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IMPROVING SEED GERMINATION IN *ABIES*

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Abstract. A procedure is described for drying stratified *Abies* seeds that allows stratified seeds to be safely stored and which promotes higher germination rates. Seeds, dried to a moisture content of 35%, were stored for 12 months without losing the beneficial effect of stratification or without their viability being adversely affected. After certain storage periods, germination of dried seeds was increased well above that in routinely stratified samples. The use of this procedure in nurseries is discussed.

Seeds of many north-temperate Coniferae plants germinate more rapidly, and sometimes more completely, following a cold-moist treatment period known as stratification (or pre-chilling). This treatment is designed to overcome germination blocks caused by internal seed dormancy mechanisms or poor germination conditions (7), the within-seeds processes that remove such blocks being collectively referred to as after-ripening (4). There are three main prerequisites for successful stratification: a) a moisture source to hydrate the seeds so that the necessary biochemical changes occur, b) near freezing temperatures that favor certain biochemical changes and morphological developments, but delay sprouting of individual seeds that have completed after-ripening, reduce microorganism activity, and prevent damage from respiratory overheating, and c) adequate aeration to allow respired carbon dioxide to escape and also to help minimize heat accumulation (4). To this should be added a fourth criterion, the correct period of treatment. This study concerned primarily the first and last of these factors.

In many forest tree nurseries, the method of stratification is usually some version of the so-called "naked stratification" technique described by Allen and Bientjes (1), in which seeds are soaked in water at room temperature. After hydrating for 1 to 2 days, excess water is drained off, and the seeds are placed in plastic bags and refrigerated at 1° to 5°C for periods of a few weeks to several months, depending on the species, before being sown. This treatment results in relatively high seed

moisture levels (40% or more of fresh weight) and so differs from seed storage which, by definition, promotes seeds longevity, where moisture levels between 5% and 10% of fresh weight (as well as freezing or subfreezing temperatures) are required for most gymnosperm seeds (12). Whereas the role of moisture in seed storage and germination processes has been intensively studied and is well known, its effect in stratification is less well understood.

By design, stratification promotes germination. A few published reports have indicated that stratified seeds can be redried and stored at lower temperature (3,8,11), but the effect of the stratification treatment (germination stimulation) was lost, and dormancy reinduced, when moisture levels fell below 10%. Hellum (9) observed that repeated wetting and drying of *Picea glauca* (Moench) Voss seeds caused reductions in rate and amount of germination and in seedling dry weights. McLemore and Barnett (10) observed that dormancy was greatest in *Pinus taeda* L. seeds stored at 10% to 18% moisture, and was less at both higher and lower moisture contents, being least when seeds were stored above 20% moisture content.

Using a different approach, Danielson and Tanaka (5) found that stratified *Pinus ponderosa* Laws. seeds could be air-dried to a moisture content of approximately 26% and stored at 2°C for 9 months without losing the stratification effect. Stratified seeds of *Pseudotsuga menziesii* (Mirb.) Franco, air-dried to 37% moisture, could be stored only for 3 months, since germination occurred in the refrigerator during longer storage periods. In both species, stratified seeds that had not been air-dried germinated during the third month of storage because of their higher moisture levels (33% in *Pinus ponderosa*, 50% in *Pseudotsuga menziesii*). I was interested in determining if the seeds of other conifer species important in this region's reforestation programme could also be redried and stored after stratification. Research has concentrated on three *Abies* species — *A. amabilis* (Dougl.) Forbes, *A. grandis* (Dougl.) Lindl and *A. lasiocarpa* (Hook.) Nutt., although other tree species have also been investigated.

MATERIALS AND METHODS

Seeds of *Abies amabilis*, *A. grandis* and *A. lasiocarpa* were stratified, then redried to moisture levels of 35, 25 and 15% of fresh weight, and stored at 2°C for up to 12 months. A fourth moisture level, 45%, in undried stratified seeds, was also tested. The experimental procedure and seed sources have been described elsewhere (6).

Throughout this research, seeds were hydrated in distilled

water at room temperature for 2 days, drained, then refrigerated in plastic bags at 2°C for 4 weeks. This is similar to the stratification used for seedling production of true fir species in British Columbia nurseries. Water uptake was regulated by the time of soaking, and actual moisture levels in the seeds during stratification varied between 40% and 60% fresh weight, according to seedlot.

