COLLECTION, PROCESSING, TESTING, AND STORAGE OF TRUE FIR SEEDS— A REVIEW

By

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ABSTRACT

*This review of published papers, unpublished reports, and current research activities considers the major steps in procurement of true fir (*Abies*) seeds for reforestation. Cone collection timing is examined in terms of both the morphological and physiological maturity of the seeds and the feasibility of artificially ripening prematurely harvested seeds. The impact of various stages of* Abies *seed extraction, particularly that of dewinging, in causing seed injury and losses in germination quality is discussed. Principles, methodology, and equipment for laboratory measurements of seed quality are described and comparisons drawn with so-called quick tests of viability. The concept of seed dormancy is introduced to explain the requirement for prechilling (stratification) shown by various* Abies *species. Plant performance in the field is related to seed vigor, the concept and principal components of which are outlined. Both European and North American research is reviewed to outline the technological requirements for seed storage and its effects on vigor and plant production.*

INTRODUCTION

Expanding reforestation programs are increasing demands for greater amounts of high quality seeds and for more detailed and up-to-date technology concerning their procurement, processing, and efficient use. The main problems with *Abies* seeds are low quality and poor storability—problems that are frequently more severe than with other conifers. Despite considerable research on selected species, no coherent technology has been developed for overcoming these difficulties.

This paper reviews the biological requirements and mechanics of cone collection, seed processing, storage, and testing for true fir seeds. Seed maturity will be discussed in morphological and physiological terms, and the effectiveness of various ripeness indices will be compared. The practical implications and problems of artificially ripening immature cones will also be evaluated. Adding to the complications of seed procurement, the soft, fragile seedcoat is easily damaged during processing; the nature and causes of such injury and their effect on seed quality are discussed in some detail.

Seed testing will be described in relation to standardized, almost worldwide, procedures. The reasons and procedures for performing purity, weight, and germination tests are explained, together with the advantages and disadvantages of various quick tests for seed viability. The concept of dormancy will be introduced as the basis for the prechilling (stratification) treatment without which many *Abies* seeds fail to germinate. Seed vigor and its relationship to plant performance in the field is also described.

Since germination is usually poor, much effort has been expended to conserve what little seed viability is available. The principles and technology of seed storage will be examined and recommendations made from North American and European experience.

Throughout the review, attempts are made to identify the more glaring deficiencies in information and the limits to the transfer of technology from one species to another. Although the paper is primarily a review of published literature, numerous unpublished reports are included and references are made to several current research investigations.

CONE COLLECTION

Flowering and Fruiting

Flowers (strobili) of the true firs are unisexual and typically form high in the tree crown, with the seed-bearing female strobili being found singly or in small groups on the uppermost branches. Eis (1970) gave the minimum age for production of megasporangiate (female—seed) strobili in *Abies grandis* (Dougl.) Lindl. and *A. lasiocarpa* (Hook.) Nutt. as 20 years, in contrast with 35 years for production of microsporangiate (male—pollen) strobili. After pollination, the female strobili remain upright on the branches and, at maturity, the cones are between 8 and 25 cm long.

Pollination, fertilization, ripening, and seed dissemination all occur in the same growing season, and in as little as 90 to 120 days after pollination mature seeds may begin to disseminate (Franklin and Ritchie 1970). In all *Abies* species, the mature cone scales separate (abscise) from the spike-like cone axis and seeds separate from the scale. In *A. procera* (Rehd.), seed dissemination requires wind action or other branch movement to disturb the cone, whereas in *A. amabilis* (Dougl.) Forbes, the scales become greatly distorted as they dry out and actively tear themselves from the axis. This may account for the tendency for *A. amabilis* seeds to be shed earlier than those of *A. procera*. The cone disintegration characteristics of other species such as *A. grandis* and *A. lasiocarpa* are intermediate (Franklin and Ritchie 1970). While most seeds are disseminated in the fall, some may continue to be released well into winter and become trapped in snow banks. Such seeds will germinate within the snow bank, but the germinants have little or no chance to become established (Franklin and Krueger 1968; Irmak 1961; Stein 1951).

Maturation

Since *Abies* cones disintegrate at maturity, they must be collected some time in advance. Judging the best time to do so can be a major difficulty, especially since cones do not all mature simultaneously. Maturation date varies for cones on the same tree, from tree to tree within the same stand, from stand to stand in the same year, and from one year to the next (Edwards 1978a). The extent to which advance collections can be made is governed by the fact that seeds cease development if the cones are detached too soon from the parent tree, regardless of how the cones are handled after collection. This is because the food reserves necessary for continued development are not then available in the cone. Once the critical point has been passed, seed ripening will continue within the cones even when detached from the parent tree, the amount depending largely on how the cones are handled. Whereas early cone collections are more sensitive to the handling method, this sensitivity decreases in later collections (Edwards 1978a). The critical point, beyond which seeds continue to develop despite removal of the cones from the parent tree, is usually marked when the seeds reach maximum dry weight. In crop plants this completion of organic accumulation signifies the attainment of physiological maturity (Harrington 1972) and marks the point where the seeds begin to age; seed vigor is then at its highest and begins thereafter to decline into senescence.

However, even as Harrington (1972) points out, maturation may not be complete when maximum dry weight has been reached, since the embryos in some species may be immature and may continue to develop after removal from the parent plant. Their further maturation involves a reduction in seed moisture content and a usually demonstrable increase in seed germinability. The latter may be masked by an increase in seed dormancy (Edwards 1978a).

Rediske and Nicholson (1965) recorded such observations in their study of seed maturation in *A. procera* and described two phases of ripening. The first, termed "maturation," was a period of organic accumulation in the seeds, indicated by a rise in seed weight and an increase in radiographic density of the so-called "endosperm" (the megagametophytic tissue), at the end of which the seeds were barely germinable. The second phase was a period of "after ripening," achieved by artificially storing intact *A. procera* cones for several weeks before the seeds were extracted. During this second phase, marked increases in germination were accompanied by a further increase in seed dry weight and a discernible reduction in seed moisture content.

Physical Indices of Maturity

Increasing germination capacity in maturing seeds is an indicator that ripening is progressing. In *A. procera*, Franklin (1965) and Rediske and Nicholson (1965) observed that germination increased to a peak and then levelled off (suggesting that maturity had been achieved) before seed dissemination began. In contrast, Pfister (1966) and Snyder (1976), studying *A. grandis*, and Speers (1962), studying *A. fraseri* (Pursh) Poir., found that germination continued to increase right up to seed dispersal. Therefore, peak germination cannot be relied upon as a satisfactory index of ripeness in all species. Not only is germination testing an unwieldy method of assessing maturation, some species (including *A. procera*) may enter partial dormancy as they mature (Edwards 1978a). Other methods of estimating the development of maturity and the proper time to begin collecting cones have had to be devised.

Maturity indices based on physical parameters of cones and seeds have become widely used since they lend themselves to field estimation. Regrettably, many have been merely subjective estimates, the success of which has depended largely on the experience of the collector. Stoeckeler and Jones (1957) suggested that the cones of *A. balsamea* (L.) Mill. are mature when they begin to turn purple, while Strawn (cited by Franklin 1974) suggested a change from blue-green to brown in the cones of *A. fraseri*. In the Japanese true firs, two species, *A. firma* Sieb. et Zucc. and *A. homolepis* Sieb. et Zucc., show a change in color from green to yellowish brown, while three others, *A. mariesii* Mast., *A. sachalinensis* Fr. Schm. and *A. vietchii* Lindl., turn from bluish purple to brown, such changes being indicative of approaching maturity (Asakawa—cited by Franklin 1974). Dalskov (1960) reported that by starting to collect Danish sources of *A. procera* when the cones have begun to turn from green to yellowish brown, and have begun to bend the branches down because of their weight, some two to three weeks would be available before natural seedfall. At the

first signs of cone scale separation, the cones could also be considered collectable (Dalskov 1960). *A. concolor* (Gord. & Glend.) Lindl. seeds of Yugoslavian provenance possessed superior germination when they came from violet cones rather than yellow cones (Stilinovic and Tucovic 1971), but it was not clear if these color differences were related to maturity.

Gajic (1964) observed that Serbian sources of *A. alba* Mill. produced seeds with two distinct seedcoat colors—bright ochre and opaque violet—and concluded that only one color of seed was found on any one parent tree. His observations established that bright ochre seeds possessed the best germination, and that only these seeds should be collected. No mention was made of color changes with advancing maturation. In contrast, the acquisition of a distinct coloration in the seedcoat of *A. fraseri* was recommended by Speers (1962) as the point to begin cone collections. The development of a purple coloration with a brown edge in the wings of *A. grandis* (Pfister 1967), or a uniform brown color with a deep magenta edge in the wings of *A. concolor* (Oliver 1974), were seen to be closely associated with maturity and have been recommended also as possible indices.

Two other, more objective, interrelated physical parameters are moisture content and specific gravity, and both have been reported as reliable maturity indices for numerous conifer and broadleaved species. Changes in moisture content of scales and seeds and in specific gravity of the cone are strong manifestations that ripening is progressing (Rediske 1961). This loss of water during seed maturation is more an inherent phase of seed development than is implied by the passive concept of seed drying (Pollock and Roos 1972). A summary of moisture content, cone specific gravity and other maturity indices is shown in table 1. There is some general agreement that maturity has been reached when cone specific gravity is 0.90 or less and flotation techniques, using fluids of various densities, have been devised for field measurements. Cone moisture content has been less widely applied as a maturity index, probably because it is less easily determined, requiring some laboratory facilities such as a drying oven, and it takes longer to measure. Rietveld (1978) pointed out that the specific gravity/cone maturity relationship holds true only for cones in a population of trees, not for the population of cones as a single tree. Because cones on different trees may resist moisture loss or dry prematurely owing to differences in thickness of cuticle, exposure, or surface area, the relationship in individual trees may not follow exactly the same pattern as the mean of the population. A similar caution is necessary when using other ripeness criteria. Most authors have stressed that moisture content and specific gravity must be measured only on freshly picked cones.

Finnis (1950) was perhaps the first to propose that relative development of the embryo (i.e., ratio of embryo length to length of the cavity in the endosperm) might be a good indicator of maturity, at least in *Pseudotsuga menziesii* (Mirb.) Franco seeds. Embryos do not have to be completely elongated for germination to occur, but seeds with embryos less than 50 percent extended germinate less vigorously and predictably. A relative embryo length of 75 percent is now widely accepted in British Columbia as the point at which to begin cone collections in most species, including *Abies*. Embryo development can be determined readily by field personnel equipped with only a 10X magnifying glass, a sharp knife, and minimum training (Dobbs *et al.* 1976). It can also be recorded easily on x-ray film if facilities are available. Oliver (1974) found that when cones of *A. concolor* were ripe enough to collect—some three and a half weeks before seedfall—94 percent of the em-

Table 1. Summary of seedbearing age, seed crop, frequency, and maturity criteria in North American *Abies* species.

Species	*Minimum* seed-bearing age*	*Interval between* large seed crops*	*Cone maturity index*
	years	*years*	
A. amabilis	30	3-6	Embryo 75% + developed (Dobbs *et al.* 1976).
A. balsamea	15	2	Purpling of the cones (Stoeckeler and Jones 1957). Cone moisture 60% (Bakuzis and Hansen 1965).
A. bracteata	?	3-5	
A. concolor	40	2-5	Cone specific gravity 0.85 (Oliver, see Franklin 1974) to 0.96 (Oliver 1974).
A. fraseri	15	3	Blue green cones turn brown (Strawn, see Franklin 1974). Distinct seed coat color (Speers 1962).
A. grandis	20	2-3	Cone specific gravity 0.90; seeds detached from cone scales; seed wing color changed from purple to brown (green colored cones only) (Pfister 1967). Embyro 75% + developed (Dobbs *et al.* 1976).
A. lasiocarpa			
var. lasiocarpa	20	2-4	Embryo 75% + developed
var. arizonica	50	2-3	(Dobbs *et al.* 1976).
A. magnifica			
var. magnifica	35-45	2-3	Cone specific gravity 0.75 (Oliver 1974).
var. shastensis	30-40	2-3	
A. procera	12-15	3-6	Cone specific gravity 0.90; (Franklin 1965) crude fat content of seeds 25mg/g (Rediske and Nicholson 1965).

