SEED ENHANCEMENT/UPGRADING TECHNIQUES: READ THE SEED

KIM R. CREASY

Kim Creasy is with Nature's Common Elements, P.O. Box 29003, Barrie, Ontario, L4N 7W7, Canada; (705) 323-9098.

nces@bconnex.net

Creasy, K.R. 2002. Seed Enhancement/Upgrading Techniques: Read the Seed. In: Dumroese, R.K.; Riley, LE.; Landis, T.D., technical coordinators. National Proceedings: Forest and Conservation Nursery Associations-1999, 2000, and 2001. Proceedings RMRS-P-24. Ogden, UT: USDA Forest Service, Rocky Mountain Research Station: 187-195. Available at: http://www.fcnanet.org/proceedings/2000/creasy.pdf

Key Words

Seed processing, IDS, seed cleaning, PREVAC, seed quality, seed purity

To the nursery industry in Canada, seed enhancing and upgrading techniques have ever increasingly become and are now an integral part of their operations prior to greenhouse sowing. The terms "enhancing" and "upgrading" can be used interchangeably, but they essentially mean the same thing. It's the idea of improving the quality of initial processed seed, which can be accomplished in many ways. Our upgrading work encompasses a number of coniferous species, such as white, red, jack, and lodgepole pine, and white, black, Engelmann, and blue spruce.

Credit for the initial "operational Incubation, Drying, Separation (I.D.S.)" beginnings in Canada over and above the documented research goes to the former company of Western Tree Seeds of Blind Bay, British Columbia -Frank Barnard and Tom Hilman to be precise. These two gentlemen began with an idea and made it reality. Others have also had significant input, resulting in proving this technology to be a benefit for the nursery industry.

Water separation techniques applied to cleaned seeds, removing physically damaged seeds, heavy debris, light debris, and dead/empty/partially filled seeds are making notable improvements to seedlot vigor and germination capacity. Seeding efficiency and conservative utilization of the seeds are the most important and beneficial factors. When using water separation techniques, seed is more responsive when compared to air separation equipment. The upgrading techniques for this presentation center themselves by utilizing the combined effects of pressure vacuum (PREVAC®) and I.D.S. Important scientific principles and attention to detail for each of these is integral, and combined, these form the cornerstone for achieving successful results. Tracking moisture content-initially and throughout the processes-is essential and very interesting to follow. A picture is provided by this information as to what moisture levels are in the various stages of treatment.

It is very important to have a preset worksheet to record initial seedlot details as well as all pertinent and necessary information collected through all processing stages. Once treatment is completed, a wonderful snapshot is created giving an excellent reference of the seedlot dynamics and how the results were derived. Similar situations with other seedlots can be determined by comparison as to whether the results are favorable or not or even as an antidote to describe something unique.

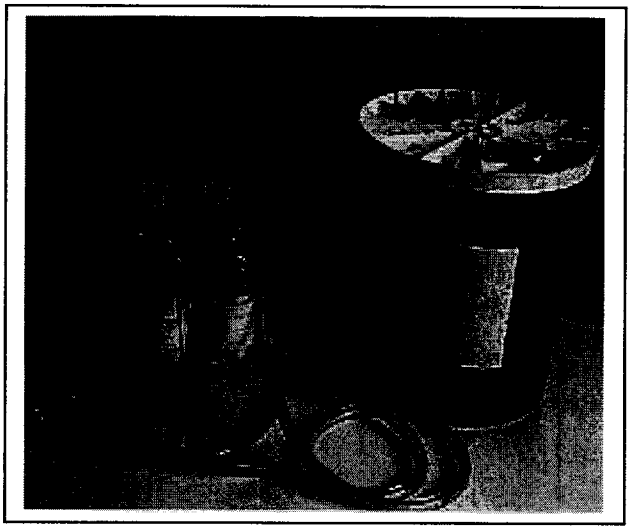


Figure 1. Prevac Unit

Vacuum Pressure Pump, Gast*

This pump is readily mounted and produces steady, quiet, pulseless air delivery. Ideal for intermittent service alternately. The pump rotor is mounted on the motor shaft. Composition vanes take up their own wear automatically. Self-adjusting shaft seals prevent oil leakage. The motor is a 1/3 hp ball bearing-type, with internal thermal-protector, fan cooled.

