

# Upgrading Seed Lots of European Silver Fir (*Abies alba* Mill.) Using Imbibition-Drying-Separation

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## Abstract

A study was conducted to determine if imbibition-drying-separation (IDS) would adequately remove dead, empty, and insect-infested seeds to improve seed-lot quality of European silver fir (*Abies alba* Mill.). The seeds were placed in water and allowed to imbibe for 48 hr, dried for 1, 2, or 5 hr, and then separated into four fractions (A–D) per drying time using an air separator. The x-ray images showed that air separation was successful. Overall, an estimated 88 percent of filled seeds were recovered in fraction A after drying for 1 hr. The germination tests confirmed that the germination capacity of the upgraded fractions was higher than the bulk seed lot. Thus, IDS can be used to improve poor-quality seed lots, which would otherwise not be commercially viable for seedling production in container nurseries.

## Introduction

European silver fir (*Abies alba* Mill.) produces good seed crops every 3 to 5 years. The seed crops often have a high proportion of empty seeds, which is largely because of poor pollination and seed pests such as chalcids (*Megastigmus suspectus*) and midges (*Resseliella piceae*) (Edwards 2008, Skrzypczyńska 1998, Wolf 2003). In most species, these empty and insect-infested seeds are removed during processing and cleaning of seed lots. Removing nonviable seeds, however, is possible only if filled and empty seeds differ in some physical characteristic that can be detected by mechanical or electrical means (Copeland and McDonald 1995). In *Abies* species, empty seeds sometimes have thickened seed coats or contain brown-black material that makes separation of filled and empty seeds by density very difficult. In addition to having a high proportion of empty seeds, *Abies* seeds also have resin vesicles that are susceptible to damage during processing. These resin vesicles contain terpenes that inhibit germination (Edwards 2008, Kolotelo 1997). In *Abies alba*, germination is often poor, ranging from 5 to 80 percent (Edwards 2008), which makes seedling production expensive and inefficient, particularly in container nurseries.

A three-stage process known as imbibition-drying-separation (IDS) can be used to improve tree seed-lot quality. This process is based on the principle that live seeds retain imbibed water more tightly than dead seeds when subjected to drying (Simak 1984a, 1984b). Thus, IDS exploits differential drying, which results in transient density differences so that live and dead seeds can be separated by various means (Gosling 2006, Karrfalt 1996). These include flotation in water, alcohol, or ether or by using an air separator or gravity table (Edwards 2008, Kolotelo 1997). IDS has been used successfully with several species, including lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) (Simak 1984a), Scots pine (*P. sylvestris* L.) (Bergsten 1988), loblolly pine (*P. taeda* L.), and slash pine (*P. elliotii* Engelm.) (McRae et al. 1994). The IDS process is used operationally in Sweden (Karrfalt 1996, McRae et al. 1994). IDS has also been used successfully to remove seeds infested with chalcids (*Megastigmus spermotrophus*) from seed lots of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Sweeney et al. 1991). Some species, such as longleaf pine (*Pinus palustris* Mill.), are not suited to separation by IDS because of their seed and wing characteristics (McRae et al. 1994). Seed processing is not a precise science, and tradeoffs are often made among seed quality, seed yield, and the amount of processing required to meet targets. The aim of this study was to evaluate the use of IDS to remove dead, empty, and insect-infested seeds and to determine its efficacy in improving seed-lot quality and increasing the germination capacity of European silver fir.

## Materials and Methods

The key to IDS is to establish the baseline seed-lot quality, and then to track changes using a range of seed tests to determine if, how, and when the three-stage process improves poor seed lot quality.

## Seed Source

In January 2014, a seed lot (250.0 kg [551.2 lb]) of European silver fir (aal.13[498]D1) from an elevation of 615 m (2,017 ft)

was imported into the United Kingdom from the Brad-Poiana Neamtului region in Romania. The International Seed Testing Association, or ISTA, test certificate noted that the purity of the seed lot was 98.1 percent with the inert matter comprising wings, scales, needles, and resin. In addition, the seed lot contained 23 percent empty seeds and 64 percent viable seeds. Seed tests (described in the following section) were done on the bulk seed lot to establish a baseline for measuring the efficacy of IDS.