* Data from Franklin (1974). It is estimated that commercial-sized cone crops do not form for at least 5 to 10 years after seed-bearing begins.

bryos were fully elongated, while for *A. magnifica* var. *magnifica* A. Murr., 84 percent of the embryos were fully elongated two weeks before seedfall. Recent experience with true fir species in British Columbia indicates that embryos tend to be 90 to 100 percent extended by mid-August, well before the seeds are ripe, and that the condition of the endosperm must also be taken into account.[1] A watery, translucent tissue indicates immaturity despite the degree of embryo extension. When the seeds are sufficiently developed, the tissues show little or no shrinkage and curling and will retain a relatively firm, fresh appearance when longitudinally sliced seeds are left uncovered overnight at room temperature (figures 1 and 2).

Nearly all of these studies have sought a single cone or seed parameter that would indicate the attainment of maturity. Oliver (1974) found cone specific gravity in *A. magnifica* and *A. concolor* was almost as good an index as embryo development, but recommended that a method combining several characteristics would offer a more effective, yet simple and direct, index of maturation. He suggested that cones of these two species should be ready for picking when (i) the seed wings are uniformly brown with a deep magenta edge, (ii) seeds are free or only loosely attached to the cone scale, (iii) embryos are uniformly pale yellow-green, and (iv) the embryo entirely fills the cavity within the endosperm. Snyder (1976) made a similar recommendation for *A. grandis* and *A. procera*; *viz.*, (i) the seed wings are uniformly brown and are unattached to the cone scale, (ii) cones are light brown, (iii) embryos are at least 90 percent extended and embryos are firm, and (iv) cone specific gravity for *A. grandis* is 0.85 or lower, and 0.80 or lower for *A. procera*.

Some other attempts to relate maturity and physical parameters have been unsuccessful. Ching (1960) found no relationship between cone diameter, length or dry weight, cone moisture, or seed production of individual cones with maturity (equated with germination capacity) for *A. grandis*. Likewise, Edwards (1969) discerned no relationship between any physical (or biochemical) parameter and maturity in *A. procera*. There may be many reasons for such unsuccessful correlations, but the more important ones include differences among seed sources and stands, among and within trees, and among yearly variations. Year-to-year differences may be the most significant factor. While maturity may be related to one physical index or another in a good cone year, the same relationship may be lacking in a poor cone year, or in years when there is a good cone crop but no seed crop.

Biochemical Indices of Maturity

It was not until the early 1960s that significant efforts were made to understand the biochemical changes occurring within ripening tree seeds and to seek indices based on such changes. Although data had been published on biochemical maturation in seeds used as food items (Crocker and Barton 1953), little was known about this process in tree seeds until Rediske (1961) published his study on *Pseudotsuga menziesii*. Considerable data have been compiled over the past 15 years, focusing mainly on those chemical components that remain relatively stable for the earlier part of the ripening season, then undergo significant changes in concentration as maturity is attained. Rediske and Nicholson (1965) examined seven biochemical constituents in *A. procera* seeds. Crude fat was found to be the main storage form, and this constituent increased rapidly and sufficiently uniformly to be used as a maturity indicator. Consequently, it was concluded that at a crude fat concentration of 250 mg/g in the seeds, *A. procera* cones were ripe enough to collect, but that some artificial ripening was necessary to achieve maximum seed quality. Although one major forest company, at least, uses such biochemical indices for judging ripeness in its seed collections, such indicators remain relatively impractical for small cone collection operations. The analyses require laboratory facilities, are time consuming to perform, and repeated analyses during the maturation period may add significantly to seed collection costs. Bennett (1966) included *A. balsamea* and *A. concolor* in his study of the chemical composition of conifer seeds, but did not associate his findings with the developmental stage of the seeds.

Degree-Day Summations

Until some 10 years ago, all ripeness indices were related to changes occurring within the cones and/or seeds, and surprisingly little attention had been paid to any index based upon the environment under which the seeds developed. It was known for some time that "hot" summers promoted full embryo and endosperm development, while "cool" summers delayed ripening. Simak (1972), studying *Pinus sylvestris* L., noted that the higher the heat sum, the earlier the seeds ripened. Zasada (1973) proposed that a quantitative measure of the progress of the ripening season could be obtained from a computation of average daily temperatures and degree-day summations following pollination, such summations being potentially more reliable indicators of seed maturity than calendar date. For *Picea glauca* (Moench) Voss, Zasada (1973) found that a minimum of 625 degree days (above a threshold of 5°C) produced cones that could be ripened in storage after collection. Tanaka and Cameron (1978) observed some 1000 to 1100 degree days were required for ripeness in *Pinus ponderosa* Laws. cones at high elevations in the Pacific Northwest.

There are no reported degree-day summations for *Abies* seeds. Indeed, this criterion has been largely overlooked in North America, while receiving increasing consideration in Scandinavia. Refinement and correlation with other variables

1. Bowden-Green, R. (Brit. Col. Ministry of Forests.) Personal communication.

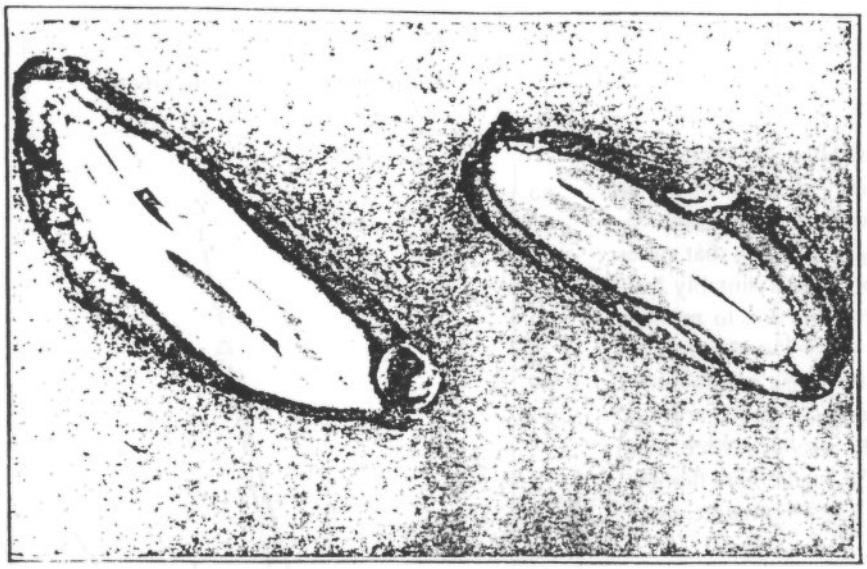

Figure 1. Mature *Abies amabilis* seeds. Embryos have fully extended, the "endosperm" is firm, white and shows no sign of shrinkage after sectioning. (Photo: B.C. Ministry of Forests.)

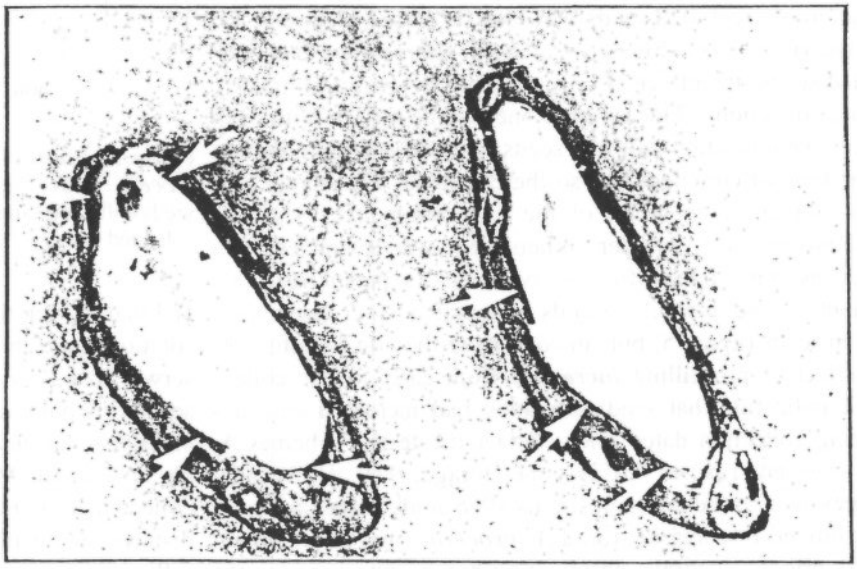

Figure 2. Endosperm condition is important in judging maturity in *Abies* seeds. Although embryos are fully elongated, the endosperm has pulled away (arrows) from the seedcoat after sectioning, indicating incomplete maturation. (Photo: B.C. Ministry of Forests.)

such as solar radiation and precipitation might lead to a singularly useful maturity index.

Artificial Cone Ripening

It was probably Haack (1905—cited by Huss 1951) who first proposed that the viability of poorly ripened seeds might be increased by storing the cones for a time before extraction. Silen (1958) added new life to the idea by suggesting that cones could be collected before they were ripe and, if suitably handled, might be ripened artificially after collection to produce high quality seeds. In the past 20 years, studies have been conducted on many species to determine the earliest possible time that seeds could be collected, since it was realized that artificial ripening of prematurely collected seeds provides three main benefits: cone collection operations can be made more flexible, the collection period can be extended, and immature cones from logging operations can be used.

Rediske and Nicholson (1965) found that if *A. procera* cones were collected when the crude fat concentration had risen to 0.25 g/g of seed, highest viability could be attained by "after-ripening" the cones for a period of at least six weeks. Cones could safely be picked on September 15, or possibly earlier, provided the required period of artificial ripening of seeds in the cones was allowed prior to seed extraction. Edwards (1969) observed that when cones of *A. procera* were stored up to six weeks before the seeds were extracted, germination of unprechilled seeds was increased, indicating that ripening had continued within the detached cones (figure 3); germination of prechilled seeds was also increased by prior cone storage (figure 4). However, in both unprechilled and prechilled seeds, the effect of cone storage *decreased* from early to late collections. This is what one would expect: with each successive collection date the seeds had become more mature at the time of cone harvest, so the effect of cone storage—in promoting the completion of the maturation processes—became progressively smaller. When seeds were extracted from the cones immediately after removal from the parent tree, i.e., without storage, prechilled seeds germinated better than unprechilled seeds (figure 5) but, in contrast to the effect of storage, the effect of prechilling *increased* from early to late collections, indicating that seed dormancy had increased with advancing collection date. These data indicate that whereas *A. procera* seeds ripened during cone storage, they also became progressively more dormant. In his 1965 study, Franklin found only limited ripening in stored *A. procera* cones collected six weeks prior to seedfall, germination being only three-fourths of that obtained from cones collected at natural seedfall. Unsuitable storage conditions were suspected, but large tree-to-tree variations in cone maturity and in responses to ripening treatments were also contributing factors.

