Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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Operational Density Separation Processing (DSP) At the BCFS Tree Seed Centre (TSC) - 1993

Introduction

The TSC initiated density separation processing (DSP) on a limited operational scale as part of the preparation for sowing program. The results of this program are the topic of this presentation.

The 1993 program objectives were as follows:

1. To establish initial seedlot selection criteria and use this criteria to select seedlots suitable for density separation processing.

2. To implement DSP operationally, on selected seedlots for which improvements in overall seedlot quality would result in the need to sow less seeds per cavity.

3. To gather information related to costs and feasibility of offering this service on a large operational scale.

Density Separation Processing (DSP) is a term coined by the Ministry of Forests to describe a method of enhanced separation under development at the Tree Seed Centre. Conventional separation methods such as, specific gravity tables, aspirators and fanning mills are also based on differences in density, but are used on seed which is within a relatively narrow moisture content range (i.e. 4-10%). Conventional methods are most efficient for seedlots where a distinct difference between size and density of filled/empty and extraneous material exists. Enhanced separation methods such as DSP or Incubation-Dry-Separate (IDS) are performed on seed at higher moisture contents; the basis for these methods is that potentially viable seed will actively bind moisture and therefore be more dense in comparison to seed which is not potentially viable. The point of separation is when the potentially viable seed are denser than water and sink, while the non-viable seed are still lighter than water and float. The use of this physiological property of viable and non-viable seeds allows for more efficient separations where discrete differences between viable and non-viable seed exist. Published information on the long term storability of enhanced separated seed is lacking and therefore, most enhanced separation is currently performed as part of the stratification treatment just prior to sowing.

Much of the interest in enhanced separation methods was based on Dr. George Edwards work on white spruce, lodgepole pine and Douglas-fir (Edwards *et al*., 1986). In 1987 the Tree Seed Centre (TSC) initiated the construction of lab and production fluidized bed dryers, separation columns and performed a feasibility study on introducing enhanced separation methods (Furber 1987). In 1991, the TSC initiated a study on the nursery performance of density separation processed (DSP) spruce seed which provided encouraging results for this upgrading method (Banerjee and Scagel, 1992).

Selection Criteria

Given the narrow time frames to process sowing requests and considering that this was our first year of service, the TSC selected seedlots rather than processing on a client requested basis. There were several levels of seedlot selection which began with the following criteria:

1. **Species:** Amabilis Fir (Ba), Subalpine Fir (Bl), Douglas-fir (Fd), interior lodgepole pine (Pli), interior spruce (Sx), Sitka X interior spruce hybrids (SxS) and western white pine (Pw)

2. **Germination Capacity:** less than 70%

3. **Active Seedlot Balance:** greater than 5000 grams for all species with the exception of western white pine where a minimum seedlot balance of 1000 grams was used.

4. **Seedlot usage:** to reduce the risk of performing DSP on provenance's for which there was no longer a need, the number of years a seedlot was requested for operational use[1] since 1986 was established as a selection criteria. The following was used:

    - more than 3 years: Ba, Sx
    - more than 2 years: Fd, Pli
    - more than 1 year: Bl, Pw

The seedlots which met this selection criteria, were then evaluated for DSP suitability. An assessment of the seedlots x-ray radiograph was made and if the seedlot was declared a potential candidate for DSP, a cutting test was then performed. The results of the cutting test in combination with the x-ray analysis enabled technicians to characterize a seedlot and its DSP suitability using the following classes:

Class 1. Empties > 20%
Class 2. Damaged and Discoloured > 20%
Class 3. Immature > 30%
Class 4. Germination < 30%

Based on discussions with those staff members who had past experience and/or were actively involved in DSP, it was suggested that DSP would be more successful on seedlots from Class 1 or 2. The exception to this was with western white pine in order to accommodate a number of client requests. At this point, technicians began routine screening of operational sowing request schedules, to identify suitable seedlots requested for use in the 1993 program. The selection process is summarized in Figure 1 as follows:

[1]operational use meant that the seedlot had been requested for crown land reforestation purposes

