

# Relating Seed Treatments to Nursery Performance: Experience with Southern Pines

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## ABSTRACT

Producing good quality seeds that perform well in the nursery continues to be challenging. High quality conifer seeds are obtained by optimizing collecting, processing, storing, and treating methodologies, and such quality is needed to consistently produce uniform nursery crops. Although new technologies are becoming available to evaluate seed quality, they have not been developed to the extent that they replace the more traditional methodologies developed over decades of trial and error. The most reliable approaches to predict nursery performance rely on obtaining high seed quality, applying appropriate treatments, and conducting germination evaluations that follow established practices.

## KEYWORDS

seed collection, seed processing, seed pathogens, seed testing

## Introduction

High quality seeds are essential for successfully producing nursery crops that meet management goals and perform well in the field. Uniformity in the production of pine (*Pinus* spp.) seedlings primarily depends on prompt and uniform germination, early seedling development, and a variety of cultural practices that are applied as seedlings develop. Most container nursery managers should be able to maintain 85% or higher germination, and 90% or higher survival after the emergence of the seedlings. Otherwise, oversowing will be necessary, and the subsequent waste of seeds will jeopardize efforts to produce high quality crops consistently and economically.

Meeting the goal of high levels of seed germination and seedling establishment requires considerable care in collecting, processing, and storing seeds, and in applying appropriate pregermination treatments. Seed maturity and dormancy, as measured by speed of germination, vary by species in southern pines. Collecting, handling, and processing affects seed quality (Barnett and McLemore 1970; Barnett 1976a). Dormancy can influence the germination pattern by slowing the initiation rate of germination, particularly in bareroot nursery beds where temperatures and photoperiods are often considerably

less than optimal (McLemore 1969). It is necessary, then, to understand the biology of the species to be produced in the nursery.

The ability to correlate seed parameters to nursery establishment and planting success has been sought by managers of forest nurseries for many decades. In recent years, new technologies to evaluate the physiological conditions of seeds have been developed and evaluated.

The objectives of this paper are to review practices needed to produce high quality, viable seeds and to evaluate current and new technologies that may increase our ability to predict seed performance in the nursery. The overall goal is to be able to consistently produce nursery crops that meet management targets.

### **Producing Seeds of High Quality**

There is a demand for large quantities of consistently high quality seeds to meet the continuing emphasis on reforestation. If seeds are not properly collected, handled, and stored, the result can be poor quality. Viability may be reduced by 20% to 30% by improper handling, making nursery management difficult.

Numerous factors during cone collection and seed processing can affect quality. The most important of these are cone maturity and storage, cone and seed processing, seed moisture content, and storage temperatures. Unfavorable conditions in any one of these areas can cause secondary seed dormancy, the reduction of storability, or the immediate loss of viability.

#### **Collecting and Processing Seeds**

The initial germination of most conifer seeds is directly related to cone maturity at the time of extraction (McLemore 1959, 1975; Barnett 1976a; Tanaka 1984). Cones are considered mature when they can be easily opened at the time of collection (McLemore 1959). In some species, however, seed maturity may occur at a somewhat different time from cone maturity. An after-ripening period may be required to improve seed yields, but after-ripening may not improve viability (Barnett

1976a). Extended cone storage may also reduce seed quality.

After extraction, seeds must be dewinged, cleaned, and dried. These operations result in frequent injury to seeds, and particular care should be used in processing (Tanaka 1984). Precision sowers, such as vacuum seeders, generally require that there be fewer wings and less trash than conventional sowing machines, so extra care should be used in seed cleaning processes, or only seeds of the highest quality should be purchased.

All empty seeds should be removed from lots before use. This is the easiest means of upgrading a seedlot. Normally, empty seeds are removed by mechanical cleaning equipment, including scalpels and gravity, or pneumatic seed cleaning equipment. When seedlots are small, however, as in lots used for progeny tests, it is often convenient to use flotation in water or organic solvents to separate unfilled seeds (Baldwin 1932; Barton 1961; Barnett and McLemore 1970). Flotation in most organic solvents should be delayed until just before use. If the solvent is not thoroughly removed in drying, seeds so treated may rapidly lose viability in storage (Barnett 1971a).

#### **Storing Seeds**

Careful control of seed moisture content and storage temperatures is essential to maintain viability (Barton 1961; Jones 1966; Barnett and McLemore 1970). General recommendations for long-term storage of conifer seeds are to dry seeds to 10% or less moisture content, and hold at subfreezing temperatures. Seeds that are damaged or are known to have low vigor can be preserved by drying to a moisture content of 8% to 10%, and lowering the storage temperatures to about -18 °C (0 °F) (Kamra 1967).