For *A. grandis*, Pfister (1966) found that cones collected within four weeks before seedfall could be artificially ripened

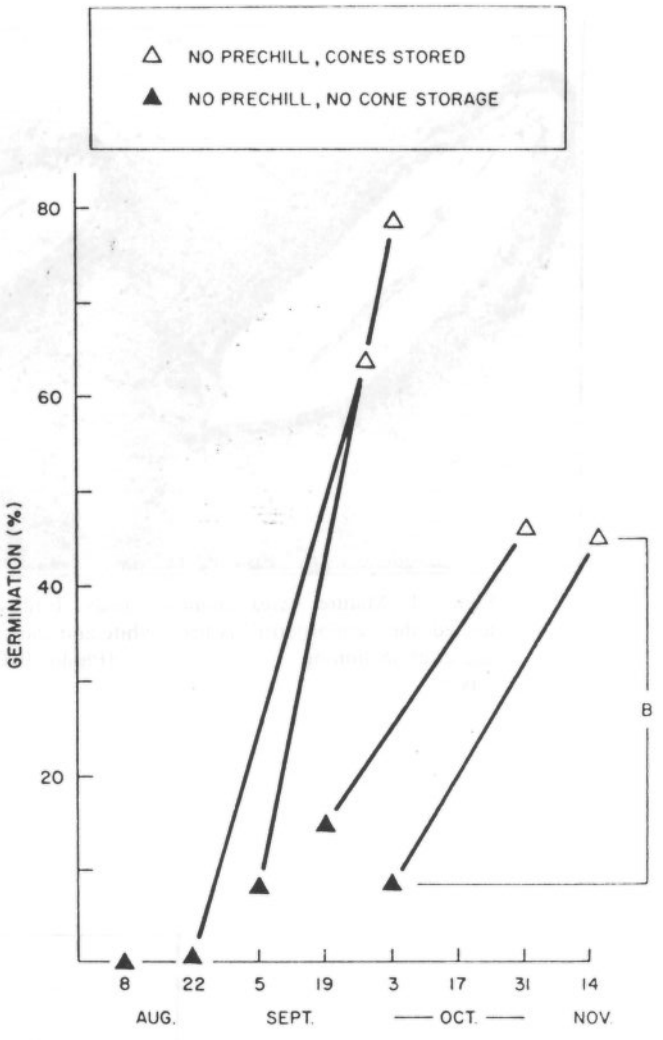

Figure 3. Effect of cone storage on germination of unprechilled *A. procera* seeds, by collection data. Lines connect stored/unstored seeds collected same data. Bracket B—effect of cone storage on seeds collected Oct. 3.

and that storing the cones with their bases in water or nutrient solution gave the best results. Likewise, Oliver (1974) observed that *A. concolor* cones collected four weeks before seedfall would also ripen in storage, but that cones of *A. magnifica* var. *magnifica* need to be collected as close as possible to seedfall. Muller (1971) recommended storage of early-collected *A. nordmanniana* (Steven) Spach. cones until they disintegrated naturally, by which time the seeds were said to be fully mature with enhanced germinability. At least one commercial seed processor in the Pacific Northwest uses this method for *Abies* collections. Cones are stored in sacks, in sheds equipped with forced air circulation, and the seeds are among the last to be scheduled for extraction. By December, full seed maturity has been achieved and the cones have completely disintegrated, making extraction simpler.

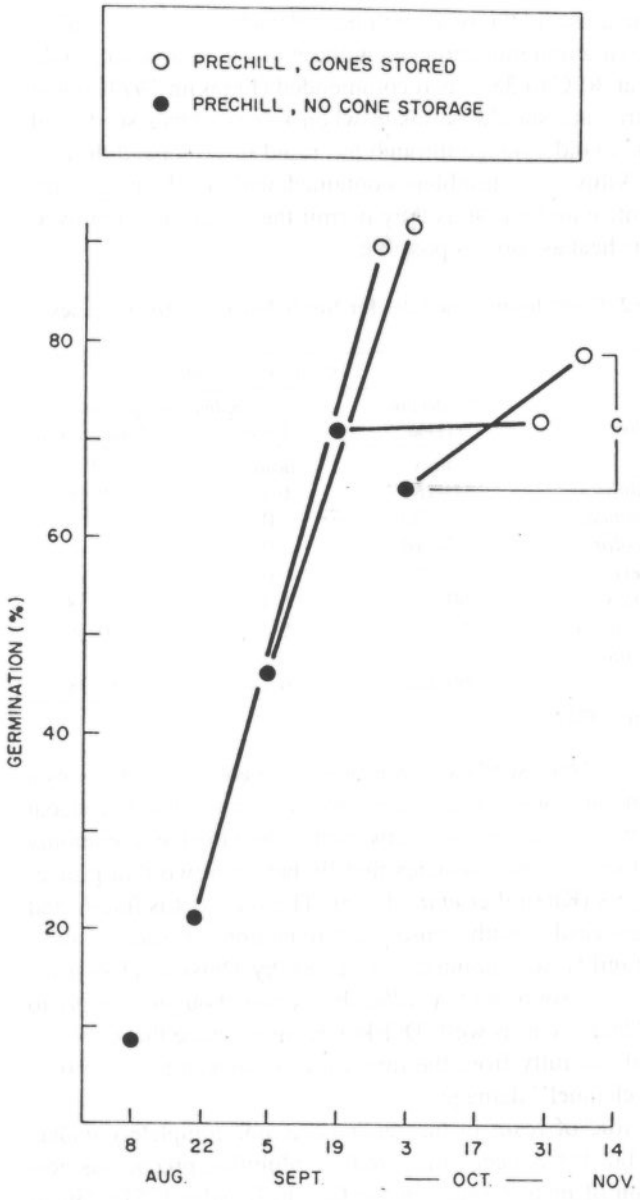

Figure 4. Effect of cone storage on germination of prechilled *A. procera* seeds, by collection date. Lines connect stored/unstored seeds collected same date. Bracket C—effect of storage on prechilled seeds from collection of Oct. 3.

Cone Collection

Cones traditionally have been collected by hand from standing or recently felled trees. Since the wood of most *Abies* tree stems is relatively brittle, considerable caution is required when climbing, as the tops may break off. Cones should not be thrown down to the ground, even in sacks, but should be lowered by rope. Even for collections made close to natural seedfall—when the cones are lighter, the seeds are riper and the seedcoats have hardened—some care is still required to avoid damage to the resin vesicles of the seedcoat (Dalskov 1960). Squirrel-cut or cached cones are much easier to collect and the seeds are more likely to be mature (Franklin 1974), although such cones may have been bruised and some of the seeds damaged on impact with the ground. Seeds collected by this means may be inferior in physical quality and the parent trees will not be known. Shooting out cone-laden tree tops with a rifle has been used from time to time with some degree of success, smaller crews collecting as many, if not more, cones than by climbing. Another technique that is still experimental is the use of an aerial cone rake, a device designed to be carried by helicopter and lowered over the crowns of cone-bearing trees. In the process of retrieval, cones are raked from branches by a series of tines and collected by a system of funnels and troughs (Dobbs *et al.* 1977).

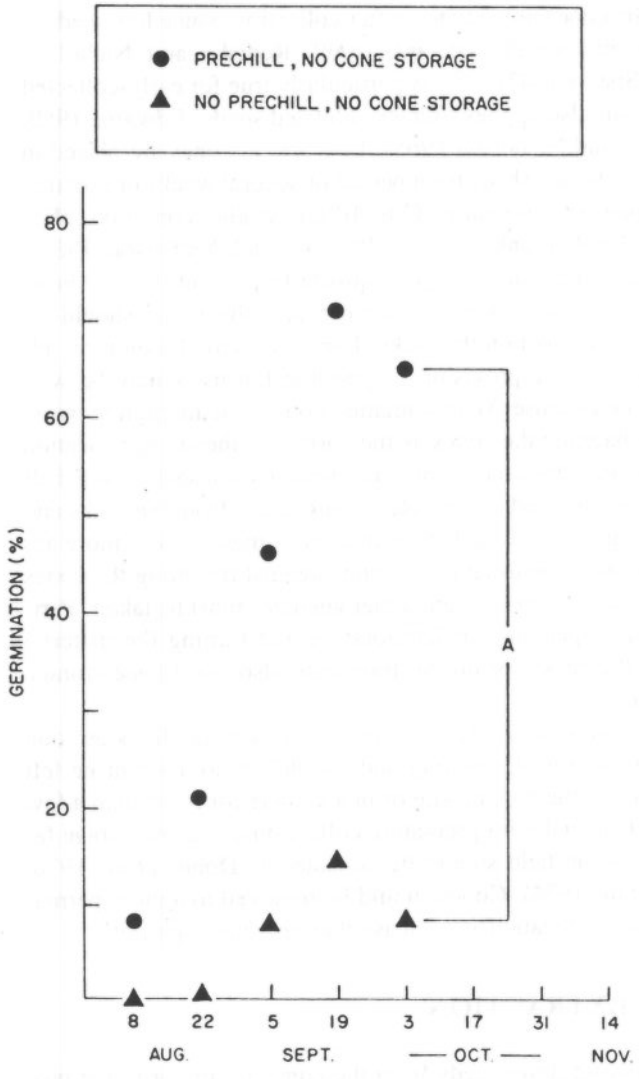

Figure 5. Effect of data of cone collection and seed prechilling on germination of *A. procera* seeds. No cone storage. Bracket A—effect of prechilling on seeds collected Oct. 3.

As already discussed, the period for cone collection is relatively short, usually no more than six weeks at the most. Calendar dates (Franklin 1974) vary with locality and weather patterns, but if cone storage facilities are available, collections usually can begin by middle to late August for several species, including *A. amabilis*, *A. balsamea*, *A. grandis*, *A. lasiocarpa* var. *lasiocarpa*, *A. magnifica* var. *magnifica* and, perhaps, *A. procera*. Knowledge of local ripening conditions (degree-day summations) and the use of the few known ripeness indices are an aid in deciding when to begin collecting.

Cone Storage

As has been described, seed ripening of *Abies* species occurs in two recognizable phases, the first being the accumulation of organic materials, and the second involving metabolic changes within the seeds. For this reason, seeds should not be removed from the cones immediately after collection, since low seed viability may result (Edwards 1969; Rediske and Nicholson 1965; Speers 1962). This is particularly true for early-collected seeds, but also applies to later-collected seeds (Edwards 1969; Rediske and Nicholson 1965). In practice, cones are placed in sacks in storage sheds for a period of several weeks or months, and ripen best in cool (5°C to 10°C), shaded conditions (Edwards 1969; Franklin 1965; Rediske and Nicholson 1965). Good air circulation is a prerequisite to preventing over-heating and the development of mold and the sacks should be spaced well apart on the racks. Forced air circulation is an advantage and the provision of portable fan units may be well worth the expense. With immature cones that are high in moisture, rebagging the cones as they arrive at the storage location and reducing the amount of cones held in each sack by one half will promote good curing. Removing cones from the sacks and spreading them on mesh-bottomed trays may be even more advantageous. The usual precautions against rewetting the cones and damage by rodents and other agencies must be taken. Periodically inspecting for deterioration and turning the material within the sacks (or on the trays) are also good cone storage practices.

Cones should be placed in proper storage facilities as soon as possible after harvesting and should on no account be left untended at the picking site or in a vehicle for more than a few hours. Especially for premature collections, interim storage facilities in the field should be considered (Dobbs *et al.* 1976; Stein *et al.* 1974). Cones should be removed to a more permanent storage location as soon as other operations permit.

SEED EXTRACTION

Extracting *Abies* seeds from the cones is similar to the procedures used for other conifers. When cones have been allowed to ripen completely in storage, little or no additional drying is necessary since the cone scales will have separated from the axis and the seeds will have detached from the scales. When extraction must proceed without this natural drying, kiln drying at 30°C to 38°C is recommended (Franklin 1974) (table 2). Extra care should be taken when kilning *Abies* seeds and cones to avoid damage through too rapid or prolonged drying. Rotary kilns, i.e., tumblers contained within a heating compartment, may be best as they permit the seeds to be removed from the heat as soon as possible.

Table 2. Cone drying schedules for North American *Abies* species

	Cone dry schedule		
	Air drying	*Kiln drying period*	
Species	*period*	*Time*	*Temperature*
	days	*hours*	*°C*
A. amabilis	60-180	6-14	30-38
A. balsamea	20- 30	0	
A. concolor	7- 14	0	
A. fraseri	30- 45	0	
A. grandis	60-180	6-16	30-38
A. lasiocarpa	60-180	6-16	30-38
A. magnifica	8- 21	0	
A. procera	60-180	6-14	30-38

(Franklin 1974).