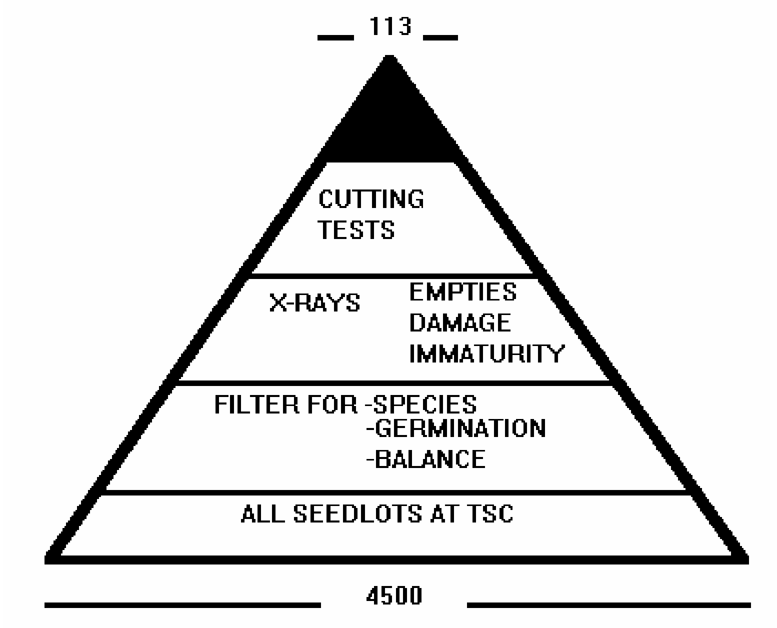

Figure 1. The reduction in number of potential seedlots, from initial number of seedlots at base, to final number of potential seedlots indicated in black.

Density Separation Processing Methods

The TSC is developing two processing methods: imbibitional and dehydrational. Neither method incorporates an incubation step and both methods are based on the ability of potentially viable seed to actively bind moisture, while non-viable seed do not, creating differences in specific gravity thereby affecting separations. The methods on an operational scale, require the ability to quickly separate viable and non-viable seed. To predict viability, seed cutting tests are used as answers are immediate, no special equipment is required and the cost is relatively low. Such tests can be time consuming and subjective, however the continuous cross-referencing of seed types usually leads to consensus for categorization among technicians.

The ability to distinguish floaters from sinkers is usually not a problem, but it is important that the seed is mixed thoroughly in the water to ensure that the surface tension of the water is broken so that viable seed are allowed to sink. Once separations occur, the fraction weight is determined and samples from each fraction are used for cutting tests, moisture content determination and germination testing. For specific details on the equipment used please contact the author directly.

Imbibitional DSP

In this method, dry seed (5-10% MC) was placed in a tank of aerated water. If a seedlot contained damaged seed coats or impurities such as resin and cone scales they were the first materials to sink. The time for this initial separation ranged from one hour for spruce to 12 hours for Amabilis Fir. These initial sinkers were removed as discard material and soaking of the seedlot continued until an acceptable separation between sinkers and floaters occurred. Acceptable separation of viable seed (sinkers) and non-viable seed (floaters) was determined by periodic seed cutting tests.

Dehydrational DSP

This method, is based on the differences in drying rates between viable and non-viable seed. Viable seed is able to actively bind and retain water much longer than non-viable seed during the drying step. The first step in upgrading with this method was to determine the 'target' of improvement. The results of cutting tests were used to estimate the proportion of viable seed. For example, if a cutting test showed that 60% of the seed was well developed, mature and in good condition, this was assumed to be potentially viable, therefore the target separation was 60% sinkers and 40% floaters.

After determining the target of improvement seed was soaked until all of it sank. If some floaters remained after an extended period they were evaluated, however this portion usually contained non-viable seed. If these floaters comprised a large proportion of the seedlot then the sinkers were reevaluated and given a new 'target' based on cutting tests. The sinker fraction was then dried back using a fluidized bed dryer. Drying times and target moisture contents varied and were influenced by seedlot characteristics, batch size and ambient conditions. During the drying step, technicians performed sink/float tests to confirm when seed was uniformly dried and moisture content differences between viable and non viable seed were such that a target separation was possible and once reached seed was removed from the dryer and placed in the separation tank and the separation completed within minutes.

A flowchart example of pathways to further separation is presented in Figure 2. This diagram is general to both methods, but the initial removal of impurities is only possible using dry seed or seed at a moisture content in which all materials float.

