Dormancy and Vigour of Tree Seeds'

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Abstract. This paper discusses tree seed dormancy and vigour with specific references to the true firs (Abies). The benefits of a modified stratification method--the stratification-redry technique--are outlined. Physiological measurements are described with some examples of how they may be applied to assess seed vigour. Especially promising for the development of seed vigour indexes are low temperature stress tests, germination rates, seed respiration, and seed reserve levels. These may soon be employed as management tools to aid nurserymen to monitor the deterioration of seeds in storage, to assess the effectiveness of dormancy release treatments, and to predict seed performance in the nursery.

TREE SEED DORMANCY

As nurserymen, you probably recognize that the dormancy of tree seeds can be a major impediment to seedling production. Simply put, if seeds do not germinate, there will be no seedlings. Seed dormancy may be defined as:

"The inability of seeds, due to a mechanical or physiological block, to germinate even when placed under favourable conditions for germination."

In this context, the mechanical block is most often the seedcoat, whereas physiological blocks are generally biochemical. Most tree seeds are dormant, and therefore require special treatment before they will germinate. The most effective dormancy-breaking treatment for many tree seeds is the chilling of moist seeds at 2°C, otherwise known as stratification. A modified method of stratification, the stratification-redry technique (Danielson and Tanaka 1978, Edwards 1986), has been shown to be effective for very dormant seeds such as amabilis fir (Abies amabilis Edwards 1980a, 1981; Leadem 1986). In the past year, the stratification-redry procedure has also been successful in overcoming the dormancy of subalpine fir (Abies lasiocarpa) seeds (Leadem 1988).

The efficacy of the stratification-redry treatment was demonstrated in an experiment in which three different seed sources of A. lasiocarpa received 10 stratification treatments (table 1). In the first 6 treatments, moisture was not controlled during stratification. Seeds were imbibed for 48 h, and moisture content (m.c.) remained about 45% during the entire chilling period.

TABLE 1. STRATIFICATION TREATMENTS @ 2°C

No. Weeks	Wks @ 45% mc	Wks @ 35% mc	Total
1.	*	*	*
2.	4	-	4
3.	8	-	8
4.	12	-	12
5.	12	-	16
6.	24	-	24
	Stratification-redry treatments		
7.	4	4	8
8.	4	8	12
9. **	4	12	16
10.	4	20	24

* Seeds were imbibed for 48 h, but received no chilling.
** Standard stratification-redry procedure.

In the remaining 4 treatments, seed moisture was reduced to 35% m.c. for part of the stratification period. The stratification-redry procedure (Treatment 9, table 1) was the standard on which all moisture-control treatments

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were patterned. In treatment 9 seeds were soaked for 48 h and stratified at 2°C for 4 weeks at 45 % m.c. Seeds were then dried to 35% m.c. and stratified for an additional 12 weeks. In the remaining treatments (7, 8, and 10), the number of weeks of moisture control are longer or shorter than in treatment 9.