Mature *Abies* seeds are oval or oblong and each possesses a large membraneous wing. The brown or tan-colored seedcoat is rather soft and contains between five and twelve (commonly seven to nine) resin vesicles that lie between two thin protective layers (Kitzmiller *et al.* 1975). The seedcoat is fragile and damages easily, with consequent reductions in seed quality. This fragility was pointed out earlier by Dalskov (1960) for Danish seed sources of *A. alba*, but it was thought to apply to other *Abies* seeds as well. Dalskov recommended that cones be handled carefully from the time they are picked to avoid seed "resin channel" damage.

The role of resin in the seedcoat is not completely understood, but it has been suggested as inhibiting precocious germination of mature seeds in the fall (Rohmeder 1951). Gunia and Simak (1970) thought that the resin might also provide some form of protection for the embryo and endosperm against excessive drying. Bouvarel and Lemoine (1958) believed the oxidation products of the resin were toxic to the embryo. Extraction of the resin and application of it to seeds of other species inhibited germination (Dassler and Zentsch 1959; Rohmeder 1951).

The inhibiting effect on germination caused by damaging the vesicles and thus permitting the resin to flow out was demonstrated with fresh seeds of *A. alba* by Gunia and Simak (1970), with stored seeds of *A. concolor* by Kitzmiller *et al.* (1973), and with fresh seeds of *A. concolor* and *A. magnifica* by Kitzmiller *et al.* (1975). In every instance of resin vesicle damage, germination capacity was reduced. In one seedlot of *A. concolor*, germination was reduced from 72 percent to eight

percent. The inhibitory effect of the resin leakage was greater when damage to the vesicles occurred before the seeds were prechilled. Lowering of germination was believed to be connected with increased damage by molds, since the leaking resin, once oxidized in the air, becomes a good medium for fungal development (Gunia and Simak 1970; Kitzmiller *et al.* 1973). Prechilled seeds were far less affected by molds than unprechilled seeds, even after damage.

Damaging the resin vesicles of prechilled seeds of *A. magnifica* had a greater detrimental effect on germination than when the same damage occurred to prechilled seeds of *A. concolor*, perhaps because *A. concolor* has a thinner seedcoat and more resin vesicles. Despite the vesicle damage, prechilled seeds of both species germinated better than undamaged seeds without prechill, leading to the speculation that the resin played some role in seedcoat dormancy (Kitzmiller *et al.* 1975). Zentsch (1960) found that removal of the resin from the seedcoats of *A. pectinata* DC., using a low temperature vacuum distillation technique, increased germination, adding support to the idea that the resin is a germination inhibitor in undamaged seeds. The reasons for the rapid lowering of germination when resin vesicles are damaged remains uncertain.

Whatever the exact role of the resin in the seedcoats of *Abies* seeds, the need to eliminate or at least minimize vesicle damage has been substantiated. Dewinging the seeds by gently hand rubbing small amounts in a linen bag has been recommended for *A. balsamea* seeds as a means of minimizing injury (Roe 1948). In the writer's experience, even this method causes the rupture of some resin vesicles in *A. procera* seeds. For larger seedlots, various types of mechanical dewingers are in use, some probably inflicting more damage than others, although no thorough investigations have been made in this area. Prolonged dewinging, or dewinging seeds that included a considerable amount of hard, sharp debris such as cone scales can cause considerable injury. Allen (1957a) observed that *A. lasiocarpa* seeds run through a brush dewinger three times lost 50 percent of their original viability. Kitzmiller *et al.* (1975) studied a number of seed-processing devices to see if they contributed to low seed quality, and recommended a very simple yet efficient two-step process comprising a scalper treatment followed by pneumatic separation. The scalper inflicted less damage to seeds of *A. concolor* and *A. magnifica* than hand dewinging, whereas the pneumatic separator inflicted some damage but eliminated most of the impurities remaining after the scalper treatment, and also removed most of the empty seeds. An improved air seed-sorter, that works well for *A. amabilis*, *A. grandis*, and *A. lasiocarpa*, has been described by Edwards (1979a).

Very little research has been conducted on other methods of separating filled and empty seeds of *Abies* species. Lebrun (1967) recommended using petroleum ether (specific gravity 0.657) in a flotation technique for *Abies* and other species. No loss of viability was observed; but, because of its flammability, petroleum ether is not particularly safe to use. Simak's (1973) technique using absolute alcohol has been tested on British Columbia sources of *A. amabilis*, *A. grandis*, and *A. lasiocarpa* (Edwards 1979b). Although separation was very rapid, some filled seeds remained floating on the surface, while some empty seeds sank. Germination was seriously reduced in all species following immersion in alcohol. In water, seeds that sank in between six to 24 hours were generally much superior in germinability to those that sank earlier or that remained floating after 24 hours (Edwards 1979b). Again, it was not possible to separate all the filled seeds by this method and this research is continuing.

Virtually nothing is known of the influences of other seed-processing devices, such as fanning mills and vibratory separators, on *Abies* seed quality, although the view is widely held that true fir seeds are very sensitive to damage at all stages of processing. This damage is probably a significant contributing factor to losses of viability during storage (Rediske 1967).

SEED TESTING

Laboratory tests provide a basis for seed evaluation, for the selection of lots to be stored, and for the determination of sowing rates in the nursery or field. Such tests play a vital role in facilitating commercial trading and reforestation uses of tree seeds (Stein 1967). Standard testing procedures have been developed for 10 *Abies* species by the Association of Official Seed Analysts (of North America) (Anon. 1978) and for 16 species by the International Seed Testing Association (Anon. 1976a). The Western Forest Tree Seed Council published those portions of the AOSA rules pertaining to tree seeds, including descriptions of seed handling practices as well as procedures for seed sampling for viability, moisture content, and germination testing (Anon. 1966). Other general descriptions of testing methods can be found in Bonner (1974), Copeland (1976), and Justice (1972).

Procedures for any given test are similar irrespective of their publication source. Germination prescriptions and those for other tests have been developed from research investigations and from "referee testing," i.e., comparative tests of the same seedlots using the same treatments at the same time at a number of laboratories. The treatments or combinations of treatments that consistently produced the highest germination results with the least variation have been adopted as the recommended procedures. The germination requirements of many *Abies* species have been reviewed earlier (Edwards 1962).

Types of Tests

All tests are conducted on small samples drawn at random from the seedlot. The correct sampling procedure and intensity

is required to ensure that the sample is representative of, and accurately describes, the characteristics of the seedlot. Sampling procedures are explained in detail in all seed testing manuals (Anon. 1966, 1967a, 1978).

Three or four basic types of laboratory tests are routinely conducted on the samples: the purity test, the 1000-seed weight test, the germination test, and a moisture content determination. In the purity test, the percentage (by weight) of pure seeds of the species in question is determined by separating, on a visual basis, other crop seeds and inert matter. "Pure seeds" include all whole, cracked, broken (but more than one half their original size), underdeveloped and seed-like objects of the species under consideration. Conifer seeds with their seedcoats completely removed are counted as inert matter. The purity test is simple and straightforward and there is little chance for misinterpreting or misapplying results if the rigid definitions used in classifying the components of the sample are understood (Stein 1967).

Usually eight replications of 100 seeds each, taken from the pure seed component, are used for the 1000-seed weight test. (As will be explained later [table 3], many germination tests on *Abies* seeds must compare a prechilled sample with an unprechilled sample. Since each sample comprises four replications of 100 seeds, it is convenient to count out the eight replications for the double germination test and use these same replications for the weight test.) The replications are individually weighed and, from their average weight, the weight of 1000 seeds is calculated. Together with the purity test percentage, this procedure provides an estimate of the number of seeds present in a unit weight of seedlot material. Once the germination percentage is known, these weight data are useful in calculating sowing rates for nursery beds or for direct sowings.

The purity and 1000-seed weight tests provide no information on the tree-producing capability of the seedlot. This is evaluated in a laboratory growth test, the conditions of which are selected to maximize germination. Since germination tests, by design, are conducted under optimum conditions, it is reasonable to anticipate some reduction in seed performance when used in this field. The value of germination tests lies in their predictive relationship to the performance of the seeds in the nursery or in other field sowings.

Large volumes of high-quality seeds are lost each year because of excess moisture during storage (Copeland 1976). Measurement of seed moisture content is vital in the preparation of seeds for prolonged storage, and is useful when seeds are removed from storage as a check on their retention of viability. The moisture content test is made on a separate sample from that used for other determinations and should be conducted as soon as possible after the sample has been taken, since respiration could change the moisture level of the sample if left for an extended period. In international seed commerce, the fresh-weight basis has been adopted as the standard expression for moisture content, although the dry-weight basis is preferred for research. Moisture levels based on dry weight are higher than those based on fresh weight.

As the germination test is the most important from a reforestation point of view, the major points of this test as it applies to *Abies* seeds will be covered here.

Table 3. Summary of official prescriptions for testing germination of *Abies* seeds. ISTA prescription given first, then AOSA prescription (if different) in parentheses.

Species	*Substrate*	*Temperature*	*Test duration*	*Additional directions*
		℃	*days*	
A. amabilis	TP*	20-30 (15-25)	28(21) (28)	Light: double tests** (Light: prechill† for 14 days)
A. balsamea	TP	20-30	28(21)	Light: prechill 21(28) days
A. concolor	TP	20-30	28	Light: double tests
A. fraseri	TP	20-30	28(21)	Light, prechill 21(28) days
A. grandis	TP	20-30	28	Light, double tests (Light: prechill 14 days) (No light: prechill 21 days)
A. lasiocarpa	TP	20-30	28	Light, prechill 21 days (Light)
A. magnifica	TP	20-30	28(21)	Light: prechill 21 days (No light: prechill 28 days)
A. procera	TP	20-30	28	Light: double tests (Light: prechill 14 days) (No light: prechill 21 days)

*TP—Top of Paper. IN AOSA rules this is indicated as TB (Top of Blotter)
**Double tests—no prechill and prechill 21 days
†Prechill—refrigerate at 3-5 C for the prescribed period

SEED GERMINATION TEST

Testing Equipment

Equipment used for testing germination varies widely, but the type is really not important so long as the required temperature, light, and moisture conditions are satisfied. In Europe, the traditional Jacobsen germinator is still in use. Its major drawback is the large amount of laboratory space required for the number of samples that can be tested at one time. Nevertheless, it is particularly useful for investigating light effects (intensity, duration, photoperiod) on germination, since all samples can be more uniformly exposed. A modified, individual Jacobsen-type germinator was described by Edwards (1974). Cabinet germinators are popular in North America, primarily because a large number of test samples can be accommodated at one time in a relatively small space. While it is claimed that temperature and humidity controls are superior in cabinet-type germinators, there is usually a considerable compromise with lighting, since illumination is from the sides and/or rear of the incubator. The use of clear plastic boxes with tight-fitting lids is preferred by some analysts since this permits a very good degree of humidity control. High humidities are essential not only in laboratory testing of *Abies* seeds but also in field germination (Colaone and Giannini 1971; Gabellini and Screm 1968; Gregori 1967). Comparisons of different types of equipment were made by Justice (1972).

Testing Method

The basic germination test is the same for all *Abies* species. Seed replications (from the pure seed component) are placed on a moist substrate and incubated at the prescribed temperature and light conditions for a given period of time. Periodically throughout the test, germinants are counted and classified according to rigid rules.

An alternating daily temperature regime is prescribed for most *Abies* species (table 2); that is, a temperature of 30°C is maintained for eight hours and 20°C for 16 hours. A sharp change-over (lasting one hour or less) is recommended when the seeds are likely to be dormant. All seed-testing manuals prescribe that light, evenly distributed and measuring 750–1250 lux at the seed surface, should be provided to coincide with the period of higher temperature; i.e., for eight hours. Cool-white fluorescent tubes are preferred to daylight, which is difficult to regulate and reproduce. On no account should tungsten (incandescent) bulbs be used, since these emit strongly in the far-red wavelengths (710–750 nm) which are inhibitory to germination. Cool-white fluorescents weakly emit far-red also, but they are proportionally richer in red wavelengths (660 nm) which promote germination. There is little or no evidence that light is required or favors the germination of true fir seeds, but some light is desirable to allow the analyst to evaluate more easily the essential structures of the germinants. The growth of some microorganisms may be retarded in light, although mycelia and spore bodies are not always visible to the naked eye.