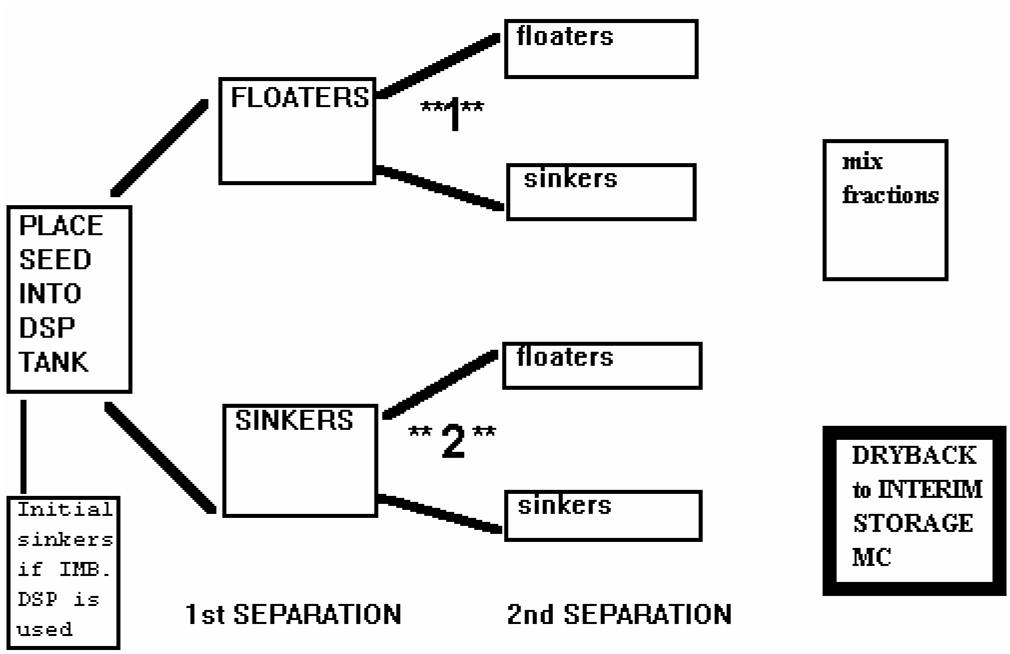

Figure 2. The pathways of seed upgrading to arrive at multiple fractions of seed.

The first separation may not produce an adequate separation and further separation is required due to:

1. too many potentially viable seed in the floating fraction**

In this situation we can (i) continue soaking the seed and separate it imbibitionally or (ii) soak the seed until it all sinks, determine the target for this fraction, dry the seed back until we reach our target and perform the separation.

2. too many non-viable seed in the sinking fraction**

In this situation we would perform a cutting test to determine the target ratio for these sinkers. They would then be dried back until we reach our target and perform the separation.

The number of separations required was linked to the types of seed present in a seedlot. The easiest separations occurred where only one type of non-viable seed was present and usually resulted in two fractions. For most seedlots there were many classes of non-viable seed: empty, immature and various types of deteriorated seed with differing abilities to retain moisture or different specific gravities depending on the level of deterioration. The economics of further separation to isolate as many viable seed as possible was balanced by the additional time required and placed into context by comparing the weight of the fraction to be further separated to the total request weight.

If numerous fractions were obtained and of similar germination they were mixed if they did not differ in the sowing factor (number of seeds per cavity) prescribed by the Ministry of Forests (MOF) sowing rules. Feedback from clients indicated that it was desirable to minimize the number of fractions sent to the nursery.

DSP Results and Discussion

A total of 131 Kg of seed was Density Separation Processed during the TSC 1993 preparation for sowing program as detailed in Table 1. This volume represented 28 seedlots over 42 sowing requests of which the dehydrational to imbibitional ratio was 33 to 9. The volume processed was significantly less than originally estimated due to normal start up difficulties and DSP staff were frequently reassigned to standard preparation activities to meet operational demands.