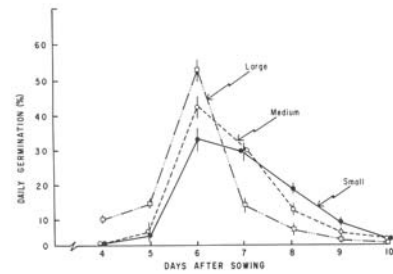
#### **Seed Sizing**

The reported effects of seed size on germination and early seedling growth are conflicting. The operational objective of sizing is to produce a uniform crop of seedlings (Owston 1972). Medium to medium-large seeds have been

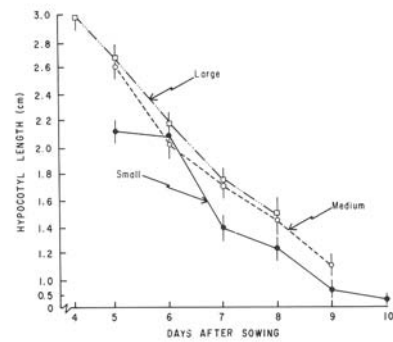
reported to produce larger and more uniform seedlings than smaller seeds (Ghosh and others 1976). Larson (1963) reported that, although seed size can influence subsequent seedling size when seedlings are grown under uniform conditions, as in greenhouses, seed size has a more pronounced effect on germination. Uniform speed of germination may therefore be the most important consideration in sizing. Recent tests under laboratory conditions of minimal environmental stress have shown that germinant size after 28 days of growth was strongly correlated with seed size (Dunlap and Barnett 1983). The faster germinating seeds in each size class produced larger germinants after 28 days of incubation (Figure 1). All seeds reached a maximum germination rate by the sixth day, but smaller seeds were slower to initiate germination (Figure 2). These results are in agreement with Venator (1973), who found that faster growing Caribbean pine (*P. caribaea* var. *hondurensis*) seedlings tend to develop from early germinating seeds. Consequently, seedling size, and possibly uniformity of growth, are primarily functions of germination patterns, which are partially determined by seed size.

#### Reducing Seed Coat Pathogens

Seeds of some conifers carry large quantities of microorganisms that may be pathogenic when seed vigor is low. For example, the seeds of longleaf pine (*Pinus palustris*) are large and have fibrous coats, and are often populated with pathogenic spores (Pawuk 1978; Fraedrich and Dwinell 1996). Results have shown that longleaf seedcoats carry pathogenic fungi that not only reduce germination, but also result in significant seedling mortality (Barnett and others 1999). Study results have shown that treating seeds with a sterilant or fungicide prior to sowing can improve both germination and seedling establishment (Barnett 1976b; Barnett and Pesacreta 1993; Littke and others 1997).



**Figure 1.** Mean daily germination of loblolly pine seeds from the large, medium, and small size classes (Dunlap and Barnett 1983).



**Figure 2.** Mean hypocotyl length of loblolly pine germinants of different germination dates 28 days after germination (Dunlap and Barnett 1983).

### Presowing Treatments

#### Conventional Seed Treatments

After seeds of high quality have been obtained and stored, they must be properly prepared before sowing. Overcoming seed dormancy is one of the major steps to ensure prompt and uniform germination. Presowing treatments to speed germination are discussed in detail by several authors (Allen and Bientjes 1954; Bonner and others 1974; Tanaka 1984). Typically, moist chilling (stratification) is done after an 8- to 24-hour period of moisture imbibition. Fully imbibed seeds are placed in polyethylene bags and held at temperatures of 1 to 5 °C (34 to 41 °F). Temperatures below freezing may injure imbibed seeds (Barnett and Hall 1977), while those above 5 °C

(41 °F) may result in germination during stratification.

The duration of stratification varies by the extent of dormancy present in the seeds. The recommendation for most species is a period of 30 days or less (Krugman and Jenkinson 1974). However, chilling of some pine species beyond 30 days markedly increases the speed and uniformity of germination (Allen 1960; McLemore and Czabator 1961; Boyer and others 1985). Longer chilling periods have been resisted by many nursery managers because some seeds may begin to germinate before the chilling needs of others are met. This precocious germination can be minimized by carefully controlling the treatment temperature. Under proper conditions, germination of more dormant pine species will not occur during 45 to 60 days of moist chilling, and the longer treatments can markedly increase the speed and uniformity of germination.