Substrates for germination testing must be: a) non-toxic to the germinating seeds and seedlings, b) free of microorganisms, c) untreated by the manufacturer against microorganisms, and d) able to provide adequate aeration and moisture to the seeds. Natural materials such as sand, peat, and soil have been used for many years and still are employed for specific applications, but paper (filter paper, blotter, or towelling) and porous mineral materials (perlite, vermiculite) are now more frequently used, particularly as cabinet germinators have gained in popularity. Paper media have certain disadvantages, prominent among which is the tendency to favor the spread of fungi, but there are procedures for counteracting this (Bonner 1974).

At least 400 seeds are used in an official test, normally in four replications of 100 seeds each. These may be broken down to eight 50-seed replications (or even smaller) if the test substrate is overcrowded by large seeds. A test of less than 400 seeds frequently raises questions of representability of the seedlot, but it may be necessary to test a smaller number, particularly for high value seedlots such as these produced in seed orchards. More than 400 seeds per test may be used in research studies.

Test Duration and Counting Germination

Germination tests run for at least 21 days (Anon. 1978) and usually 28 days (Anon. 1976a) for all *Abies* species (table 3). Germinants are counted at least once per week, more frequently if possible. In research studies, counting is usually done at the same time of day every day. The seed analyst must distinguish normal seedlings from abnormal seedlings. Normal seedlings may be defined as those that "show the capacity for continued development into normal plants when grown in good quality soil and under favorable conditions of water supply, temperature and light" (Anon. 1976a). Official testing rules require that seeds of tree species demonstrating epigeal germination (the cotyledons appear above ground) are counted as having germinated only when "the primary root and hypocotyl together exceed four times the length of the seed, provided all structures which have developed appear normal" (Anon. 1976a). (This rule was under revision at the time of writing; seed analysts should consult testing manuals published since 1980.) Several analysts have found the "4x rule" difficult to apply when testing true fir seeds, since there is a tendency for considerable mold to develop on the sample. Spread of the mold is usually worse on paper substrates than in sand or soil and it may affect the health of the developing seedling. For this

reason, some laboratories count *Abies* germinants when the primary root plus hypocotyl reach a length equal to one or two times the length of the seed. Germination evaluations before the primary root has reached a length equal to the seed do not permit the analyst to detect abnormalities in the seedlings. This is especially important if the seeds have been treated with pesticides that may not damage the ability of the seeds to sprout but may ruin plant production in the field. Diagnostic features for abnormal germinants are described in seed testing manuals (Anon. 1976a, 1978).

At the conclusion of a germination test, ungerminated seeds should be examined. These may be: a) empty seeds lacking an embryo and endosperm; b) fresh, apparently viable seeds; and c) dead seeds which are neither empty nor fresh and have not produced seedlings. Viability of fresh, ungerminated seeds is usually assessed on the basis of visual appearance of the tissues when the seeds are sliced in half longitudinally. A better check is to immerse such seeds in a one-percent solution of tetrazolium chloride and incubate at 30–35°C for four hours in the dark. Living tissues will stain a deep carmine red, while necrotic tissues stain very weakly or not at all.

Expression of Results

Germination results are expressed as a percentage of the seeds in the sample. When empty seeds are included—a common occurrence in *Abies* seedlots—the number of germinants as a proportion of all seeds in the sample is known as the "apparent" germination percentage. Not only for research work but also when comparing one seed treatment against another, or one seedlot with another, the number of seeds that germinate should be expressed as a percentage of *filled* seeds in the sample. This is referred to as the "real" germination percentage.

Consider some hypothetical results for three different seedlots (table 4). Assuming that no fresh, ungerminated seeds remained at the end of the test, it would appear that there was no major difference among the three lots (top row) until empty seeds were accounted for, when it becomes clear (bottom row) that Lot 2 was the best and Lot 1 the worst. Similarly, empty seeds may create a false treatment effect, as shown by the example in table 5. It would appear that stratification promoted germination (top row), but when empty seeds were accounted for it became clear that stratification had no effect (bottom row).

"Apparent" germination percentage is used when calculating sowing rates and for evaluating the plant production capability of a given seedlot. Care must be exercised, as described above, when choosing among several seedlots or when evaluating the effects of different seed treatments such as prechilling or the application of fungicides or other chemicals.

Table 4. Hypothetical germination results for 3 seedlots.

Lot 1	*Lot 2*	*Lot 3*	
80	81	79	Observed germination
99	85	90	Filled seed percent
81	95	88	Germination of filled seeds

Table 5. Hypothetical germination results following different pretreatments.

Not stratified	*Stratified*	*Analysis*
70	76	Observed germination
81	88	Filled seed percent
86	86	Germination of filled seeds

The germination capacity of *Abies* seeds is typically low, averaging 20–50 percent: a reflection of the presence of empty or only partially filled seeds not removed during seed sorting. There may be some difficulty in determining what should be counted as filled seeds in cutting tests or x-ray analyses. The embryo or endosperm may appear shrunken to varying degrees, a condition often found in prematurely collected lots.

Dormancy and Seed Pretreatment

The low quality of *Abies* seedlots also may be due to seed dormancy. The concept and practical implications of dormancy have been thoroughly reviewed in a number of publications (Amen 1963; Evenari 1965; Villiers 1961; Wareing 1961, and others) and was reviewed in relation to *A. procera* by Edwards (1969). In its broadest sense, seed dormancy means that condition of viable seeds that makes them resistant to germination in environments favorable for quick germination. The development of, and principles and methods of overcoming, germination resistance have been described by Gordon (1972).

At least for *A. procera* seeds, and perhaps for other species also, the term "controlled" or "blocked" germination (as coined by Evenari 1965), rather than dormancy, should be applied. In seeds with controlled germination, the non-dormant embryo becomes a physiological prisoner of its envelopes (the seedcoat and endosperm) as it ripens. Because of their properties at seed maturation, the envelopes around the embryo hold control over germination. Edwards (1969) found that the embryos of *A. procera* seeds were not dormant but that they responded to prechilling, a treatment normally used to overcome dormancy. This response was shown to be related to the mechanical restraint to germination provided by the seedcoat, a condition that progressively increased as the seeds ripened.

Whatever the physiological reasons for germination resistance in *Abies* seeds, the condition varies among species and among seedlots. Of the 16 species covered by the ISTA rules (Anon. 1976a), a routine prechill (stratification) is prescribed for eight regarded as being consistently "dormant." For the other eight species, double tests are prescribed, with and without prechill, since dormancy varies from one seedlot to another. The AOSA rules differ from the ISTA rules for four species in that either prechill is not prescribed, or prechill is routinely recommended when the ISTA rules prescribe double tests. Both sets of prescriptions are summarized in table 3. A notable divergence between the two sets of rules in the prechilling prescription occurs with *A. lasiocarpa*. Whereas the ISTA rules recommend routine prechilling since seeds of this species are usually dormant, the AOSA do not prescribe prechill treatment at all. Although it might appear logical for North American seed analysts to follow the AOSA rules, some doubt must be cast in this instance. In the writer's experience, *A. lasiocarpa* seeds have always been deeply dormant and only prolonged prechilling has stimulated germination. When seedlots are tested for the first time, the performance of double tests will clearly establish whether or not the seedlot is dormant. Double tests should also be considered on seedlots that have been stored for more than two years to determine whether the dormant state has altered. Although these tests may appear superfluous as well as time- and seed-consuming, they will add immeasurably to the information bank for *Abies* species.

Prechilling is usually accomplished by soaking the seeds in water at room temperature for 24 hours, after which excess water is drained off, the seeds are placed in plastic bags (four-mil thickness or heavier) and refrigerated at 3–5°c for between 14 and 28 days. Freezing should be avoided. The ideal duration of prechilling is controversial, some workers arguing that if one month produces a certain amount of germination, two months or more will result in higher germination levels. This has been demonstrated experimentally for *A. concolor* by Stilinovic and Tucovic (1971), who observed that four months of treatment was better than two months, while three weeks prechilling produced no germination at all. However, the approach that "more is better" in this situation should be taken with caution, since fungal development may damage the seeds more than the prolonged treatment benefits them. Different prechilling temperatures and treatment durations were tested on two seedlots of *A. grandis* and one of *A. lasiocarpa* (figure 6). Treatment for 60 days generally resulted in better germination than 30 or 90 days. Prechilling at +2° or +5°C produced better results in most instances than either −2° or +7°C (Edwards 1978b).

Germination of *A. procera* seeds buried in snowbanks, reported by several workers (Franklin and Kureger 1968; Irmak 1961; Stein 1951), was mentioned earlier. Low temperature germination has also been studied in the laboratory, seeds being placed in a cold chamber as for prechilling and left for a period of more than five months. Germination began in the refrigerator after 70 to 90 days, depending on seedlot, and was complete after 160 days. Once started, the rate of germination was similar to that for unstratified seeds at normal temperatures (Edwards 1969). Allen (1960) similarly noted that germination of *A. grandis* and *A. procera* began at temperatures of 0–2°C, while Roe (1948) reported sprouting of *A. balsamea* after some eight months in cold storage. Stone (1957, 1958) also observed low temperature germination in pine seeds and suggested that prechilling might be considered as a process of slow germination. At least one commercial seedsman in the Pacific Northwest prepares *Abies* seeds for sowing by prechilling in the refrigerator until germination just begins. Various studies have reported that prechilled seeds germinate more readily over a wider range of germination temperatures than do those without prechilling (Allen 1960; Wang 1960). Where its beneficial effects are known, prechilling has been recommended and used in commercial practice because it avoids the necessity of fall sowing and prepares the seeds for germination under field conditions. In certain nurseries, taller seedlings of *A. grandis* and *A. procera* were obtained from prechilled seeds, the increase in height being proportional to the length of prechill in some cases (Faulkner and Aldhous 1959).

One aspect of seed germination studies that has been almost totally neglected is the effect of prechill moisture level on subsequent germination. Danielson and Tanaka (1978) reported that prechilled seeds of *Pinus ponderosa* were air-dried and returned to cold storage (2°C) for nine months without losing the benefit of the previous prechill and without their viability being adversely affected. A poorer response with *Pseudotsuga menziesii* seeds was attributed to a too-high seed moisture content during storage. The practical significance of this finding is considerable. In 1977, work was initiated to study the possibilities of redrying and safely storing prechilled seeds of *A. amabilis, A grandis*, and *A. lasiocarpa* of British Columbia provenances (Edwards 1980a). The seeds of four seedlots were routinely prechilled (soaked for 48 hours, drained of excess water, then refrigerated at 2–4°C) for four weeks. Samples from each were then dried to 35 percent, 25 percent, and 15 percent moisture, based on fresh weight of the seeds. Control samples were not dried and contained the natural moisture level of between 45 and 55 percent after prechilling. Standard germination tests were conducted immediately after drying, and again after one, two, three, and four weeks, three, six, nine, and twelve months of storage in the same refrigerator used for prechilling (i.e., at 2–5°C).