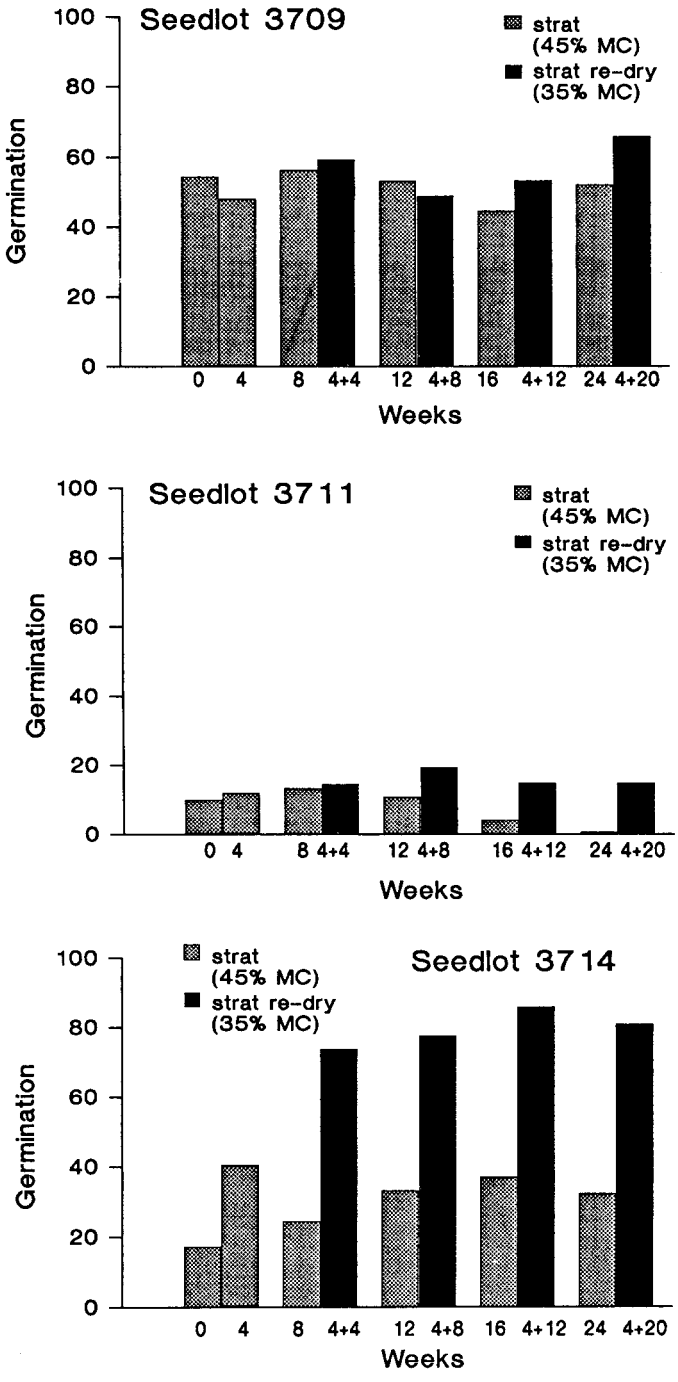

FIGURE 1. Germination of three Abies lasiocarpa seed sources to duration and stratification treatment with and without moisture control.

Germination tests of treated seeds were conducted by incubating the seeds under a daily alternating 30°C/20°C for 4 weeks (ISTA 1985). Eight hours of light were given during the high temperature period.

The responses of three A. lasiocarpa seed

uest Seed Sale/ Withdrawal

Nov. 1-Dec. 31 25 workdays 25+ workdays Jan. 1-Aure 1. Lot 3709 exhibited the response of a nondormant seed source. Seeds germinated about 55% with no chilling, and none of the stratification treatments appeared to substantially increase germination above that of unstratified seeds. Although moisture control during stratification did not increase germination, the stratificationredry treatments were not detrimental to the germination of this nondormant lot. This is an important consideration in any nursery situation where efficient management is essential. Because the stratification-redry procedure enhances the performance of dormant seed sources but does not adversely affect nondormant seeds, only a single method is necessary to prepare seeds for sowing.

Seedlot 3711 consisted of poor quality seeds that did not respond very well to any of the treatments. This behaviour is characteristic of seeds that have been harvested prematurely or improperly handled in the field. These seeds do not germinate well, and deteriorate rapidly in storage (Edwards 1980). The quality of such seeds cannot be improved, regardless of treatment, and the best solution is usually to dispose of the lot.

In seedlot 3714, only 18% germination was achieved with unchilled seeds, but 4 weeks stratification substantially increased germination. This definite response to stratification is typical of dormant seed sources. Moisture control (i.e., reducing seed m.c. to 35%) during stratification had a major effect on germination of seedlot 3714. All the stratification-redry treatments (4 + 4, 8, 10 or 12 wks) significantly improved germination compared to that of seeds stratified for the same length of time without moisture control. The maximum response was 86% germination, observed in seeds which received treatment 9 (4 + 12 weeks). Seeds with no moisture control, and stratified for the same length of time, germinated only 37%.

Radicle emergence is generally taken as evidence of the breaking of dormancy, but more than just radicle emergence is necessary to demonstrate that dormancy requirements have been satisfied. Dormancy release is, in actuality, a composite of a number of qualities which enhance seed performance. In this regard, the stratification redry treatment effectively breaks dormancy of true firs (Abies), and also has been shown to enhance a number of qualities not ordinarily associated with the breaking of dormancy. Abies seeds are often susceptible to mold (Leadem 1986) and are poorly geotrophic. When given stratification-redry treatment,

however, the seeds not only germinate more rapidly and exhibit less fungal contamination, but the radicles are more likely to grow directly down into the substrate (unpublished data).

To date, the stratification-redry treatment has been found to be effective for releasing dormancy of Abies amabilis (Pacific silver fir); Abies rag ndiss(rand Abies lasiocarpa subalpine fir); and Abies rop cera (noble fir) (Edwards 1981, 1982; Tanaka and Edwards 1986; Leadem 1986, 1988). Unfortunately, the use of stratification-redry on other species has been limited (Danielson and Tanaka 1978).