### Imbibition-Drying-Separation

A 3.0-kg (6.6-lb) sample from the bulk seed lot was placed in a bucket of water, imbibed for 48 hr at 3.0 to 5.0 °C (37.4

to 41.0 °F), and then spin dried to remove excess water. The imbibed seeds were then split into three subsamples (1.2 kg [2.6 lb] each), spread in boxes with mesh bottoms, and then dried in a warm air stream for 1, 2, or 5 hr at 26.0 to 28.0 °C (78.8 to 82.4 °F). After drying, each subsample was randomly selected and separated by specific gravity into fractions using an air separator (Damas Lasti, Denmark). The air separator uses light suction, which is controlled by adjusting apertures in successive aspiration chambers, to separate seeds into three fractions comprising a clean fraction and two reject fractions. Because the air separator requires relatively large sample to operate, drying time was not replicated. For each drying time, the clean fraction was sent through the air separator two more times (figure 1). The corresponding reject fractions were

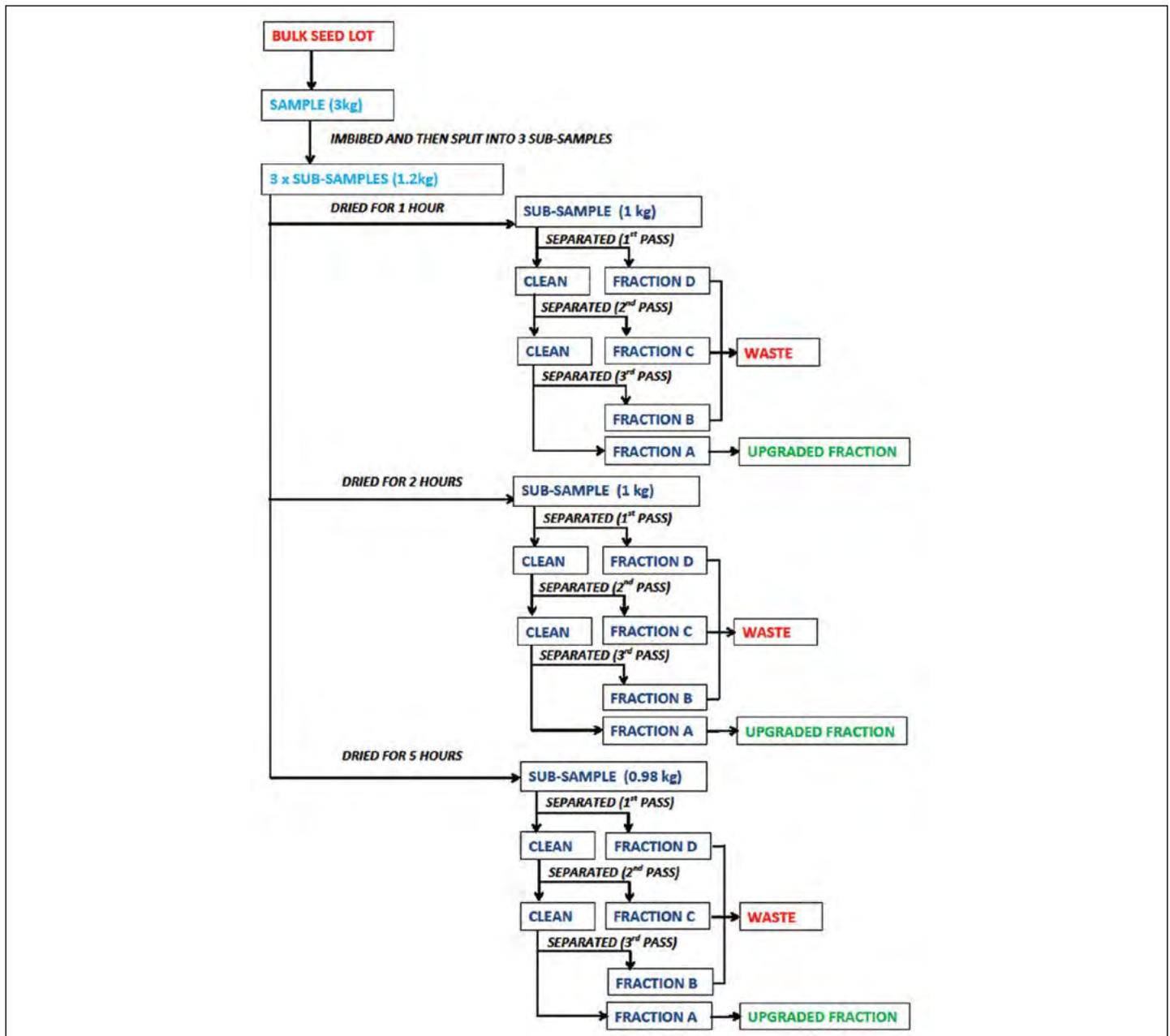


Figure 1. Flowchart of the imbibition-drying-separation (IDS) process.

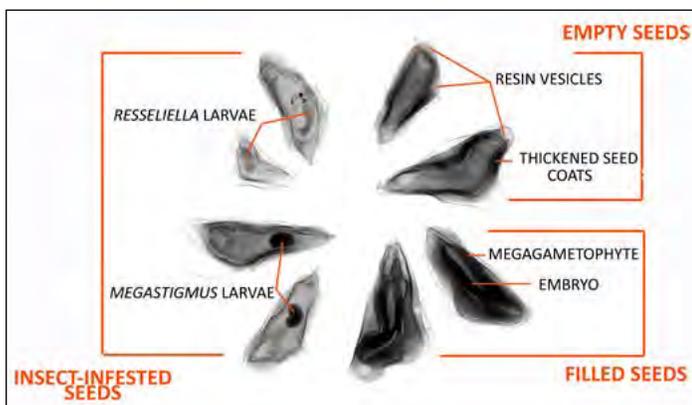
combined to form fraction D after the first pass, fraction C after the second pass, and, finally, fraction B after the third pass, while the last clean fraction was fraction A. This process resulted in a total of four fractions (A–D) per drying time. Twenty seeds were then removed from each combination of drying time and fraction and weighed individually, confirming that fraction D had the lightest seeds and fraction A had the heaviest seeds.