A schematic procedure for drying stratified seeds is shown in Figure 1. Several standard-sized samples (usually 50 seeds, as used in the germination tests) are oven-dried at 105°C to constant weight (usually 24 h) to provide an average expression of the dry weight of a given number of seeds. This average expression is substituted in formula I (used to calculate moisture content percentage), which permits calculation of the fresh weight of a stratified, 50-seed sample at any given target moisture content (formula II = formula I transposed). In the laboratory, changes in fresh weight are monitored after the seeds have been uniformly exposed to the air in a thin layer spread on an absorbent surface. Repeated weighings, at progressively shorter intervals as the target fresh weight is approached, are made on at least 6 samples from random positions among the drying seeds. The same samples must be weighed each time. Provided all the seeds have been dried uniformly, when the average fresh weight of the monitor samples reaches the new fresh weight, all the seeds are rebagged, using fresh containers, and returned to the refrigerator in which they were stratified. Target moisture levels within $\pm 2.5\%$ can be reached using this procedure.

RESULTS AND DISCUSSION

The interactions between storage period and moisture level have been described by Edwards (6), who concluded that optimum germination in *A. grandis* occurred when seeds redried to 35% moisture content had been stored for 3 months (Figure 2). Four major effects were discerned:

a) Seeds dried to 35% germinated best for all storage periods, the differences at day 14 (upper set of bars) being statistically significant in almost all storage periods (Figure 2). These seeds stored well for 6 months, then germination began in the refrigerator. Even after 12 months' storage, they germinated 75% to 80%, better than routinely stratified seeds that had been neither dried (45% moisture content) nor stored (0 weeks storage). Thus, seeds at 35% moisture content had retained the stratification effect throughout the longest period of storage.

b) In seeds dried to 35%, the difference between germination at day 14 (upper bars) and at day 28 (lower bars) de-

REDRYING PROCEDURE

- 8 - 10 SAMPLES OF 50 SEEDS EACH OVEN-DRIED TO CONSTANT WEIGHT
- CALCULATE AVERAGE DRY WEIGHT FOR A 50-SEED SAMPLE
- USE AVERAGE DRY WEIGHT TO CALCULATE WHAT NEW FRESH WEIGHT MUST BE AT SPECIFIED MOISTURE CONTENT

i) SINCE $M.C. \% = \frac{\text{FRESH WEIGHT (FW)} - \text{DRY WEIGHT (DW)}}{\text{FRESH WEIGHT (FW)}} \cdot 100$

ii) THEN $\text{NEW FW} = \frac{\text{AVERAGE DW} \times 100}{100 - \text{SPECIFIED M.C.}}$

FOR EXAMPLE, SUPPOSE $\text{FW} = 50 \text{ G}$ AND $\text{DW} = 40 \text{ G}$

$$M.C. \% = \frac{50 - 40}{50} \cdot 100 = \frac{10}{50} \cdot 100 = 20$$

WHAT MUST FW BE FOR $M.C. \% = 15$?

$$\text{NEW FW} = \frac{40 \times 100}{100 - 15} = \frac{4000}{85} = 47.1 \text{ G}$$

$$\begin{aligned} \text{CHECK: } M.C. \% &= \frac{\text{FW} - \text{DW}}{\text{FW}} \cdot 100 = \frac{47.1 - 40}{47.1} \cdot 100 \\ &= \frac{7.1}{47.1} \cdot 100 = \underline{\underline{15.07 \%}} \end{aligned}$$

- AFTER STRATIFICATION, AIR-DRY SEEDS TO SPECIFIED M.C. % USING 5 - 6 50-SEED SAMPLES TO MONITOR DRYING
- RE-BAG SEEDS AND RETURN TO REFRIGERATOR (2°C)
- STORE IN REFRIGERATOR FOR SPECIFIED PERIOD

Figure 1. An outline of the procedure used for drying stratified seeds to a known moisture level