The study revealed that prechilled seeds air-dried to a moisture content of 25 percent could be stored for 12 months without any significant reduction in germination (figure 7). Tests on *A. amabilis* were limited to three months' storage (figure 8). *A. grandis* and *A. lasiocarpa* seeds air-dried to 35 percent were

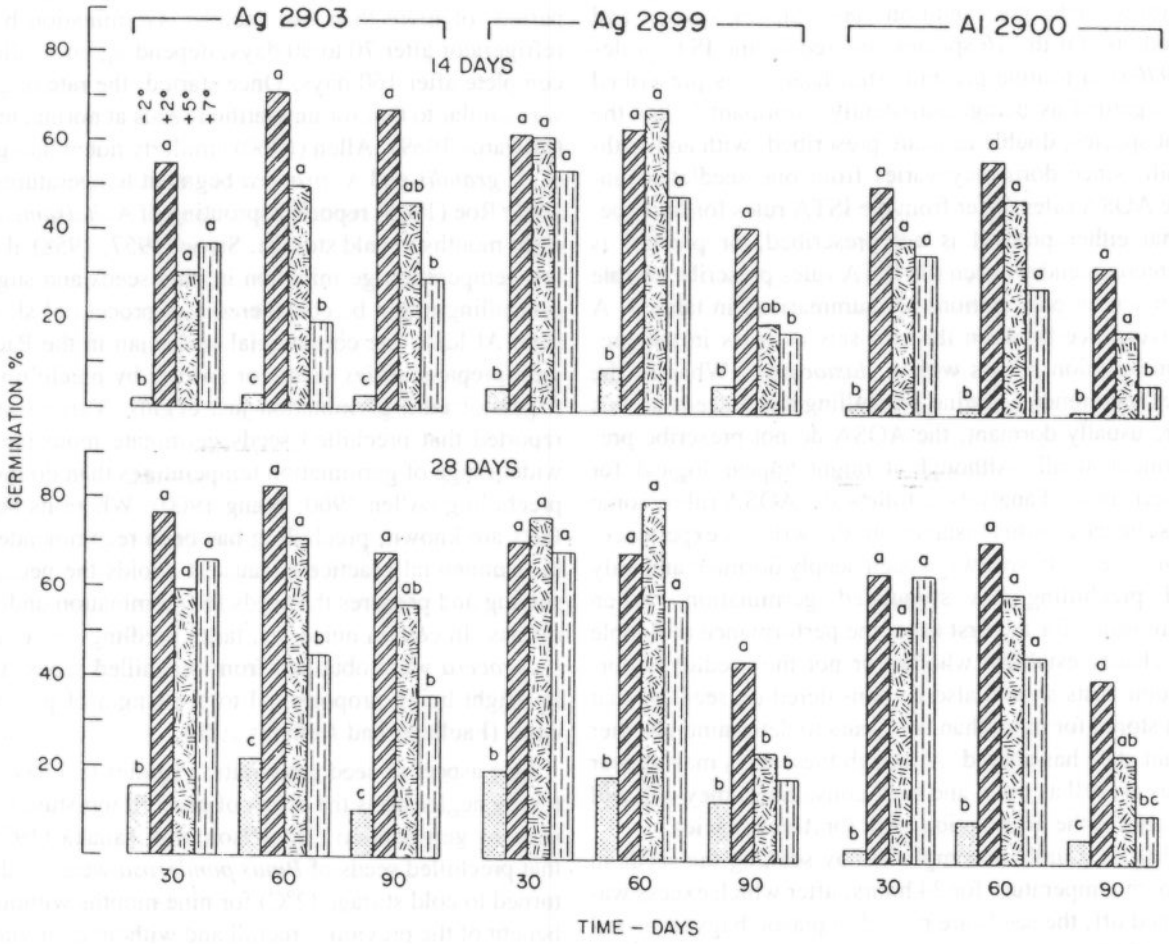

Figure 6. Effect of four temperatures (−2, +2, +5, +7°C) and three durations (30, 60, 90 days) of prechilling in *A. grandis* (Ag 2903 Ag 2899 and *A. lasiocarpa* (Al 2900 seeds. Within each treatment duration, means followed by the same letter are not significantly different (P = 0.05)(B.C. Ministry of Forests Registered Seedlot).

successfully stored for six months, but emergence during storage occurred when they were held for nine and twelve months. At 25 percent and 35 percent, the beneficial effect of prechilling was retained but was lost in seeds dried to 15 percent, presumably because at this moisture level dormancy had been reimposed. In seeds not dried, storage amounted to prolonging the prechill, producing erratic germination behavior and losses in seed viability (Edwards 1980a). Subsequent trials have shown that *A. amabilis* and *A. procera* seeds also can be stored for periods similar to those for *A. grandis* and *A. lasiocarpa* seeds.

Not only were the seeds successfully stored, but significant increases in germination over the controls were obtained, especially in seeds dried to 35 percent. At this moisture level, the effect was clearly more on germination rate, as all germination occurred within the first 14 days of the test for seeds stored longer than one month. Maximum effect occurred in seeds stored for three months at 35 percent, the response in *A. lasiocarpa* seeds (figure 7) being most marked. Other trials on approximately 20 additional seedlots have confirmed that air-drying prechilled *Abies* seeds to 35 percent moisture, followed by three months' storage, produces better germination than prechilling alone (Edwards and Leadem, unpublished data) and the procedure has been suggested as an alternative technique for preparing these species for nursery sowing (Edwards 1980b). This research continues.

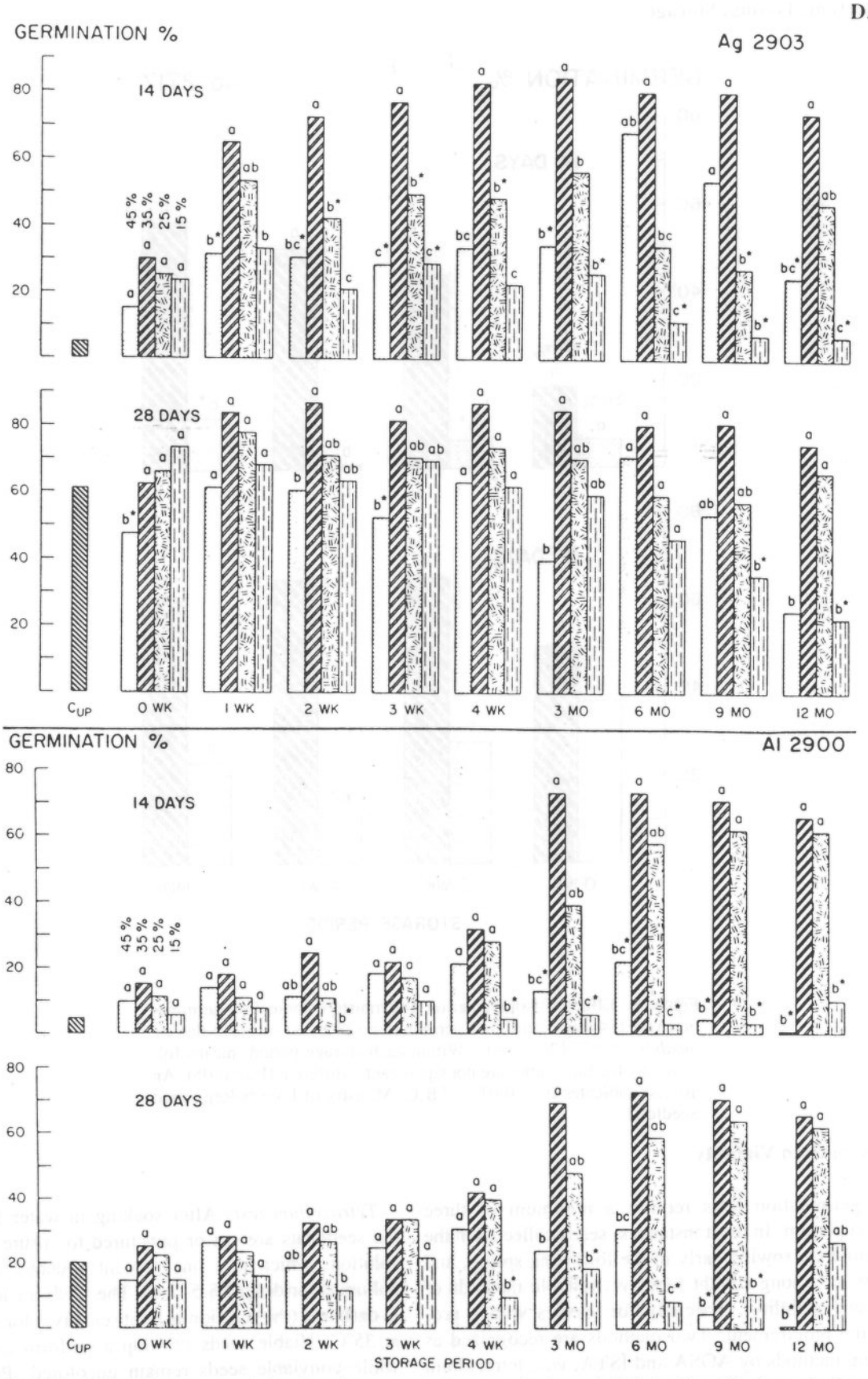

Figure 7. Effect of storage period and moisture content on germination rate (14 days) and final germination (28 days) of prechilled *A. grandis* (Ag 2903(c) and *A. lasiocarpa* (Al 2900(c) seeds. C_{up}—unprechilled sample. Within each storage period, means followed by the same letter are not significantly different (P = 0.05). An asterisk indicates P = 0.01. ((c)B.C. Ministry of Forests Registered Seedlot.)

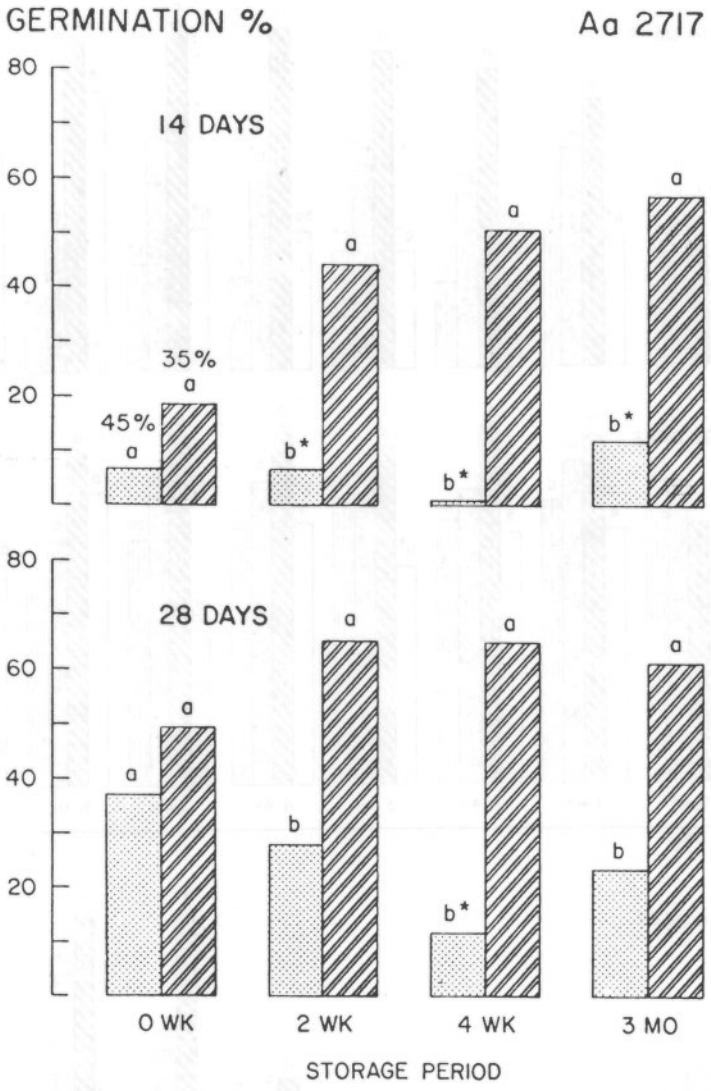

Figure 8. Effect of storage period and moisture content on germination rate (14 days) and final germination (28 days) of prechilled *A. amabilis* (Aa 2717(c) seeds. Within each storage period, means followed by the same letter are not significantly different (P 26 0.05). An asterisk indicates P = 0.01. (B.C. Ministry of Forests Registered Seedlot.)