## Moisture Content

Before and after each drying time, moisture content (percentage fresh weight) was determined on four replicates of 10 seeds using the low-constant-temperature oven method (17 hr at 103.0 °C [217.4 °F]) (ISTA 2009). The change in moisture content over time provided an indication of the seeds' drying rate.

## X-ray Tests

Six subsamples of 25 seeds were randomly selected from the bulk seed lot (control, before IDS) and each fraction (A–D) after each drying time and then x-rayed (20 kV for 10 sec). Seeds were scored into three categories (determined after assessing the x-ray images): filled, empty, or insect infested (figure 2). This scoring provided an indication of the separation accuracy resulting from the differential seed drying. Using these x-ray data, a recovery rate was calculated for all combinations of drying time and separation fraction. The recovery rate is a measure of the IDS success based on the amount of filled seed discarded in the reject or waste fractions; in this case, combined fractions B, C, and D. A recovery rate of 95 percent means that only 5 percent of filled seeds were lost as waste. Recovery rate was calculated using a formula by Jones et al. (2002), which was modified to account for differences in separation processes. An example of the formula used for the recovery rate of fraction A is given in the following equation:



**Figure 2.** X-ray image showing filled, empty, and insect-infested seeds of European silver fir. (Photo by Shelagh McCartan, 2015)

$$\text{Recovery rate (\%)} = \frac{[P_{f=A} \times W_{f=A}]}{\sum [P_{f=A} \times W_{f=A}] \dots [P_{f=D} \times W_{f=D}]} \times 100$$

where  $P_f$  = percent filled seeds in a fraction,  $W_f$  = weight of fraction.