Free Air Displacement:	4.5 cfm
Ultimate Vacuum:	26" Hg
Pressure:	15 psi
Electrical:	115V, 60Hz
Weight:	15 kg (33 lbs.)

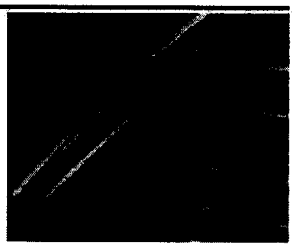
CSA listed.

Figure 2. Catalogue information

290 PUR Tubing, Ether-Grade, NALGENE*

For high-purity work, pressure applications, metering pumps, degreasing lines, acid lines, gas and oil lines, slurry transfer, solids and granular transfer.

Contains no plasticizers and low level of extractables. Clear, flexible clean polyurethane tubing has excellent chemical resistance. Offers good resistance to hydrolytic degradation. Often used with distilled, deionized, demineralized or reverse-osmosis treated water. Durometer hardness: Shore A, 75. Operating temperature range: -56° to 80°C (70° to 175°F). Not autoclavable, but can be gas sterilized. Imprinted every 12 inches with "NALGENE 290 PUR" and one-foot markings. Available in 50' coil lengths.



Vacuum Chambers, NALGENE*

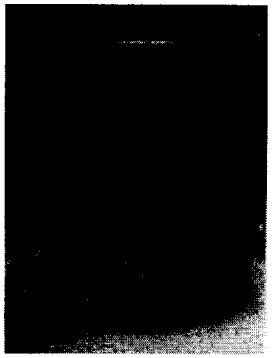
Vacuum chamber consists of transparent jar, neoprene gasket and vacuum plate with adapter for ¼" I.D. tubing.

Vacuum Chamber, **54929-051**, has a polycarbonate (PC) jar and white polypropylene plate. It is intended only for applications using non-aggressive chemicals, e.g., student demonstrations of vacuum procedures. Vacuum Chambers, **54929-062** and **-084**, have pale amber polyetherimide (PEI) jars with excellent chemical resistance to acids, bases, aliphatic alcohols and hydrocarbons and saturated halogenated hydrocarbons; plates are white polycarbonate.

Replacement parts are available. Polyetherimide vacuum jars are chemically-resistant and have an amber cast. Vacuum plates, for use with jars or glass bell jars up to 12½" in diameter, are supplied with 3/32" thick gaskets. Tubing adapter that fits ¼" I.D. tubing is conveniently located on rim of plate. Replacement gaskets are available.

Note: Do not autoclave these jars when used for a vacuum service. Do not use with unsaturated halogenated hydrocarbons.

Ordering Information: Vacuum chambers include jar, gasket and plate.



54929-051

Figure 3, 4. Catalogue information

PREVAC®

The principle for PREVAC® is that vacuum pressure is created within a pressure vessel containing the appropriate amount of seeds and water: "air and airspace are replaced by water." (Figures 1 through 4.) Damaged seeds (whether cracked, abraded, or chipped) and heavy debris therefore become heavier than the water and sink. Species tolerance to vacuum pressures and the amount of time needed must first be established before the proper protocol can be set for operational routines. With some species-for example, black spruce-modified Glycerin based (C3H302) solutions, may need to be used to achieve a proper sink/float pattern. Seed density can improperly represent damaged seed and cause sound, healthy seeds to end up as part of the sunken fraction.