Uniformity in the production of seedlings is determined primarily by prompt and uniform seed germination and early establishment. Seeds germinating over an extended period have greater mortality during establishment and result in lower grade seedlings (Table 1).

### Less Common Presowing Treatments

Through the years, techniques other than stratification have been investigated in an attempt to accelerate the dormancy-breaking process or obtain desirable germination patterns in a more efficient manner. Many of these techniques have shown little practical application. Three methods that have been used to meet particular needs will be discussed.

#### *Aerated Water Soaks*

Soaking seeds in aerated water is a technique that is frequently used for overcoming dormancy. Soaking pine seeds in continuously aerated water at 5 °C (41 °F) has increased the speed of germination as well as conventional moist chilling (Barnett 1971b). Aerating seeds at 10 °C (50 °F) stimulated germination as much as colder soaks, and did so in less time. Although very dormant seeds can be soaked at low temperatures for nearly 5 months without harm, periods of up to 60 days are usually sufficient. With less dormant seeds and higher soaking temperatures, it may be necessary to shorten periods to 2 to 3 weeks to prevent premature germination or induction of secondary dormancy. The water must be aerated continuously to keep the oxygen content near saturation.

**Table 1.** Mortality and seedling size of shortleaf pine (*Pinus echinata*) seedlings at lifting, as related to time of germination, averaged across 6 half-sib lots and stratification treatments (Barnett 1993).

Time after sowing <i>Days</i>	<i>Week</i>	Germination per week <i>Percentage</i>	Mortality of germinants <i>Percentage</i>	Seedling size <sup>z</sup>	
				<i>Height (mm)</i>	<i>Diameter (mm)</i>
8-10	1	6	12	157	4.1
13-17	2	52	13	160	4.0
20-24	3	30	18	144	3.5
27-31	4	10	27	118	3.1
34-38	5	2	56	115	2.7

<sup>z</sup>25.4 mm = 1 in

Although lengthy aerated soaks are feasible, they are not usually used because there is less confidence in the system. Lengthy treatments are best applied by moist chilling techniques. Aerated soaks are used in instances where insufficient time is available to stratify seeds.

### *Priming*

Another technique that may result in more prompt and uniform germination is priming. In priming, seeds are incubated at optimum germination temperatures, but prevented from germinating by limiting the amount of moisture imbibed. Osmotic solutions, particularly polyethylene glycol (PEG), have been widely used to prime agricultural seeds (Bodsworth and Bewley 1981). Seeds are allowed to absorb moisture, but not to the level required for germination. Although Simak (1976) found that an 11-day treatment of Scots pine (*P. sylvestris*) seeds with a PEG solution (-800 kPa) improved germination, similar experiments by Fleming and Lister (1984) and Haridi (1985) provided variable results depending upon species and seed sources. Malek (1992) found that priming of black spruce (*Picea mariana*) seeds using PEG solutions was less effective than soaking in aerated water.

Solid matrix priming (SMP) uses another approach to limit moisture imbibition by seeds. In SMP, seeds are typically exposed at low temperatures to solid matrices with different water-holding capacities, such as sphagnum, cat litter, peat moss, or combinations of fine grades of sand. The results are again mixed, depending on species and seed source. Wu and others (2001) found that SMP treatments to loblolly pine (*Pinus taeda*) seeds after moist chilling improved the rapidity, synchrony, and completeness of germination. Ma and others (2003) reported that 4 fir species (Pacific silver fir [*Abies amabilis*], sub-alpine fir [*A. lasiocarpa*], grand fir [*A. grandis*], and noble fir [*A. procera*]) responded positively to SMP treatments. Feurtado and others (2003), however, found that western white pine (*Pinus monticola*) seeds did not respond to SMP as

much as to conventional cold-water soaking. Consequently, due to inconsistent results, priming has not become a widely accepted presowing treatment for forest tree seeds.

### *Germinant Sowing*

The principles involved with presowing treatments to speed germination are carried farther in the concept of germinant sowing. Managers of most container nurseries attempt to produce one tree per container cell. Sowing a germinant into each cell significantly improves the efficiency of the operation. Unless seed quality is very high, the traditional approach is to sow multiple seeds per cell, and thin cells that have more than one seedling. Germinating seeds prior to sowing and sowing only germinated seeds is one approach to increasing uniformity of seedling establishment. Barnett (1983, 1985) adapted the concept of fluid drilling (sowing germinants in a viscous gel) to southern pine species. The purpose of the gel is to protect the radicles of seedlings during sowing.