## Germination Tests

A germination test was conducted to determine the proportion of seeds capable of producing normal seedlings under laboratory conditions. Two subsamples of 50 seeds from the bulk seed lot (control, before IDS) and each fraction (A–D) after each drying time were spread over filter papers, which were suspended above reservoirs of water in germination boxes. The seeds were chilled for 6 weeks at 3.0 to 5.0 °C (37.4 to 41.0 °F) and then incubated at 20.0 °C (68.0 °F) for a further 3 weeks. Germination was assessed two or three times per week for 21 days or until no further germination occurred over three consecutive assessments. Seeds were considered germinated when the radicle was 10.0 mm (0.4 in) long. Abnormal breached seedlings or those seedlings with stunted or necrotic roots (figure 3) were recorded but not included in the calculation of germination capacity (ISTA 2009). Germination capacity was calculated as a percentage of germinated seeds in each sample. After 21 days, ungerminated seeds were cut and scored as filled, empty, or insect infested.



**Figure 3.** Germinants included (a) normal seedlings, (b) "twin" seedlings, (c) abnormal breached seedlings, and (d) abnormal seedlings with stunted primary roots. (Photos by Shelagh McCartan, 2014–2015)

## Data Analyses

The effect of drying time (three levels) and separation fraction (four levels) was determined on the number of seeds scored in each category (filled, empty, or insect-infested seeds) in x-ray and germination tests using the FITMULTINOMIAL procedure in Genstat 13 (Payne et al. 2009). Because the interaction between drying time and fraction was not replicated, only main effects of drying time and fraction were analyzed statistically by fitting a generalized linear model with multinomial distribution. Because subsamples were pseudoreplicates,

the data were pooled for each combination of drying time and fraction. Sums of the three seed categories were constrained to the multinomial total and individual category counts modeled with a poisson error distribution and logarithm link function. Using the x-ray data, the significance of differences in the numbers of filled, empty and insect-infested seeds between fractions A, B, C, and D and the original bulk seed lot were then tested in turn using chi-square.

Because the effect of drying time was nonsignificant, counts were pooled across drying time for each of fractions A, B, C, and D. For each chi-squared test, the goodness-of-fit for the numbers of seeds in each category between the fraction and the bulk (control) was analyzed as a one-way table with the expected numbers of seeds per fraction calculated from the proportions of filled, empty, and insect-infested seeds in the bulk seed lot (control). A Bonferroni correction was applied to adjust the cutoff of significant p-values to correct for the four comparisons.

## Results

### Moisture Content

At time 0, the bulk seed lot (control, before IDS) had a moisture content of 13.4 percent, increasing to 36.1 percent after imbibition (48 hr). Following drying treatments, seed moisture content decreased to 21.5 percent after 1 hr, 21.9 percent after 2 hr, and 14.7 percent after 5 hr.

### Imbibition-Drying-Separation

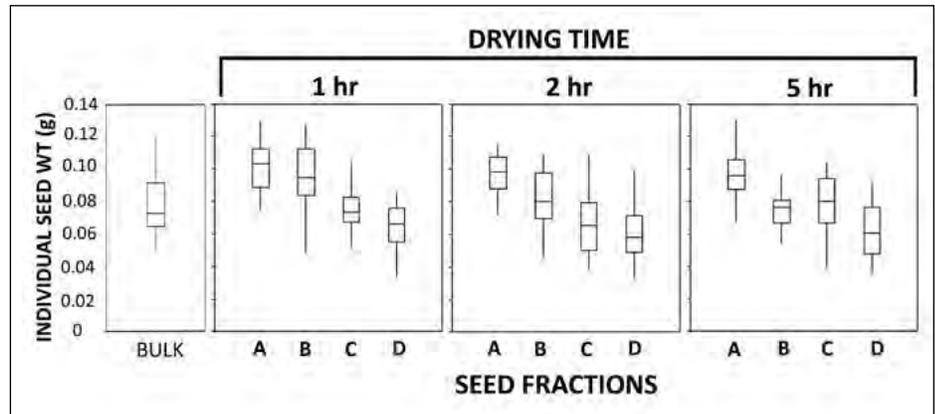
After IDS, there were four fractions per drying time (12 in total). Based on weight, fraction A had significantly more seeds for each drying time than the other fractions, which were similar to one another (table 1). The largest fraction A (by weight) resulted after drying for 1 hr, which represented about 80 percent of the original subsample (table 1). Within each fraction, the weight of individual seeds varied significantly, with heaviest seeds usually occurring in fraction A and lightest seeds in fraction D (figure 4).