Quick Tests for Seed Viability

Standard germination tests require a minimum of three weeks for completion. In most instances, seeds collected in the fall are required for sowing early in the following spring, and seed analysts have long sought rapid yet reliable methods of measuring seed viability, especially for species with a prolonged chilling requirement. Two methods are recognized as official testing methods by AOSA and ISTA; *viz.*, tetrazolium staining and embryo excision. Other tests—*e.g.*, x-ray, hydrogen peroxide and cutting tests—are also in use.

Tetrazolium test. After soaking in water for 24–48 hours, the seedcoats are cut or punctured to ensure entry of the test solution, which is a one percent aqueous solution of tetrazolium chloride (pH 6.5–7.0). The seeds are left in the solution in darkness (the reaction is light sensitive) for six to eight hours at 35°C. Viable seeds develop a uniform carmine-red stain, while nonviable seeds remain uncolored. Procedural details may be found in the official seed testing rules (Anon. 1976a, 1978).

Excised embryo test. This test is primarily on hardwood seeds and some very dormant conifer species; there are no prescriptions for *Abies* species. In general, after the seeds have been soaked in water to soften them, the seedcoats (and endosperm if present) are cut and the embryo removed for culturing (usually on moist filter paper). Excision of embryos without damage is difficult and the test, like tetrazolium staining, requires skilled personnel for correct interpretation of the results.

X-ray tests. Interest is developing rapidly in the use of soft x-rays for estimating seed viability. The method was first proposed by Simak and Gustafsson (1953) and has been studied for more than 25 years, but so far no testing prescriptions have been formulated. X-rays are frequently used for checking filled, empty, and abnormal seeds (figure 9). X-ray contrast techniques, in which the seeds are exposed to the vapors of organic solvents or soaked in solutions of heavy metals such as barium chloride, have shown promise for expanding testing capabilities. The contrast agents penetrate dead or damaged tissue but not intact cells, and the impregnated tissues appear more clearly on the radiograph. Although *Abies* seeds impregnate well with the vapors of organic solvents (chloroform, trichloroethylene), all agents tested so far have reduced germinability, making it difficult to relate the degree of penetration to natural ability to germinate (Edwards 1979b); this work continues.

Hydrogen peroxide test. Seeds are soaked in one percent hydrogen peroxide for 24 hours; then the micropylar end of the seed is cut away so as just to expose the white tissues within. The seeds are returned to fresh peroxide solution and incubated in the dark for four to five days at 20–30°C. Seed quality can be estimated from the percentage of seeds showing "evident" elongation (5 mm or more) of the embryo, "slight" elongation (1–2 mm) or no growth. Seeds showing "slight" growth are returned to fresh solution for another day or two and reassessed.[2]

Several researchers (Barnett 1976; Carter and Jones 1962; Ching and Parker 1958; Riffle and Springfield 1968; Trappe 1961) have attempted to stimulate conifer seed germination or reduce seedcoat microflora, or both, by using hydrogen peroxide. A recent study on British Columbia sources of *A. amabilis* and *A. grandis* seeds found no benefit from hydrogen peroxide treatments in increasing either rate or capacity of germination. Certain treatments effectively reduced the numbers of seedborne fungi, but there were no corresponding increases in germination (Edwards and Sutherland 1979).

Cutting test. Although the cutting test is the simplest to perform, it is also the least reliable. Seeds are halved longitudinally, using a sharp blade; those with firm, healthy-looking tissues that are fully developed with the proper color are judged

2. H. R. Danielson, Personal communication.

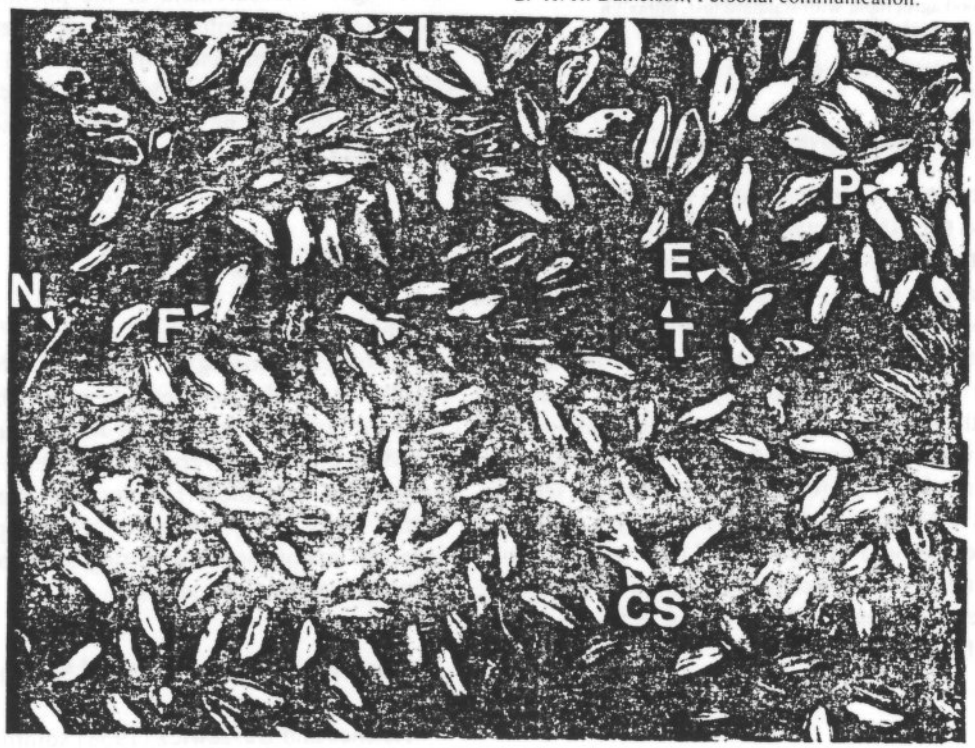

Figure 9. An x-ray radiograph of a typical mixture of filled seeds (F), empty seeds (E), seeds containing an insect larvae (I), and some inert matter such as a needle (N), twig fragment (T), pitch (P) and cone scales (CS). *Abies amabilis* (approx. life size).

viable. The test usually fails to detect seeds damaged during handling and processing or that have died during storage.

Test comparisons. Tetrazolium, hydrogen peroxide, and x-ray tests were compared by Leadem (1979) for *A. amabilis* seeds. The x-ray contrast method, using chloroform as the contrast agent, gave better agreement (with the standard germination test) than hydrogen peroxide for low quality (below 30 percent germination) lots; while hydrogen peroxide was the better of the two quick tests for lots with 30–50 percent germination. Overall, the x-ray contrast method tended to overestimate slightly and the hydrogen peroxide test to underestimate germination capacity. From this, it was suggested that a combination of the two methods might have a "best" estimate. The tetrazolium chloride test gave good agreement with the standard test in three of five seedlots examined but did not perform overall as well as the other two methods. A summary of these comparisons for several conifer species is shown in table 6.

Table 6. Comparisons of three quick tests for forest tree seeds

	A. X-ray Contrast Test
(i)	Quick to perform
(ii)	Requires little training
(iii)	Good agreement with standard test
	B. Tetrazolium Chloride Test
(i)	Tedious, time-consuming
(ii)	Requires experienced technician
(iii)	Fairly good agreement with standard test, although tends to overestimate
	C. Hydrogen Peroxide Test
(i)	Quick to perform
(ii)	Technically simple
(iii)	Tends to underestimate germination capacity

Whereas quick tests enable the analyst to obtain some data on a seedlot in less time than a standard germination test, many analysts find that they are unable to perform as many quick tests in, for instance, a month, as they could standard tests; in other words, most quick tests require more analyst-hours to perform. In addition, they require a high degree of skill and experience to perform them consistently and well. It must be emphasized that these tests are only estimates of viability; and the relationship with actual germination, particularly in the field, frequently is only an approximate one. Also, the technology of quick tests is particularly unreliable for *Abies* seeds. Reforestation planners should realize that if data based only on quick tests are available, seed use in the spring following cone collection may be inefficient, with too many or too few seeds being withdrawn from storage.

Seed Vigor

Even after carefully controlled germination tests, some aspects of seedlot quality may remain unknown. Rapidity of germination, germination under unfavorable conditions (high or low temperatures, drought, or water-logging), resistance to disease, and other factors are all related to seed vigor. In the germination test, normal and abnormal germinants are recorded separately from dead and empty seeds. Only the normal germinant component is used to assess seed vigor.

Vigor is all those qualities that make for fast emergence under a wide range of field conditions (Grabe 1979). Several performance characteristics are affected by seed vigor, including storability (seeds entering storage with low vigor are more likely to deteriorate), germination under stress conditions, speed of crop establishment, the number of plants established, their uniformity, and seedling growth rate. A low vigor seedlot could perform well in the nursery if conditions are exactly favorable; if not, low vigor will be manifested in non-uniform emergence, skips in the seedling rows, and variation in seedling size. Seed vigor includes genetic and environmental components. Seed maturity and development, seed size, chemical composition, and degree of deterioration resulting from damage in the field before processing; mechanical damage during processing; aging in storage: all are factors to be considered in the environmental component.

A number of vigor tests have been devised for agricultural and vegetable seeds (Anon. 1976b), but none have been adapted or are widely used for conifer seeds. Two or more of these tests, such as the "cold test," "the accelerated aging test," and/or a "stress test" (Anon. 1976b) are needed to evaluate vigor. Measurement of the phenomenon is becoming a growing concern with some nurserymen, but there has been little research conducted on this subject for tree seeds to date. Detailed reviews of seed vigor for agricultural and other non-forest species can be found in Heydecker (1972) and Pollock and Roos (1972).

SEED STORAGE

The storage of tree seeds has been intensively researched and storage technology is well established. Improvements are still being sought, but methods are available for conserving the seeds of most *Abies* species for at least the period between good seed crops. Several detailed reviews of storage methods have been published (Holmes and Buszewicz 1958; Magini 1962; Wang 1974) which describe the principles of seed storage, the major factors affecting seed storage, and the storage requirements of tree species.

Storage Temperature and Seed Moisture Content

Holmes and Buszewicz (1958) summarized published information on seed longevity under various storage conditions for seven *Abies* species. Most authorities agree that unless special precautions are taken, true fir seeds quickly lose their viability. This is believed to be related to their high oil and resin contents which, when oxidized, are toxic to the embryo (Bouvarel and

Table 7. Summary of European recommendations for storage of *Abies* seeds.

Species	*Moisture Content*	*Storage Temperature*	*Possible Storage Period*	*Source of Data*
	percent	*°C*	*years*	
A. alba	5-7	−3 to −7	2+	Giannini and Murazio (1972)
	10-12	−4 to −8	2	Gradi (1963)
	9	−6	1−2†	Gradi (1966)
	7-8	−6	2+†	Gradi (1966)
	9-10	<0	2+†	Machanicek (1965)
	6	−3 to −7	1−2	Magini and Cappelli (1964a,b)
	<9	−15	4−5	Muller (1977)
	9-10*	−3 to −17	2+	Rohmeder (1953)
	8-9	−4 to −15	3+	Schonborn (1964)
	7-12**	<0	2+	Tocci (1967)
	78-80 R.H.‡	0 to −7	0.5	Tokarz (1974)
A. cephalonica	9−11	+4	1−2	Bouvarel and Lemoine (1958)
A. grandis	9−11	+4	1−2	Bouvarel and Lemoine (1958)
		−10 to −15	2+	Hofman and Vackova (1966)
	9−10	<0	2+	Machanicek (1965)
	<9	−15	4−5+	Muller (1977)
A. lasiocarpa		<0		Huss (1967)
A. nordmanniana	9−11	+4	1−2	Bouvarel and Lemoine (1958)
	<9	−15	4−5+	Muller (1977)

* Dry weight basis
** Fresh weight basis
† Sealed containers
‡ Relative humidity

Lemoine 1958). Summaries of recommended storage conditions for *Abies* seeds based on European and North American Research are listed in tables 7 and 8. Throughout most of these studies, the superiority of sub-freezing temperatures has been amply demonstrated. Recent research indicates that seed moisture content is probably the most important single factor in maintaining germinability. The general relationship between storage temperature and seed moisture level was enunciated by Barton (1961). At any given moisture level, seeds deteriorate faster as storage temperature rises (within limits), and the lower the storage temperature, the greater the tolerance to high moisture content. Magini and Cappelli (1964a, b) emphasized the same points: the importance of storage temperature varies according to the moisture content of the seeds, being of greater significance when moisture is high. Conversely, if moisture content is low, temperature has less effect on the outcome of seed conservation; at low enough moisture content, storage becomes almost independent of temperature.