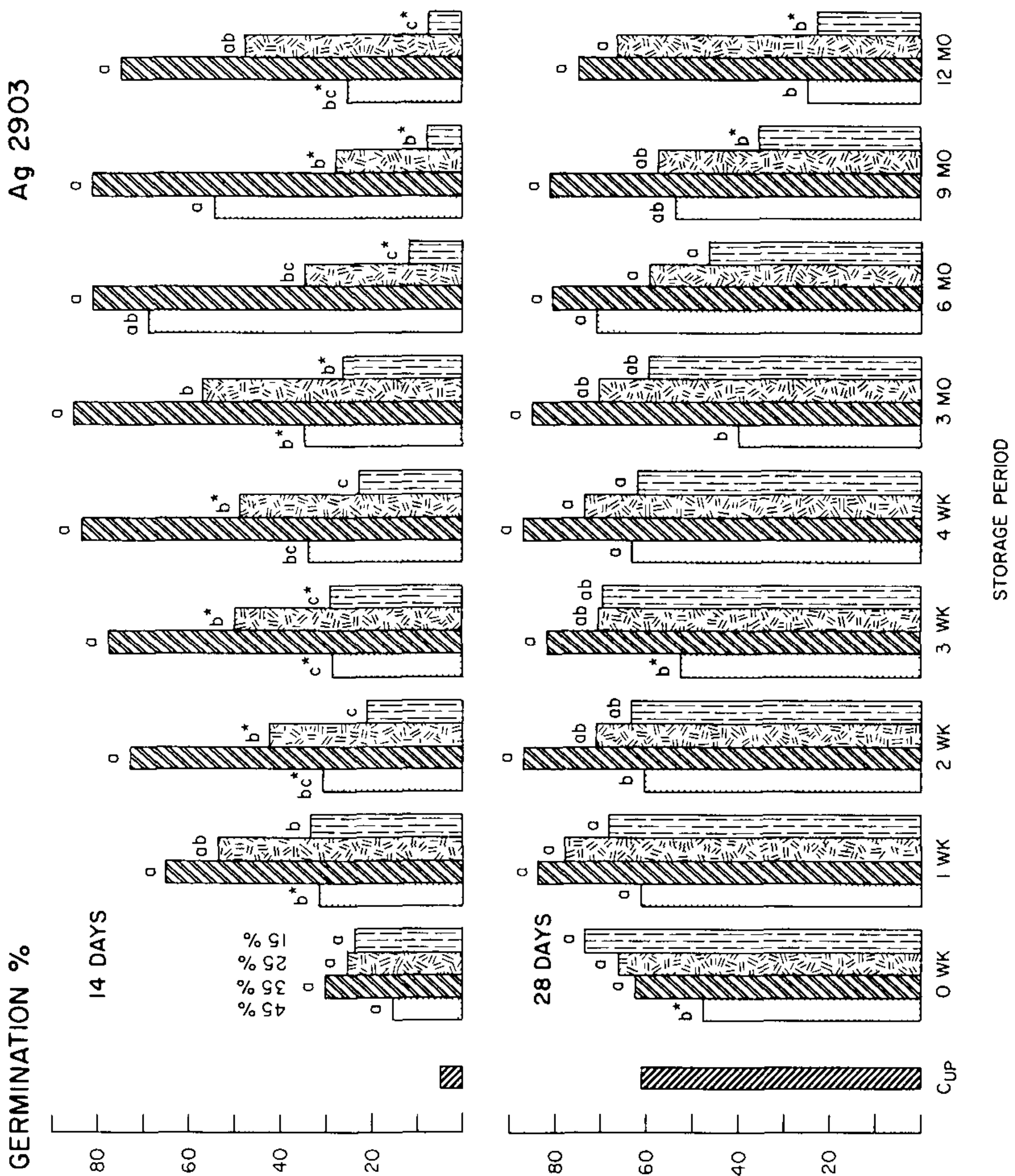


Figure 2. Effect of length of storage at 2°C and seed moisture content on germination rate (14 days), and on final germination percentage (28 days) of *Abies grandis* seeds that had been stratified for 4 weeks C_{UP} — unstratified sample Within each storage period, columns topped by the same letter represent germination means that were not significantly different ($P = 0.05$) An asterisk indicates $P = 0.01$

creased as storage period increased, until at 3 months' storage there was no difference. That is, all the germination that would occur had taken place within the first 2 weeks of the test (Figure 2). For some storage periods, germination was as complete as it would be by day 10

c) For seeds not dried (i.e., at 45% moisture content), germination fluctuated from one storage period to another, suggesting that moisture levels were also varying. Variation between replications was also greatest at this moisture level. At 6 months' storage, non-dried seeds germinated relatively well, perhaps because these samples had lost some moisture.

d) Seeds at 15% moisture content germinated as well as or better than non-dried seeds through 3 months of storage, then their germinability decreased with longer storage periods (Figure 2). Tetrazolium staining showed that viability in ungerminated seeds at this moisture level remained high even after 12 months, so their apparent decline was likely due to the reimposition of dormancy. Ungerminated seeds at 45% moisture showed little staining activity after 12 months' storage, indicating that they were almost completely dead.