### X-ray Tests

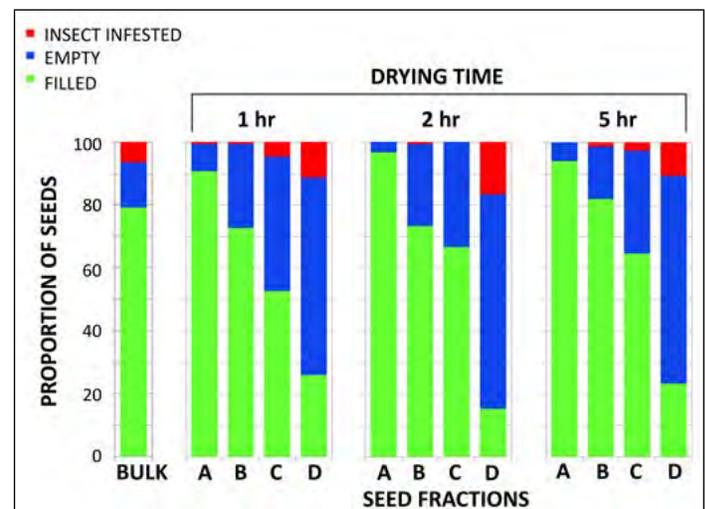
Before IDS, the original bulk seed lot contained 79 percent filled seeds, 14 percent empty seeds, and 7 percent insect-infested seeds (figure 5). After IDS, fraction had a significant

**Table 1.** Weight (g [lb]) of each fraction after different drying times.

Seed fractions	Drying time (hours)		
	1	2	5
A	853 [1.9]	605 [1.3]	614 [1.4]
B	84 [0.2]	103 [0.2]	87 [0.2]
C	51 [0.1]	228 [0.5]	130 [0.3]
D	82 [0.2]	87 [0.2]	144 [0.3]



**Figure 4.** Box-and-whisker plot showing individual seed weights (g) for bulk seed lot (before imbibition-drying-separation [IDS]) and fractions (A–D) after drying for 1, 2, or 5 hr (n = 20). Fraction D separated out on the first pass, fraction C separated out on the second pass, and fractions B and A separated out on the third pass in the air separator.



**Figure 5.** Proportion of filled, empty, and insect-infested seeds in the bulk seed lot (before imbibition-drying-separation [IDS]) and fractions after IDS determined from x-ray images. Fraction D separated out on the first pass, fraction C separated out on the second pass, and fractions B and A separated out on the third pass in the air separator.

effect on the number of seeds within each category ( $p < 0.001$ ), but drying time did not have an effect ( $p = 0.871$ ) (figure 5). Each fraction had significantly different overall proportions of filled, empty, and insect-infested seeds compared with the original bulk seed lot ( $p < 0.001$  for all four fractions). Fraction A had more filled seeds (91 to 97 percent) but fewer empty and insect-infested seeds; fraction B had similar levels of filled seeds as the original bulk seed lot but it had more

empty and fewer insect-infested seeds; fractions C and D had fewer filled seeds than the original bulk seed lot. Fraction A had the highest recovery rate of filled seeds (88 percent) after drying for 1 hr (table 2). Fraction D contained the highest proportion of insect-infested seeds, regardless of drying time.