TREE SEED VIGOUR

It is generally acknowledged that seed vigour declines more rapidly than germination (Heydecker 1969). Accordingly, vigour tests have been employed as more sensitive indicators of deterioration and other qualities not as easily detected in germination tests. While several definitions of seed vigour have been proposed (Assoc. Off. Seed Anal. 1983), the following working definition is preferred:

"Seed vigour is that property which enables seeds to germinate quickly under a wide range of conditions, and endows germinants with the ability to establish quickly and resist disease."

This definition of vigour emphasizes the broader, more practical aspects of biological function as it relates to the health and performance of young germinants in the nursery. The period of early germination and emergence is critical to successful nursery production. Germinants which are endowed with those qualities referred to as vigour are better able to overcome the hazards inherent in the susceptible early emergence phase, and thus are more likely to become successfully established as healthy, freegrowing seedlings.

Measurement of seed vigour

Although most nurserymen intuitively recognize vigour in seeds, the concept of vigour has been difficult to express quantitatively. The inability to quantify vigour has been a major obstacle to widespread acceptance of the seed vigour concept. However, some examples of previous attempts to quantify seed vigour are given in table 2. Germination value (G.V.) is a well-known measure devised by Czabator (1962) in which total germination and the speed of germination are combined into a single value. The G.V. index has only had limited use, however, because standard values must be empirically determined for each species. Germination value is expressed as a single value without units, and thus is also difficult to relate to other measures of germination performance.

TABLE 2. SOME INDEXES OF SEED VIGOUR

Index	Description
Germination value	Speed X total germination (Czabator 1962)
Stress tests	Temperature extremes (AOSA 1983)
Growth models	Mathematical expression (Tipton 1984)
Respiration	Biological function Carver and Matthews 1975)
Seed Reserves	Storage protein, lipids

Several seed vigour indexes, however, may have potential for testing tree seeds. Stress tests, usually conducted under high or low temperatures, have been widely used to test the vigour of agricultural species (AOSA 1983), and could be extended to tree seeds. Other examples of measures used to assess seed vigour are growth models (Tipton 1984), seed respiration (Carver and Matthews 1975) and seed reserves. Forest tree seed vigour based on low temperature stress tests, growth models, respiration, and seed storage products are considered more fully in the following sections.

Low temperature stress tests

Standard conditions have been prescribed for testing the germination of tree seeds (AOSA 1978, ISTA 1985). Although standard tests are essential when making comparisons between several seed lots, and between laboratories, the results of such tests may not be relevant to the nursery situation, because tests conducted under optimum conditions do not necessarily reflect how seeds will perform under less than optimal circumstances in the nursery.

Another consideration is that the benefits of seed treatments may not be apparent when comparisons are made under optimal conditions. Nursery tests conducted on Abies rop cera (Rehd.) by Y. Tanaka (personal communication, 1982) showed little difference between the stratification-redry technique and 2 months stratification when seeds were sown under warm conditions, but during cold, wet conditions, seeds that received the redry treatment germinated significantly better. Data obtained for Abies amabilis incubated under low temperatures (15°C/10°C) also illustrates that tests conducted under stress conditions may better indicate the efficacy of different stratification treatments in situations that are more similar to those encountered in the nursery (fig. 2). Similar data were obtained by Davidson et al. (1985) who also incubated Abies seeds under low temperatures.

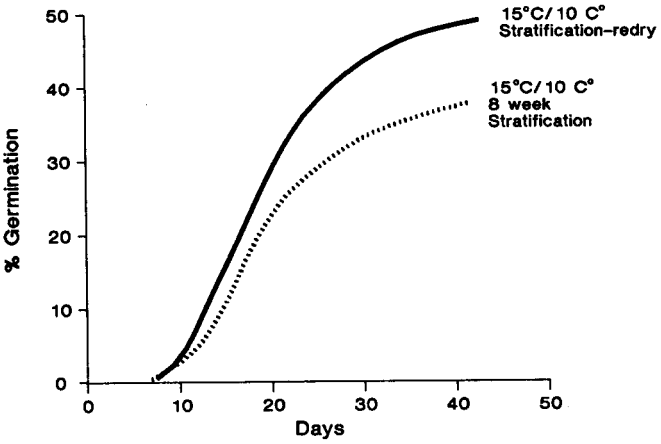

FIGURE 2. Germination of Abies amabilis seeds at low temperatures after receiving different stratification treatments

Growth Model Vigour Index

Mathematical expressions of growth known as growth models, can be employed to transform standard germination curves into a more useful form (Tipton 1984). Growth models use the same basic data used to generate standard germination curves, but the data are mathematically transformed to create other expressions of biological performance, such as germination rate curves. Such curves graphically depict growth characteristics that are not otherwise detectable, and thus may be more sensitive indicators of seed performance (fig. 3).