Table 1. The number of requests, seedlots, type of upgrading, and proportion of total weight processed for each species which received Density Separation Processing (DSP)

SPECIES[2]	**#REQUESTS**	**#SEEDLOTS**	**DEHY. DSP**	**IMB. DSP**	**% WEIGHT**
Ba	18	12	14	4	68.1
Bl	4	4	4	0	13.5
Fdi	4	2	3	1	3.2
Pli	2	2	1	1	4.4
Pw	6	3	3	3	1.9
Sx	6	4	6	0	7.7
Sxs	2	1	2	0	1.2
	42	28	33	9	100.0

[2]Species definitions: Ba - Amabilis fir; Bl - subalpine fir; Fdi - interior Douglas-fir; Pli - interior lodgeploe pine; Pw - western white pine; Sx - interior spruce and SxS - Sitka X interior spruce hybrids.

The success of DSP was evaluated using three criteria:

I. The gain/loss in germination % (best fraction vs. average)

The germination of the best fraction versus the control has been used as a measure of the success of DSP. A density separation was considered a success if the germination of the best fraction compared to the most recent test of this seedlot was such that the number of seeds sown per cavity could be decreased. The gain or loss in germination is especially informative if only two fractions exist, but can be misleading if many fractions are present after DSP or if the best fraction represents a minor small proportion of the request. In these situations it may be useful to look at the germination of the usable fractions weighted by their mass to arrive at a mean germination. In this paper the germination of the best fraction is used in conjunction with the number of potential seedlings to evaluate DSP.

II. The gain/loss in potential number of seedlings

The Ministry of Forests Container Sowing Rules provide information to growers on seedlot sowing oversow and seeds per cavity by germination and genetic class. The Seed Planning and Registry System (SPAR) uses these rules to describe the seedling potential of registered seedlots. DSP success was declared if the number of potential seedlings increased after processing. The number of potential seedlings is an integral part of the current sowing system and provides an estimate of gains or losses in easily understandable terms.

III. The Efficiency of the separation

The third criteria, efficiency, is useful in evaluating the success of the separation from the opposite end (i.e.) how good are the floaters or what proportion of filled seed is being wasted? The efficiency of the separation is calculated as, 100% minus the germination% of the waste seed fractions. In a successful separation, the less seed wasted during processing the higher the efficiency. The separation efficiency can only be calculated if all waste fractions are tested for germination.

The results of our experience with DSP are presented in Table 2 which presents the gain or loss of DSP in relation to the above mentioned criteria as well as some ecological information on each seedlot. The discussion of results to follow will be presented on a species basis.

Interior spruce (Sx)

All separations were performed dehydrationally and resulted in increases in germination averaging 20%. The best fraction was above 80% germination for all DSP'd seedlots. The average increase in potential seedlings was 18 %. Spruce was considered a success story as all requests increased the Germination capacity (GC) and number of potential seedlings however, the efficiencies were slightly lower (mean=88%) than other species. The gains with spruce could have been higher with further separation of viable seed from floaters; it was felt that such gains would not be significant to warrant time and labour required.

Sitka X white spruce

For Sitka X interior spruce only one seedlot was processed twice dehydrationally. Both separations resulted in increased germination averaging 14%, but one had a loss in potential seedlings, the other a gain.

Western White Pine

The other extreme of the DSP program was western white pine in which all six sowing requests processed resulted in a loss of potential seedlings and a reduction in germination of the best fraction in five of the requests. Half of the requests were performed imbibitionally and the other half dehydrationally. All seedlots were graded as class 3 and the high proportion of immaturity is perhaps responsible for the lack of DSP success. The class 3 seedlots were DSP'd because of a great deal of client interest in improving western white pine germination. Seedlots with a large proportion of immature seed appear to also exhibit a high degree of variability in maturity and specific gravity. Processing such lots resulted in continuous (non-discrete) separations of sinkers and floaters and therefore lower gains and efficiencies compared to fully mature seedlots.

Cutting tests were not reliable for western white pine as many seedlots had discoloured, thought to be damaged megagametophyte tissue. The use of DSP on white pine was discontinued during the 1993 sowing season.

Amabilis fir

A total of 15 out of 18 requests were performed dehydrationally. In six of the 18 requests the separations resulted in a loss of potential seedlings. For the remaining 12 sowing requests there was an average gain in germination of 24% and an increase in potential seedlings of 48%.

Subalpine fir

Of the four requests performed dehydrationally two had an increase in both germination and potential seedlings. The cutting tests were not satisfactory for this species due to the mushiness of the seeds after a 48 hour soak. Shorter soaking times will be investigated for operational soaking of Bl.