Redrying without additional storage immediately produced an increase in germination that persisted through the first 20 days of the germination test (Figure 3). Increases were larger the more the seeds had been dried, but the differences had almost disappeared by the end of the test. For seeds dried to 35% moisture and stored for 3 months, i) countable germinants (2) were obtained by day 5, at least 3 days earlier than without storage, ii) more than 80% had germinated by day 9, and iii) as already mentioned, germination was as complete as it would be by day 14, with 85% of the seeds sprouted (Figure 3).

An additional set of samples that had been newly stratified, but which had not been dried or stored, were germinated concurrently with seeds stored for 3 months. Their final germination (approximately 65%) agreed closely with same treatment tested at the start of the experiment (Figure 3). In contrast, non-dried seeds stored for 3 months germinated less than 40%, demonstrating that storing seeds at high moisture level is detrimental to viability.

Seeds stored at 25% moisture germinated more slowly than those stored at 35%, reaching 70% at the end of the test (Figure 3). At 15% moisture, germination was rapid for the first few days, but fewer total germinants were produced than in the unstored control.

The greatest response to redrying and storage was ob-

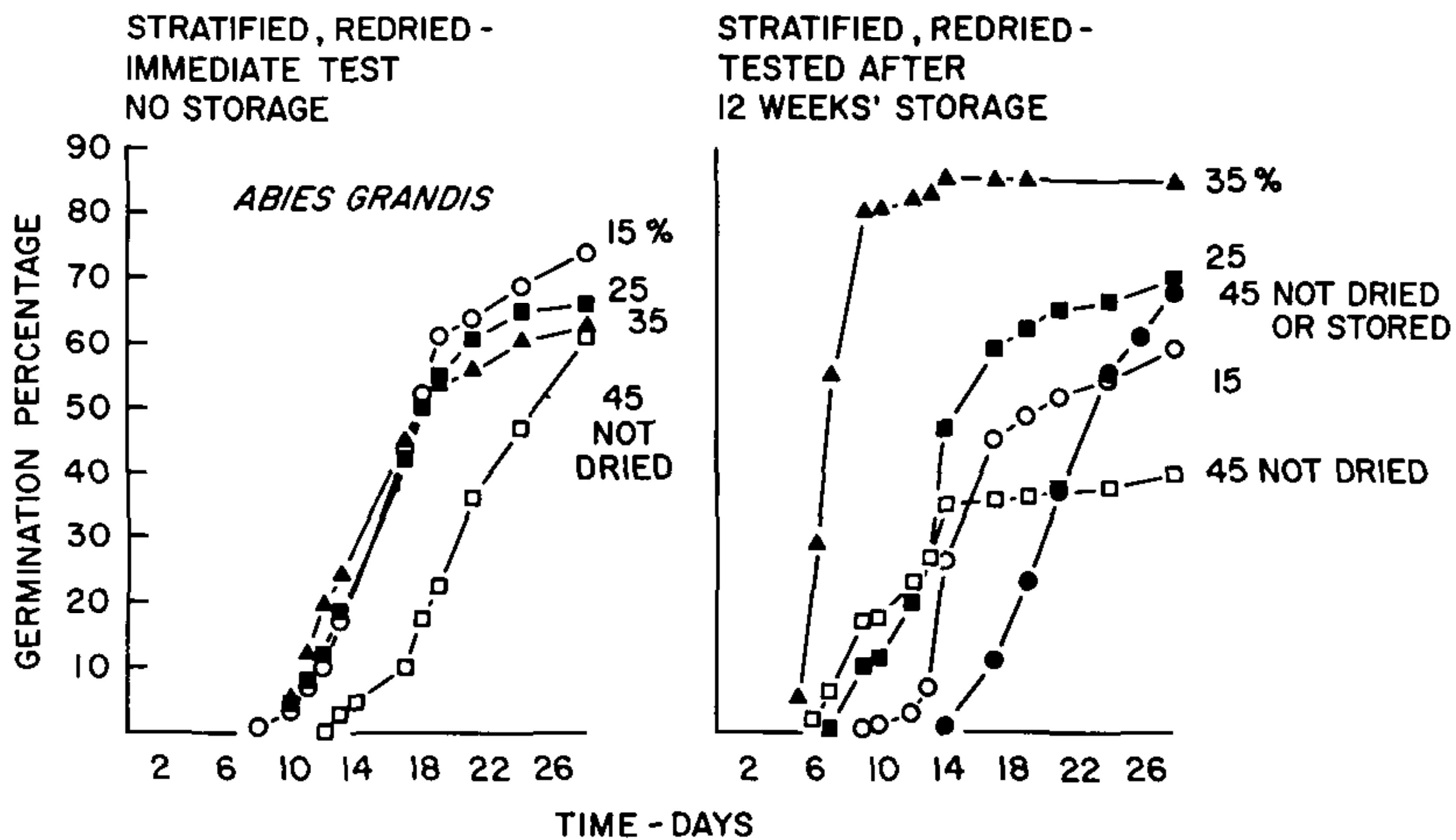


Figure 3. Cumulative germination during a 28-day test of stratified *Abies grandis* seeds following redrying to four moisture levels. Seeds were tested immediately, without any storage (left) and retested after 12 weeks' storage at 2°C (right)