The benefits of the stratification-redry treatment, for example, are readily seen if germination data is transformed and graphically expressed in the form of germination rate curves. After examining the curve characteristics of the three treatments, it is apparent that seeds given stratification-redry treatment begin germination earlier, that more germinants emerge each day, and that the germination is completed within a shorter period. By this means it is possible to describe those desirable, although generally subjective, attributes which are collectively known as vigour (table 3).

TABLE 3. CHARACTERISTICS OF VIGOUROUS SEEDS

Vigourous seeds have the ability to:

- germinate under a wide range of temperatures
- resist disease
- germinate rapidly - establish quickly
- function with greater physiological efficiency

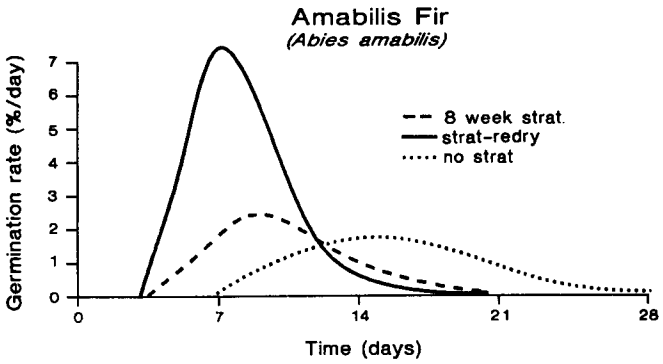

FIGURE 3. Germination rate curves of Abies amabilis seeds which received different stratification treatments.

Levels of Seed Reserves

Since the germinating embryos are solely dependent upon seed reserves, the absolute amounts of reserves stored in the seeds might be expected to directly affect seed vigour. Conifer seeds store reserves mostly in form of fats and protein (Kozlowski 1971, Leadem 1987). With this thought in mind, the relationship between storage proteins and the seed quality was examined in Abies lasiocarpa to see if this could be developed into a useful indicator of seed vigour.

Seeds were sampled from three seed sources at various times during stratification. Seedcoats were removed and fresh weights were measured prior to grinding each sample in 0.05 M potassium phosphate buffer, pH 7.0. Ground samples were then frozen at -20°C until protein extractions, based on procedures previously described by Gifford et al. (1982), were performed. Protein content was assayed by the Lowry method (Lowry et al. 1951).

Germination tests were conducted at 30°C/20°C with 8 h light. Significant differences between protein concentrations and germination percent were tested using analysis of variance. The results are given in table 4.

For any one seedlot, protein values did not vary significantly during stratification. Significant differences in the total seed protein levels between the three seedlots were found ($F=23.39$, $P<0.001$).A direct relationship could also be seen between total protein and germination percentage; the greater the total protein values, the better the seeds germinated. These results are preliminary, but are encouraging to the extent that a vigour index based on protein reserves might exist. Ultimately, it may be possible to find specific proteins whose presence is highly correlated with seed vigour and quality, but further studies are necessary to determine the identity of the candidate proteins.

TABLE 4. TOTAL PROTEIN

Seedlot	Total Protein (mg /10 seeds)	Germination (%) (4wk @45% + 12wk@35%)
3714	26.16 a	86.0 a
3709	23.98 b	53.3 b
3711	18.56 c	14.7 c

Within each column, means with the same letter are not significantly different at p = 0.05 based on Duncan's Multiple Range Test.

Respiration as a Vigour Index

Seeds generate energy for growth and development by respiring stored reserves. The rate at which seeds use their reserves is an indication of their physiological efficiency, and, potentially, also a measure of vigour.

Seed respiration is easily monitored with an apparatus known as the Clark oxygen electrode (Yellow Spring Instruments, Yellow Springs, Ohio) (Murphy and Noland 1982). Seeds are placed in the cuvette with a phosphate buffer to retain peak physiological activity. Temperature is maintained at 30°C with the constant temperature water bath assembly.

Using the Clark oxygen electrode, the respiration of A. lasiocarpa seeds was monitored while applying the stratification treatments described in table 1. The primary objective of the experiment was to study the effects of stratification, both with and without moisture control, on seed respiration and germination.