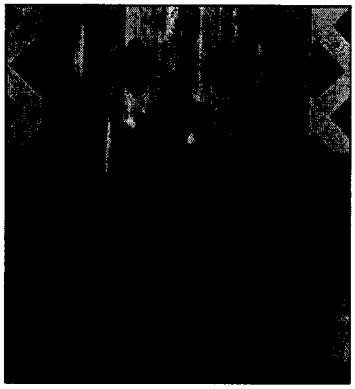


Figure 5. Cone

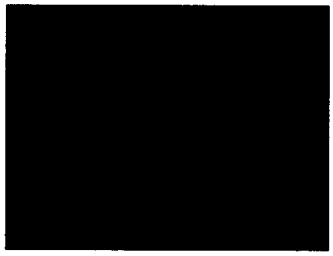


Figure 6. Glycerine Label

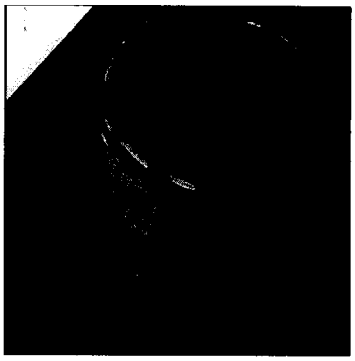


Figure 7. Spin Dryer

A transparent "cone" constructed from acrylic and lexan material with a bottom valve is the unit used for separating the good and damaged portion of the seedlot after the pressure treatment has been applied. Remember-the floating seeds in this stage represent the good fraction; the sunken is the removed or discarded fraction. In order to achieve the best separation, the floating seeds will need careful, frequent stirring and poking with a small dowel stir stick while in the "cone" to allow the damaged seeds and debris to move through the seed mass and settle to the bottom.

To collect the damaged seed fraction, a nylon mesh bag is placed over the valve outlet and opened. The water is drained until the good seed fraction just reaches the valve inlet and then the valve is closed quickly. Water is refilled and the process repeated; it may be necessary to repeat this step a couple of times in order to gain the desired result. Initially, the water may be very murky due to resin dust being removed from the seed, so a close eye must be kept as to where the base of the good fraction is and to be sure that no funneling is occurring within the cone as water is being drained. Once finished, the good fraction is also collected into a nylon mesh bag. Catching the seeds in separate mesh bags then allows the water to be spun from the seed using a "spin dryer" unit (Figure 7). To determine if the process is done well, tip the mesh bag from side to side. The good seeds will have a nice rustling sound as the seeds move within the bag. Some floating debris may be remain, but this is something that is of little concern, as it will come off with the floating fraction through the I.D.S. treatment. But be sure to watch that it does not become a pathogenic source through the next stage. Spin the seeds until the water draining out slows to just a slight drip. To prevent equipment damage or premature wear, ensure that the centrifuge is balanced while spinning.

Collecting a sample for moisture testing after this separation is completed is very necessary for both the good and removed fractions for two reasons: 1) to draw a comparison of the actual percentage of seed/debris removed from the seedlot through the dry weight calculation; and 2) calculating the dry weight of the good fraction as this is the basis for determining moisture content right up to the point of separation and is a very important primary function. It is always interesting to note the difference in moisture content between the two fractions. The good fraction will range in moisture content in an area of 12% to 15%, while the removed fraction will range broadly from a low of 16% to a high of about 30%. Species types and certainly individual seedlots have interesting resultant moisture contents.

PREVAC® EQUIPMENT: BUILD YouR OWN

The supplier of PREVAC® equipment is VWR Canlab, whose Internet address is: www.vwrcanlab.com, or telephone: 1-800-932-5000

Equipment Details.

- Gast Pressure/Vacuum Pump-Model #0323V4AG582DX; Cat. No. 54907-057
- Vacuum Chambers, Nalgene-8 3/4-inch outside diameter by 10=inch height; Cat. No. 54929-62

• 12-inch outside diameter by 12-inch height; Cat. No. 54929-084
- 290 PUR Tubing, Ether-Grade, Nalgene-1/4-inch inside diameter by 3/8-inch outside diameter; 1/1s-inch wall thickness; Cat. No. 63014-228

The separation cone and stand are items built; these could be constructed very simply if you are handy with design and fabrication work.