served in stratified *A. lasiocarpa* seeds that, until they had been stored for 3 months at 35% moisture, germinated less than 40% (Figure 4). With 3 months' storage, germination reached 70%, nearly 5 times that of a routinely stratified (no storage) sample, germination was as complete as it would be by the 14th day of the test. Optimum germination in this seedlot occurred following 6 months' storage at 35% moisture content. Seeds stored for 12 months at 35% and 25% moisture levels germinated over 60%, whereas non-dried seeds barely germinated when stored for 1 year.

All the results showed that not only is drying and storage of stratified seeds possible without losing the stratification effect, but that additional germination can also be obtained, especially in seeds dried to 35% moisture and stored for 3 months. To determine whether redrying stratified seeds to 35% moisture content followed by 3 months' storage would consistently improve germination of *Abies* seeds, this combination of treatments was applied to some 30 lots of *A. amabilis*¹. Without fail, all seedlots responded by germinating faster and more completely than routinely stratified controls. Increases in final germination ranged from approximately 5% to over 45%². To date, the treatment has been tested in the laboratory on

¹ In collaboration with Dr C L Leadem, B C Ministry of Forests

² Edwards and Leadem, unpublished

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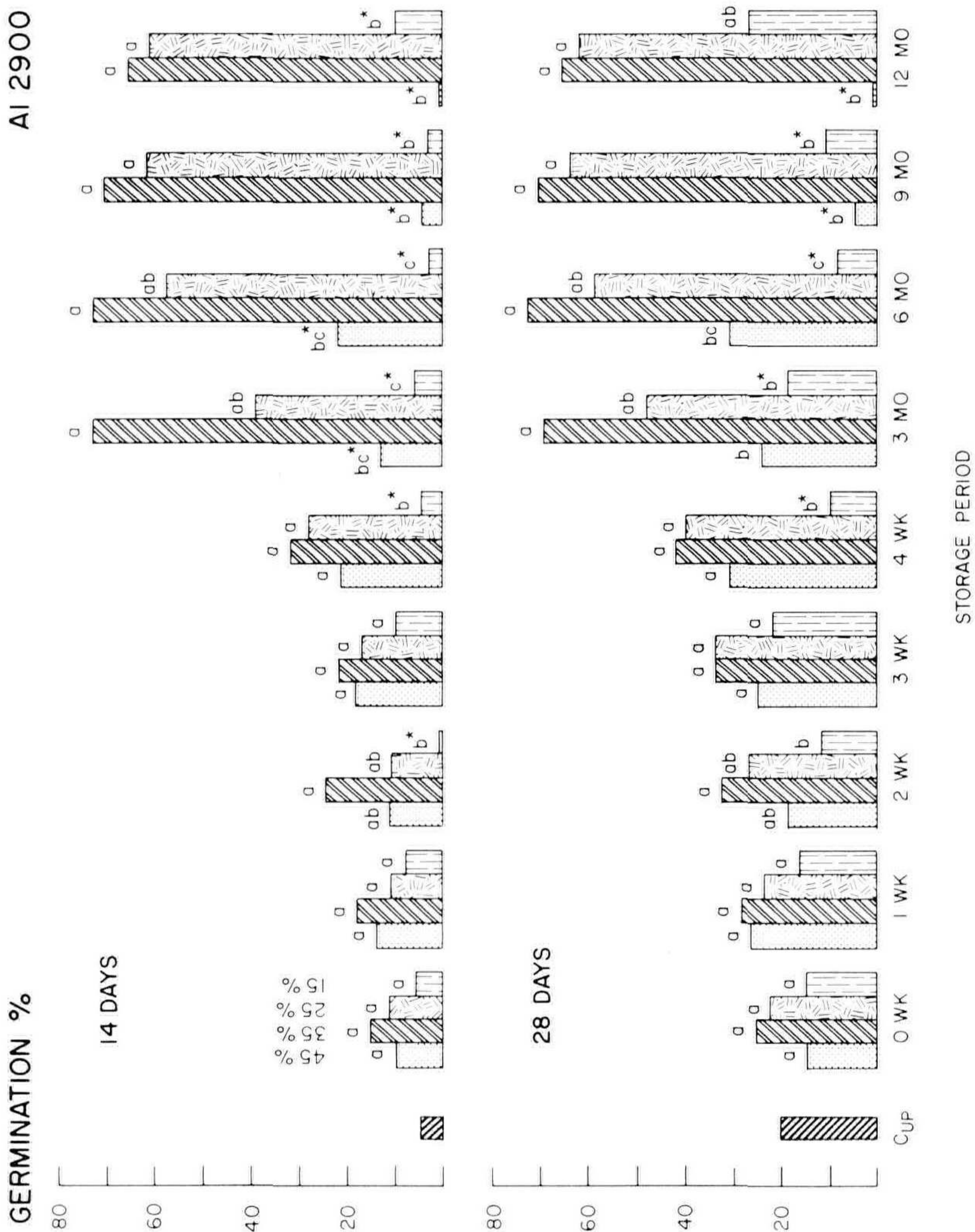


