37% can be stored for 9 and 3 months respectively without losing the beneficial effect of stratification or having their viability adversely affected. Subsequently, Edwards [48] tested the efficacy of surface-drying true fir seed after 1 month of stratification at saturation moisture, followed by 3 months of storage at 35% moisture content; this treatment not only prevented premature germination but also improved total germination and germination rate.

The exact mechanism behind the benefit of surface drying is not completely understood. It may be related to improved gaseous exchange brought about by removing the water film from the seed surface, which increases oxygen availability to the seed and facilitates the release of any accumulated toxic gases. Although the surface-drying technique provides the option of storing stratified seed for prolonged periods without losing stratification effects, the lower limit of seed moisture content should be determined for each species. Seeds can be stored safely below certain thresholds but seem to then require restratification after storage [11, 90]. This induction of secondary dormancy suggests that seed moisture and dormancy are closely related.

4.6.5 Other treatments to improve germination

Although stratification is an effective method to break dormancy, it is often time consuming. Past research has shown that hydrogen peroxide [28], gibberellic acid [110], ethylene [24], microwave irradiation [69], or osmotic agents [128] can stimulate germination of conifer seed. However, these studies were usually conducted under optimum germination conditions and may not be effective under the suboptimal temperature conditions frequently encountered in the field in early spring when seed is sown at Northwest bareroot nurseries. Further work is required to develop a quick, effective method that would facilitate germination under a wide range of temperatures and that would either eliminate the need for stratification or shorten the stratification requirement.

4.7 Future Research Needs

Technology of seed procurement and utilization has advanced significantly in the past 20 years. Further refinements are deemed necessary, however, especially because we are now moving into a transition period in which more valuable seed from seed orchards will be preferred to seed from natural stands. Some suggestions for these refinements follow:

- **Cone collection:** Though a great deal is known about seed-maturation characteristics, relatively little is known about the timing of seed dissemination. To maximize the

yield of high-quality seed in cone collection, a complete picture of the pattern of seed retrievability—which is influenced by both seed maturation and dissemination—is essential, especially in seed orchards where individual clones can be closely monitored.

- **Processing:** Currently available seed-cleaning procedures remove all of the empty seed and some of the partially developed seed. A method is needed for separating all the nonviable from viable seed, even including seed that looks fully developed but does not germinate. This is particularly important as precision sowing and uniform spacing of seedlings are introduced to maximize utilization of seedling-production areas. Each seed should have the potential of germinating, emerging through the soil surface, and forming a healthy seedling. Some effort is being made toward achieving this goal [129].
- **Testing:** Currently used seed-testing methods for western conifers provide information on germination potential of seed under optimum laboratory environments; however this often correlates poorly with nursery-bed emergence. A procedure should be developed by which germination potential in the nursery bed can be accurately assessed to improve predictability of crop establishment.
- **Seed storage:** Some true fir species, such as noble fir, produce infrequent cone crops, with large crops occurring at intervals of 3 to 6 years depending on location. There has been some concern that the viability of true fir seed deteriorates during storage within a relatively short time; current storage procedures have shown inconsistent results [3, 14]. Seed condition before storage and storage environment need to be more closely examined. The National Seed Storage Laboratory is investigating the feasibility of using liquid nitrogen to store noble fir seed for periods up to 50 years. Such an approach may be necessary to maintain the viability of a large crop of true fir until the next crop is available.
- **Seed treatment:** Coniferous seeds are generally characterized by deep dormancy requiring prolonged stratification of 60 to 90 days. Unfortunately, this requirement reduces planning and scheduling flexibility of nursery crops. Developing a quick seed treatment that would shorten or eliminate stratification requirements would be most beneficial.

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