This inverse relation was clearly demonstrated in a two-year storage trial of *A. procera* seeds by Danielson and Grabe (1973). When seed moisture level was above 12 percent, viability declined rapidly, irrespective of storage temperature (20°, 5°, −18°C). At 12 percent, viability was maintained for two years when the seeds were stored at −18°C, but not at 5° or 20°C. At 6 percent to 9 percent moisture, viability was maintained at −18° and 5°C, while in seeds dried to 4 percent moisture, viability was maintained at all three storage temperatures.

Critical Moisture Range

Whereas low moisture contents are desirable, there is evidence that overdrying can cause as much deterioration during storage as excess moisture (Harrington 1972). In extremely dry seeds—less than 5 percent—a portion of the monomolecular-bound water has been removed from the macromolecules, thus

Table 8. Summary of North American recommendations for storage of *Abies* seeds.

Species	*Moisture Content*	*Storage Temperature*	*Possible Storage Period*	*Source of Data*
	percent	*°C*	*years*	
A. balsamea	5–8	+0.5 to +4	5	Jones (1962)
A. concolor	6–9	0 and −18*	7	Allen (1957b)
	5–8	−6.7	1–2+	Jones (1962)
	5–8	−6.7	1–2+	Jones (1962)
	6–10	−18	3	Schubert (1952, 1954)
		+4		
A. grandis	11	+5	5**	Barton (1953)
	11	−4	10+**	Barton (1953)
	5–8	−6.7	1–2+	Jones (1962)
A. magnifica var. *shastensis*	11	+5	5**	Barton (1953)
	11	−4	10+**	Barton (1953)
A. procera	6–9	0 and −18*	7	Allen (1957b); Allen and Bientjes (1953)
	11			
	11	+5	5**	Barton (1953)
	6–9	−4	10+**	Barton (1953)
		+5 to −18	2+	Danielson and Grabe (1973)
	5–8			
		−10	5	Isaac (1934)
		−6.7	1–2+	Jones (1962)

* The lower temperature produced slightly better results
** Sealed containers

eliminating a layer that protects against oxidation. Seeds stored in this condition may age rapidly because of autoxidation (Harrington 1972). There is, therefore, a critical moisture level or range above or below which deterioration in germinability is rapid. For the true firs in general, the critical (safe) level appears to be between five percent and eight percent of seed fresh weight (Wang 1974), although Danielson and Grabe (1973) dried *A. procera* seeds to four percent (fresh-weight basis) and found the seeds stored very well for two years, even at +20°C.

The Main Principles

A summary of the main principles prepared by Gradi (1966), for commercial storage of seeds, adopted in the light of European experiences with *A. alba*, is relevant to fir seed storage technology today. Cone collections should be well-timed to provide ripe or artificially ripenable seeds, and the cones should be placed in storage as soon as possible after harvest. Care must be exercised to ensure continued ripening in cones collected prematurely and seed extraction should not begin until the cones have reached 14–16 percent moisture. Once seeds are extracted, moisture levels should be reduced to nine percent for storage of one to two years, or seven to eight percent for more than two years, by gradual drying at 15°C (the seeds must not be heated). Seeds are then stored in sealed containers (glass jars, polyethylene bags, or sacks) and refrigerated at −6°C.

Preconditioning

Careful preconditioning of the seeds cannot be over-emphasized. Air drying without elevating the temperature will suffice to reduce the moisture levels of most *Abies* seedlots to critical levels. If air is moved over the seeds, the relative humidity need not be very low to achieve the desired results. Magini and Cappelli (1964a, b) emphasized that the drying should be *gradual* and at no higher than 20°C. They observed that heating seeds with dry air at 50°C for three hours completely extinguished germinability, while heating to 50°C in water caused only a slight reduction in germinability.

Storage Containers

Magini and Cappelli (1964a, b) stressed that sealed containers provide better maintenance of seed moisture content over long periods, protect from losses caused by insects or pathogenic organisms, and minimize the effects of malfunctions

of the refrigerating equipment. Sealed containers also avoid the need for humidity control in the storage room, thereby eliminating the need for costly equipment.

Various types of sealed containers can be used. Glass bottles, although breakable, are better than tin cans that corrode. Screw-top plastic bottles, heavy-weight polyethylene bags, and fiberboard drums are in extensive use, and all are light and unbreakable. A combination, such as a plastic bag in a fiberboard drum lined with aluminum foil, forms an ideal container, particularly if it is filled completely to leave a minimum air space (Jones 1962). Limiting the containers to 10–20 kg avoids the waste of space of storing larger, partly filled containers and also avoids their repeated opening and resealing after each withdrawal of seeds (Holmes and Buszewicz 1958); additionally, small containers minimize the danger of the lowermost seeds being crushed. Plastic and polyethylene containers are not completely impermeable to moisture and may not be adequate in conditions of high external humidity for long-term storage of seeds requiring low moisture content.

Removal from Storage

To prevent fluctuations in seed moisture content, seals of containers should not be broken until the time of use. It is particularly important that sealed containers removed from cold storage be permitted to reach room temperature before being opened to avoid condensation of water within the container, as this would cause an increase in seed moisture level (Wang 1974). Alternatively, seed withdrawal might be accomplished within the cold storage room.

Beyond Seed Longevity

A recent report by Muller (1977) has emphasized a dimension frequently neglected in seed storage research; *viz.*, the influence on seed vigor and the proportion of usable seedlings produced by the nursery. In comparing storage temperatures for *A. nordmanniana* seeds (at five to nine percent moisture), she found −15°C superior to +4°C in: a) the higher percentage of plants surviving one and two growing seasons in the nursery, b) the lower percentage of diseased and dead seeds, and c) earlier bud set in the fall and earlier flush in the following spring. These differences tended to be emphasized in seeds stored for more than five years. Her general conclusion was that *A. nordmanniana* seeds will show the least signs of aging and yield more plants of high vigor if they are stored in sealed containers at −15°C and at a moisture level below nine percent. Giannini and Murazio (1972) also pointed out that long-term storage causes a greater reduction in plant percentage than in germination percentage.

SUMMARY AND CONCLUSIONS

It should be relatively clear from the foregoing that knowledge of procurement and handling of true fir seeds is far from complete. Certain conclusions, tentative though they may be, can and should be drawn at this time, if only that they might spur further investigation and development of improved procedures. The following points, therefore, are meant to be accepted with caution.

Cone Collecting

Cones of most *Abies* species appear to be collectable several weeks in advance of natural seedfall. The exact period is uncertain, but four weeks—perhaps six or more—may be available in most crop years. Cones collected this early contain immature seeds and require very careful and gentle handling to realize high seed quality. Cones should be stored intact under cool, well-ventilated conditions. The longer the storage period, the better the chances that ripening will go its full course, provided the cones do not mold or otherwise deteriorate. The earliest time to begin such premature collections is still very difficult to define but would probably be indicated best by the separation of a majority of the seeds from the cone scales, which signals the approach of the end of the organic accumulation phase. Development of the embryo must be considered. A minimum extension of 75 percent is suggested, and the endosperm must be firm and show no signs of shrinkage from the seedcoat. Coloration of the seed wing should be well advanced, and cone specific gravities should be 0.90 or lower. None of these parameters on its own should be taken as an indicator and at least two, probably three, should be satisfied before collections begin. The development of degree-day summations or other environmental records will be significant in refining these indices. Unless there is a particularly heavy cone crop, which may require an early start, it is safer to delay collections as long as possible. In this manner, the problems associated with cone handling after harvest are minimized.

Seed Extraction

Processors should not be in any haste to remove seeds from the cones, provided the cones are well cared for. Extraction should be delayed for four to eight weeks (or more) after harvest. Under no circumstances should extraction begin if the endosperms have not become firm. Even cones collected close to time of natural seedfall should be stored for several weeks to promote the fullest "after-ripening" possible. Cone kilning may be avoided by this procedure. Extraction procedures should be such that seeds are handled as little as possible. Dewinging appears to be a crucial step from a seed injury standpoint and must be done with great care. Large lots requir-

ing mechanical dewinging are the most likely to suffer seedcoat damage. A slotted drum dewinger is suggested as a possibly safer method than most others. Separation of filled and empty seeds can be accomplished with various devices, but again, gentleness in handling is of paramount importance in maintaining high seed quality.

Seed Testing

The basic laboratory germination test procedure requires an alternating temperature regime with light provided by cool-white fluorescents coinciding with the higher temperature. Seeds can be tested on a variety of substrates, paper being preferred. The same material should be used throughout to ensure comparability of results. Germinants should be evaluated at least once per week over a three- to four-week period, using diagnostic features prescribed in standard rules. Germination test results should be based on the proportion of filled seeds in the sample. X-ray procedures facilitate the determination of filled and empty seeds and, since the exposures are not known to be harmful, the same seeds can be germinated. Alternatively, a cutting test must be used on separate samples.

Many *Abies* seeds require or respond to prechill (stratification). Unless experience has shown otherwise, or the seedlot has been previously tested, double tests are recommended. Dormancy varies from one species to another, and among seedlots. It may also vary from one seed collection to another from the same source. Seeds should be checked at least once during prechill for mold growth. Some white mycelia may become apparent, but this is believed to be harmless and usually disappears when the seeds encounter the higher temperatures of the germinator. Quick tests for viability can be used under circumstances that do not permit a standard germination test, but the technology of such tests is particularly unreliable for *Abies* seeds.

Seed Storage

The intended period of storage should be known before the seeds are placed in the refrigerator. It seems pointless to attempt conservation of large volumes of true fir seeds for periods longer than the interval between good cone crops; since good crops are relatively frequent for most species, little will be gained from planning the storage of reforestation seedlots for 10 years when three to five years or so will suffice. If the seedlot is to be used in the nursery within one or two growing seasons after collection, preparation for storage may be less intensive than if it is to be stored over a longer period. Preparation requires a gradual lowering of seed moisture content by air-drying at temperatures no higher than 20°C. Most seeds, if dried to 10–11 percent of fresh weight, will probably retain their viability until the second sowing season after collecting if they are just frozen (0°C). For successful storage for two or three years, lower temperatures ($-3°$ to -10°C) are required, while for four to five years or more, temperatures need to be very low ($-15°$ to -17°C). Such low temperatures require expensive equipment. The alternative is to reduce moisture content to between five percent and eight percent of fresh weight, a condition that can be achieved easily and inexpensively. Drying may take several days, but the seeds can be stored safely at higher temperatures or for longer periods. Seeds should not be dried below five percent, since autoxidation may occur. Dried seeds should be sealed in moisture-proof containers that are not so large that they require repeated unsealing and resealing during the storage period. A maximum container size of 20 kg is recommended, and the containers should not be stacked vertically unless they can resist deformation. On removal from cold storage, containers should be allowed to equilibrate with laboratory temperature before opening to avoid condensation within that will raise seed moisture.

All experiences concerning true fir seeds, whether they concern seed ripening and collection patterns, extraction procedures, storage methods or requirements, or testing, should be recorded for future collation by some central agency. Coordinated research among various groups and individuals is required if true fir seed technology is to be advanced. Tree seeds, and particularly those of *Abies* species, are—or should be—regarded as invaluable resources for expanding reforestation programs. Much greater efforts are needed to ensure their proper use.

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