The key to success of germinant sowing is the capability to sort germinants from dead or non-germinating seeds. Normally, germinants will have a lower specific gravity than non-germinants, and separation can be accomplished by using a sugar solution (Taylor and others 1977). South and Young (1995) report that sowing germinants to improve seed efficiency is used operationally in South Africa. With their *Eucalyptus* and *Pinus* spp., separation is achieved by adding sugar to water until the germinants float to the top of the solution. Equipment has been developed in South Africa to mechanically sow these germinants into container cells.

A limitation of the application of germinant sowing to southern pines relates to the difficulty in sorting germinants from non-germinants. Barnett (1985) found that density sorting of seeds from species such as loblolly (*P. taeda*) and longleaf pines did not work, due either to seed density or seedcoat nature.

Although germinant sowing of coniferous seeds has not been widely accepted in the US, sowing germinants of oak (*Quercus* spp.) or walnut (*Juglans* spp.) species has been recommended and used in small scale operations (USFS 1948; Davis and others 2004). Germinating acorns and walnuts before sowing can be a beneficial practice for small-scale nursery culture and direct seeding operations.

### **Methods to Upgrade Quality of Seed Lots**

The ultimate goal of techniques to improve seed quality, such as winnowing (commonly used for millennia), is the capability to separate filled dead from live seeds. For decades, the most effective means of improving pine seeds has been to remove all unfilled seeds, and then separate damaged or poorly developed seeds from filled seeds. Technology to accomplish these tasks has been based on seed flotation or the use of mechanical equipment. Aspirator and specific gravity table techniques work well for many tree species, but the most effective quality improvements have been achieved by density separation processing.

Technology to improve seedlot performance is particularly needed for species like longleaf pine that typically have poor viability. Because container nursery production has been widely adopted for this and other species, newer technologies are being sought to assure that one live seed can be sown in each container cell.

Three different approaches have been evaluated to achieve the goal of 100% germination (Barnett and Dumroese 2006). But, is it possible to determine which of 2 seemingly identical filled seeds is dead, and which is alive?

### **Incubating-Drying-Separating (IDS) Technology**

The IDS process is based on the principle that water imbibed by live seeds is lost at a slower rate than water imbibed by dead filled seeds when both are subjected to uniform drying conditions. Ideally, seeds can then be separated in a liquid medium into a nonviable floating fraction and a viable sinking fraction based on the resulting density differences between the 2 fractions.

Certain IDS procedures have been used for a number of years, and IDS can help separate nonviable from viable seedlots of a number of northern conifer species (Simak 1984; Bergsten 1987; Downie and Wang 1992). This methodology has shown limited success with southern pine species. Karrfalt (1996) reported that his attempts using IDS to remove fungus-damaged seeds of slash pine (*P. elliottii*) failed completely. Donald (1985) achieved positive results with slash pine seeds in South Africa, but that technique was of little value for low-viability lots. McRae and others (1994) were able to separate dead from live seeds of loblolly and slash pine, but questioned whether an economic advantage could be expected from the treatment. Both McRae and others (1994) and Creasey (2003) found that the wing stub of longleaf pine seeds created flotation problems that prevented successful application of IDS techniques.

### **Chlorophyll Fluorescence**

Chlorophyll fluorescence (CF) is a nondestructive and instantaneous method to measure differences in plant function by assessing the magnitude of CF signals. When chlorophyll molecules absorb light during photosynthesis, a small portion of that light is re-emitted, or fluoresced. Numerous studies have used CF to measure photosynthesis efficiency (Adams and others 1990). The same principle was used to estimate seed maturation (Ward and others 1995) and germination (Steckel and others 1989; Jalink and others 1998).

Because longleaf pine seeds have large embryos with considerable amounts of chlorophyll, we decided to evaluate CF as a method of sorting for viability improvement. Chlorophyll fluorescence was evaluated using SeedScan™ technology (Satake Corporation, Houston, TX). The SeedScan™ is a tabletop seed-by-seed maturity sorter that is designed to separate seeds based on their germination potential (Satake Corporation 2002). Although CF is related to germination in some species, no such relationship could be demonstrated when scanning longleaf pine seeds (Barnett and Dumroese 2006).

### Near Infrared Spectroscopy

Near infrared radiation (NIR) is in the wavelength range of 780 to 2,500 nm; 400 to 780 nm is visible light, and above 2,500 nm is infrared. A commercial breakthrough for NIR spectroscopy came when it was shown that this technology could be used to determine the protein content in whole grains (Williams and others 1985).