Respiration of seeds of seedlot 3714, which had been previously demonstrated to be a good quality, but dormant lot, was low when seed moisture was controlled throughout the chilling period (stratification-redry treatment)(fig. 43. For seeds stratified at high moisture levels (> 45% m.c.), respiration rates were also initially low, but they increased exponentially after 16 weeks. In seeds stratified with moisture control (35% m.c.), respiration increased only slightly with treatments longer than 16 weeks.

A different respiratory pattern was observed in the poor germinating seeds of lot 3711. Although respiration rates were relatively low for the first 4 weeks, seeds stratified at high moisture contents exhibited excessive respiration when the treatment continued past 4 weeks. By comparison, respiration in seeds with moisture control remained comparatively constant and increased relatively little during stratification.

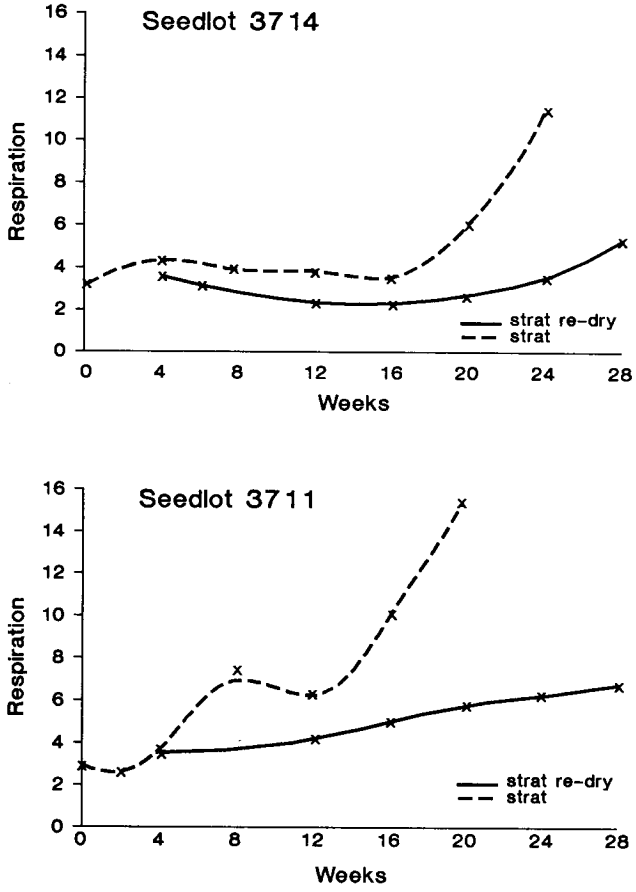

FIGURE 4. Changes in the respiration rates of Abies lasiocarpa seeds during stratification. Seeds were stratified at 2°C with or without moisture control.

It should be cautioned that respiration measured at 30°C does not necessarily reflect how seeds respond during stratification at 2°C, and also that, to date, few comparisons have been made. However, the data suggest a relationship between respiration and seed performance which may partly explain how the statification-redry treatment improves seed performance. Dormant species such as the true firs require long periods stratification to achieve optimal performance (Leadem 1986). The data in this study indicate that if seed moisture is kept at high levels, respiration tends to rise with increasing time of chilling at 2°C. Increased respiration during stratification may have significant impacts upon subsequent seed performance, for if reserves are respired during stratification, they will not be available for use during germination. If, on the other hand, stored products are slowly respired during stratification, more energy supplies will be available for the critical emergence and establishment period.

This study also indicates that changes in seed respiration may reflect changes in dormancy status. If respiration rates in figure 4 are compared to germination data in figure 1, it can be seen that the rise in respiration after 16 weeks is coincident with the length of stratification necessary to overcome the dormancy of the seedlot. This rise in respiration may be related to the breaking of seed dormancy.

The ability to identify when the breaking of dormancy occurs could prove to be valuable in assessing the physiological status of seeds. The usual method of determining stratification requirements is to subject seeds to a series of chilling times, and then germinate the seeds. The use of seed respiration as an alternate method of assessing performance would greatly simplify the search for optimal stratification treatments, since with the Clark oxygen electrode, seed respiration can be measured in 5 minutes as opposed to the 3-4 weeks required for a germination test.

CONCLUSION

Seed vigour has been shown to be related to germination rate, seed protein levels, and seed respiration, each of which have potential for the development of quantifiable indexes of seed vigour. Some day, we may have a simple, onestep vigour test which, by monitoring essential biological processes, will enable us to use our seed resources more efficiently. Physiological measurements such as seed respiration and storage protein may provide some valuable new technology for predicting seed performance, and to a time when nursery managers can truly say that "What you see, is what you get".

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