Figure 4. Effect of length of storage at 2°C, and of seed moisture content on germination rate (14 days) and final germination percentage (28 days) of *Abies lasiocarpa* seeds that had been stratified for 4 weeks. C_{UP} — unstratified sample. Within each storage period, columns topped by the same letter represent germination means that were not significantly different (P = 0.05). An asterisk indicates P = 0.01.

more than 50 lots of the three species native to British Columbia, as well as *A. procera* seeds, and germination has been increased in all instances. In local nursery trials, the treatment has not produced such consistent results, primarily because a new method of handling large quantities of seeds during the critical redrying step must be devised. Success, however, has been obtained elsewhere, notably with redried *A. procera* that germinated much better than routinely stratified seeds when sown under cold, wet conditions in the spring³. Seedling production was increased by 8 to 11% in two growing seasons.

When a suitable procedure for treating large quantities of seeds from numerous seedlots has been developed for nursery use, two practical implications need to be considered. First, stratification can begin well in advance of sowing date, and the seeds, after redrying, can be cold-stored for at least 6 months without losing the pretreatment effect or germinating in the refrigerator. Second, the sowing date becomes more flexible. Within the time limits identified, delays in sowing because of poor weather conditions, for example, can readily be tolerated. In effect, stratification could begin before sowing dates have been established.

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SEEDLING PRODUCTION IN THE EASTERN U.S.A.

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It is a distinct pleasure to share with you some seedling production techniques which I have observed, and indeed practiced, over the years. All of these observations will be geographically from Nebraska east to the Atlantic Ocean.

Assuming this is your first venture into the sexual propagation of plants, spend some time on researching the topic. Every volume in the Proceedings of our Society gives us several articles on seed propagation. In Volume 29, there were two splendid articles, one by Tom Wood (3) (GB & I Region) and one by Hugh Steavenson (2) (Eastern Region), both presented at the Western Region meeting in 1979 at Sacramento, California.

One can go back to the first meeting of the Society in 1951, and read Dick Fillmore's (1) words on this topic. After a review of IPPS papers, then purchase this book. "Seeds Of Woody Plants In The United States," Agriculture Handbook #450, Supt. of Documents, US Printing Office, Washington, DC 20402 (cost \$13.60). This book covers seed data of 188 genera and is the true epitome of seedling procedure and information. After reading this book, you will be asking questions of your fellow propagators. At any of the IPPS Regional meetings, all program chairmen allow time for any questions — on any plant propagation topic. I also hasten to add, please remember the old adage that there are NO dumb questions! Sometimes the answers leave much to be desired, but never be embarrassed in asking questions. One final comment before we discuss some specific fundamentals — please keep records on all practices. This information will be very valuable in future years. You can note, on this form, data pertaining to seed source, cost, amount sown, cutting test/percentage, density sown, seedling count, and size (as 1-0). You cannot have too