Today, NIR technology is widely used not only in chemical, pharmaceutical, and food industries, but also in agriculture and wood technology (Downy 1985). The main use of NIR spectroscopy within the field of seed science is quantifying seed moisture content and chemical constituents like proteins and oils (Norris 1988). It is now being used as a quantitative tool that relies on chemometrics to develop calibrations relating reference analysis of the seeds or plant material to that of the NIR optical spectrum. In other words, germination data have to be correlated to the measured spectrum on the same seeds.

Lestander (2003) has demonstrated the potential of using multivariate NIR spectroscopy for conifer seed classification. He found that filled viable and nonviable Scots pine seeds could be separated with an accuracy of >95%.

We evaluated NIR technology in both informal tests with USDA Forest Service forest products scientists and, more formally, with Seed Meister™ technology (Brimrose Corporation, Baltimore, Maryland). The Seed Meister™ AOTF-NIR spectrometer is specially designed for high-speed discrimination, quantification, and sorting of hybrid agricultural seeds (Brimrose Corporation 2002a). The scanning technology can determine oleic and linoleic acid content in sunflower (*Helianthus* spp.), and protein and oil content of soybean (*Glycine* spp.) (Brimrose Corporation 2002b). However, our tests with longleaf pine seeds show no relationships among scanning spectra and germination potential (Barnett and Dumroese 2006).

### Relating Results of Seed Tests to Performance

For decades, nursery managers and seed physiologists have sought techniques, generally with lit-

tle success, that would more accurately predict seed performance in the nursery. Nursery germination is often poorly related to germination in laboratory tests. This is probably due to less than ideal environmental conditions and soil pathogens. Efforts have been made to develop vigor or stress tests that would enable the nursery manager to predict performance more accurately. Germination percentages, however, have remained the accepted means of estimating performance.

In an evaluation of the problem, Barnett and McLemore (1984) found that laboratory germination tests performed on stratified seedlots provided the best predictors of nursery-tree yield for dormant-seeded southern pines. To date, no consistently reliable methodology has replaced laboratory germination testing. The Association of Official Seed Analysts (AOSA) has established standardized germination testing by conducting them under optimum light and temperature conditions. These tests do not reflect germination of dormant seeds on nursery beds where temperatures and photoperiods are often considerably less than optimal (Table 2). A technique to improve prediction of seed performance is to determine chilling needs under stress conditions that relate to the nursery conditions where the seeds are to be sown (Barnett 1993). Extension of the prechilling period will minimize the effect of the less than optimal nursery conditions on initial seedling development.

The results of the Barnett and McLemore (1984) study indicate that germination percentages provide a good prediction of nursery performance for non-dormant seeds. Other germination related values, such as Czabator's germination value (Czabator 1962) that provides an estimate of speed as well as completeness of germination, did not improve the predictability of performance.

### Conclusions

The ultimate goal of techniques to improve seed quality is the capability to sow filled, live seeds in

**Table 2.** Effect of length and method of stratification on a mixed loblolly pine seedlot in 2 testing environments (McLemore 1969).

Days of stratification	Stratified in refrigerator at 1 °C (34 °F)		Stratified outdoors	
	Percentage	Value <sup>z</sup>	Percentage	Value <sup>z</sup>
Tested at 16 °C (60 °F) with 11-hour photoperiod				
0	<1	0.0	<1	0.0
30	68	7.1	59	6.0
60	95	17.3	91	11.4
113	99	24.0	98	19.6
Tested at 22 °C (72 °F) with 16-hour photoperiod				
0	96	20.8	96	20.8
30	99	37.6	98	41.8
60	99	47.1	99	47.0
113	100	50.3	99	56.3

<sup>z</sup> Germination values represent the speed and completeness of germination (Czabator 1962).

a consistently uniform manner. Once seeds of the highest quality are produced, stored, and treated, the ability to predict nursery and field performance is greatly improved. Unfortunately, nursery managers often must use seeds of less than optimum quality. It then becomes important that nursery managers understand the biology of the tree species with which they work.

Through the last 6 or 7 decades, seed specialists and nursery managers have developed methodologies that have been proven to produce good nursery crops. Frequently, however, nursery production is adversely affected by poor seed quality. In the past decade, a number of new technologies have become available to seed specialists. Although a number of these have potential, none have replaced the basic technologies of seed processing known for decades.

It is important that the state-of-the-art seed processing information on species of interest is made available to nursery managers. It is challenging to inform and train managers responsible for growing numerous conifer and hardwood species because of the scope of knowledge needed.

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