**Table 2.** Percentage recovery rate of filled seeds within each fraction after different drying times.

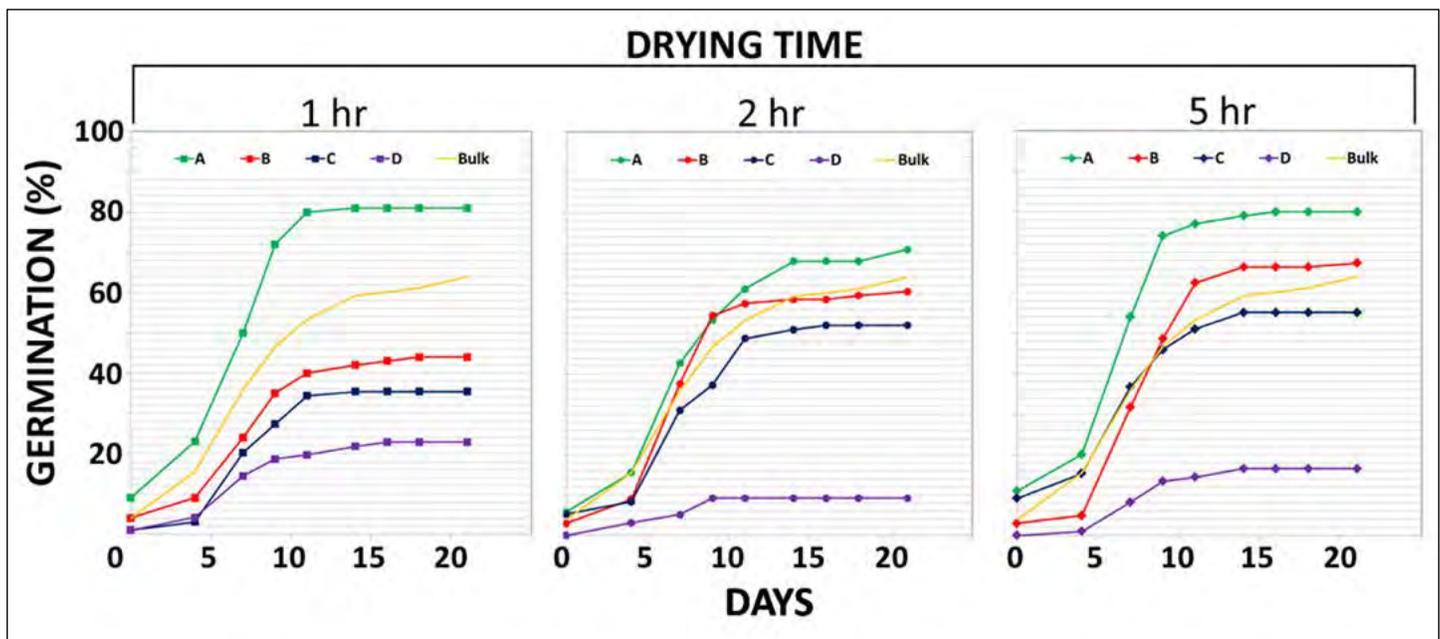
Seed fractions	Drying time (hours)		
	1	2	5
A	88	71	75
B	7	9	9
C	3	18	11
D	2	2	4

## Germination Tests

Before IDS, the bulk seed lot had a germination capacity of 64 percent (figure 6). After IDS, the number of filled (including germinants), empty, or insect-infested seeds differed significantly for fraction ( $p < 0.001$ ) but not for drying time ( $p = 0.293$ ). In all cases, the germination capacity was highest in fraction A (71 to 81 percent) and lowest in fraction D (9 to 23 percent) (figure 6). In addition, seeds within fraction A tended to germinate earlier and more uniformly than those in the other fractions. A small proportion of seeds ( $< 12$  percent) germinated prematurely during chilling, particularly in fraction A (figure 6).

## Discussion

IDS has three stages: imbibition, drying, and separation. The process, however, is not precisely defined and each stage can be done in many ways (Gosling 2006). Drying, though, is the critical stage; if too short or too long, then seeds cannot be separated effectively on the basis of transient density differences. Seeds usually lose water rapidly early in the drying process, largely because of the loss of loosely bound bulk water in seeds. The partially dried seeds then lose water more slowly due to the reduced water potential difference between the seeds and the surrounding air. The drying rate, though, depends on seed viability as live seeds retain water more tightly than dead seeds. At some point, therefore, seeds have very different physical characteristics, such as moisture content, density, and electrical conductivity, which thereby enables improved seed separation based on viability (Gosling 2006, Karrfalt 1996, Simak 1984b). In this trial, the largest weight differential among individual seeds occurred after drying for 1 hr when the moisture content had decreased from 36.1 to 21.5 percent. Longer drying resulted in confounding of fractions B and C, particularly as seeds approached storage moisture content (9 to 12 percent). Drying time, therefore, is important as the efficiency of separation determines whether IDS is a commercially viable option for upgrading poor-quality seed lots.