Douglas-fir

Three of the four requests were performed dehydrationally. The results for Douglas-fir were encouraging with germination gains in all cases and an average 10% improvement. One request resulted in a small 105 potential seedling but given the request size, this would not be considered significant at the nursery level. Seed cutting tests were poor predictors of germination in Douglas-fir; the cause is not known, but present thinking is that more reliable criteria for cutting tests is required for this species.

Interior Lodgepole pine

One request was performed dehydrationally and one was performed imbibitionally. The two requests both had a gain in germination for the best fraction, averaging 15%. One of these requests resulted in a gain and one in a loss of potential seedlings.

In comparing the evaluation criteria it is evident that while many sowing requests had gains in germination of the best fraction many of these still had losses in terms of potential seedlings. The potential seedling calculation seems to be more descriptive, as it takes into consideration all usable fractions rather than just the best fraction. A further explanation for losses of potential seedlings is due to the amount of seed required for testing purposes (i.e.) germination, moisture content determination and cutting tests. An average of 106 grams of seed was required for testing, the impact of which was proportional to the size of the request being processed.

DSP Summary

- For the seedlots investigated in 1993, dehydrational DSP appeared to be a much better technique. Imbibitional DSP had three requests with best fractions which lost more than 20% in GC and had an overall average loss in germination for the nine sowing requests processed in this manner.

- Interior spruce and Amabilis Fir were the species which responded best to DSP.

- With current techniques western white pine is not considered to be a good candidate for DSP.

- Present cutting test classification procedures were adequate for Amabilis fir, lodgepole pine, and the spruce species.

- Improvements in cutting tests are needed for subalpine fir, Douglas-fir and western white pine.

- The selection criteria for DSP which the TSC used is one of an infinite number available. The seedlots which would provide the greatest service to nurseries after upgrading will vary by nursery and their philosophy on seedling culture. The following are some factors worth considering in evaluating seedlot selection criteria for DSP.

    The ability to perform single seed sowing.
    The desire to perform single seed sowing.
    The minimum level of upgrading required.
    The maximum number of fractions desired.
    The ability to take advantage of increases in available seedlings.

ACKNOWLEDGMENTS
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TABLE ABBREVIATIONS

SP. =Species
Ba = amabilis fir = Abies amabilis (Dougl.) Forbes
Bl = subalpine fir = Abies lasciocarpa (Hook) Nutt.
Fdi = interior Douglas-fir = Pseudotsuga Menziesii var. glauca (Beissn.) Franco
Pli = interior lodgepole pine = Pinus contorta var. latifolia Dougl. ex Loud.
Pw = western white pine = Pinus monticola Dougl. ex D.Don
Sx = interior spruce = Picea glauca (Moench) Voss, Picea engelmannii Parry ex Engelm and hybrids
SxS = Sitka X interior spruce hybrid = Picea X lutzii Little

DSP TYPE =type of DSP performed — **DEHY** = dehydrational; **IMB** = imbibitional

SPAR GERM = germination capacity (%) in the Seed Planning and Registry (SPAR) system -used for the calculation of sowing request grams and potential seedlings (recommended seeds/cavity in brackets)

DSP GERM = germination capacity (%)of the best fraction following DSP.

GERM GAIN = the gain in germination of the best fraction as a result of DSP

SPAR POT. = the number of potential seedlings in this request calculated by the Ministry of Forests Sowing Rules

DSP POT. = the number of potential seedlings following DSP calculated using the Ministry of Forests Sowing Rules

GAIN POT.# = the gain in the potential number of seedlings following DSP

%GAIN POT = the percentage gain in potential seedlings relative the the initial sowing request. This allows for a comparison of changes in potential seedlings for various separations regardless of request size.

EFF % = the efficiency of the separation and is equivalent to 100 minus the germination percentage(s) of the waste fraction(s). In many cases efficiencies are unknown as germination tests were not conducted on all fractions.

ELEV (m) = the mean elevation of the collection site

LAT = the latitude in degrees and minutes

LONG = the longitude in degrees and minutes

BGCU = the biogeoclimatic zone, subzone and variant

COLL. DATE = the collection date for this seedlot

SPZ = the seed planning zone for which the seed is to be used

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