**Figure 6.** Germination capacity of the bulk seed lot (before imbibition-drying-separation [IDS]) and fractions after IDS. Fraction D separated out on the first pass, fraction C separated out on the second pass, and fractions B and A separated out on the third pass in the air separator.

In this trial, seed fractions were assessed using two seed tests. The x-ray test provided a quick, nondestructive snapshot of separation immediately after drying for 1, 2, or 5 hr. These x-ray images showed that fraction A contained mainly filled seeds, fractions B and C had varying proportions of filled and empty seeds, and fraction D had mostly empty and insect-infested seeds. It is not only the number of viable and nonviable seeds within separation fractions, however, that is important. The weight of the resulting fractions after drying also influences the percentage recovery rate. The highest recovery rate was estimated at 88 percent of filled seeds for fraction A after drying for 1 hr. This recovery rate means that 12 percent of filled seeds were discarded to produce this upgraded fraction, which therefore incurs additional costs per seed. The germination test confirmed that fraction A had a higher germination capacity than the bulk seed lot before IDS, especially after drying for 1 hr. In fraction A, the seeds also germinated faster and more uniformly than those in remaining fractions, which suggests that the resin vesicles sustained minimal damage during IDS. A small proportion of seeds produced abnormal breached seedlings, however, or seedlings with stunted, deformed roots in all fractions. This problem has been reported in other true fir species, including white fir (*Abies concolor* [Gord. & Glend.] Lindl. ex Hildebr.) (Kitzmilller et al. 1975, Kolotelo 1997).

Overall, this trial shows that IDS can be used successfully to upgrade seed lots of European silver fir. The tradeoff is that the cost per seed increases from approximately 1.0 to 1.2 cents (U.S.) because of the discarded waste (about 20 percent of bulk seed lot). A cost-benefit analysis, however, shows that the slightly higher cost per seed is offset by the improved cost efficiency of seedling production (table 3). In container nurseries, poor-quality seed lots result in a high proportion of empty cells, which is usually overcome by increasing the sowing factor (Karrfalt 2013, Kolotelo 1997). Double or triple sowing, though, not only requires more seeds but also requires more thinning of multiple seedlings per cell. Thinning, in turn, increases the risk of unintentional selection against slower germinating seed sources. In contrast, the potential benefits of single sowing the

upgraded seed lot include a higher proportion of seedlings per tray at a lower fixed cost per viable seedling compared with the bulk seed lot (table 3). The seeds also germinate more quickly and uniformly because of improved seed vigor, potentially resulting in better seedling performance (Karrfalt 2013).

## Conclusions

*Bad seed is a robbery of the worst kind: for your pocket-book not only suffers by it, but your preparations are lost and a season passes away unimproved.*

—George Washington (Brainyquote 2015).

This trial shows that IDS can be used successfully to improve the germination capacity of European silver fir seed lots. The potential benefits of these upgraded seed lots include the ability to single sow seeds, which germinate earlier and more uniformly than nonupgraded seed lots, and, therefore, potentially improve the cost efficiency of seedling production in container nurseries.

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**Table 3.** Cost-benefit analysis of using upgraded seed lots in container nurseries.

No. of seeds sown per cell	No. of empty cells per 100 containers*		No. of seedlings produced per 100 containers* (+ surplus thinned)		Total seed cost per viable seedling (U.S. cents**)	
	Bulk	Upgraded	Bulk	Upgraded	Bulk	Upgraded
1	36	19	64	81	1.7	1.5
2	13	4	87 (53)	96 (77)	2.4	2.6
3	5	1	95 (190)	99 (198)	3.3	3.6

\* Based on germination capacity of 64 percent for bulk and 81 percent for upgraded fraction A after drying for 1 hr.

\*\* £1.00 ≈ \$1.50—March 2015.

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