PROTOCOLS BY SPECIES

Black Spruce PREVAC® and Purity Enhancement Protocol Steps

PRE VA C®

- Pressure vacuum, letting vacuum pump reach a maximum vacuum pressure of 25 inches of mercury.
- Approximate run-up time is about ten seconds.
- Remove seeds from vacuum chamber by pouring seeds into cone, partially filled with water.
- Stir, drain, and collect the seed fractions into a mesh bag. Remove excess surface water using the spin dryer.

OR

- Pressure vacuum as above, remove seeds from vacuum chamber by pouring seeds into cone, partially filled with water and collect the sunken portion into a separate mesh bag from that of the floating fraction.
- Remove excess surface water using the spin dryer. Modified solution separation will only be used on the sunken fraction to recover lost seeds and can be simply done by adding glycerin to the water until the desired seed float is observed. Experience can be the best teacher.

Separation

- Prepare a glycerin (C3Hs03) solution with a specific gravity of 1.060. Check first to ensure that this is the correct specific gravity required by first treating a couple of 100 seed reps. The test separation is evaluated by germination test information received. This specific gravity is generally acceptable for black spruce seeds with 14% to 15% moisture, but expect seedlot variations.
- Pour seed into the mixed solution contained in separation cone.
- Stir to mix in well and allow to settle over the next few minutes.
- Resins, stones, other heavy debris, and cracked and damaged seeds will sink to the bottom.
- Remove debris, seeds, and particulate material collected in the bottom of the cone and those seeds suspended below the main floating fraction. Use a catchment container for glycerin liquid as the solution can be stored and reused.
- Rinse both fractions well in cold running water and again remove excess surface water in the spin dryer. This floating seed fraction can now be combined with the initial floating fraction.
- Separation is complete, and the next stages of upgrading treatments can now proceed.

Variations in the specific gravity of the solution are usually due to moisture content.

Some of these are:

Specific Gravity	Moisture Content (Percent)
1.033	Seven to eight
1.060	Fourteen to fifteen
1.100	Twenty-six and one tenth—large size fraction
1.115	Twenty-five and nine tenths—small size fraction

PREVAC® AND PURITY ENHANCEMENT PROCEDURE FOR OTHER SPECIES

Jack Pine

PREVAC®-Pressure vacuum, letting vacuum pump reach a maximum vacuum pressure of 27 inches of mercury for one minute, inclusive of run-up to pressure.

Lodgepole Pine

PREVAC®-Pressure vacuum, letting vacuum pump reach a maximum vacuum pressure of 15 inches of mercury for thirty seconds to one minute, inclusive of run-up to pressure.

White Pine

PREVAC®-Pressure vacuum, letting vacuum pump reach a maximum vacuum pressure of 27 inches of mercury for thirty seconds, inclusive of run-up to pressure.

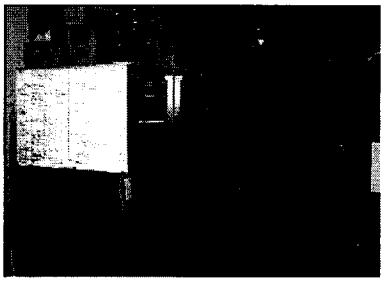


Figure 8. Misting cabinet with vapourizers

Figure 9. Flume

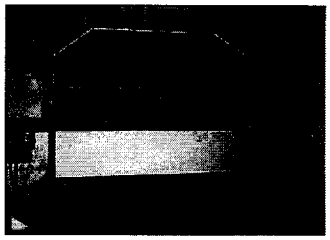


Figure 10. Seed dryer

Red Pine, White Spruce (Engelmann Spruce, Sx Spruce, Sitka Spruce, Blue Spruce)
PREVAC®-Pressure vacuum, letting vacuum pump reach a maximum vacuum pressure of 20 inches of mercury for approximate run up time of about eight seconds, inclusive of run-up pressure.

INCUBATION, DRYING, AND SEPARATION
The principle for I.D.S. is that only living tissue can retain moisture. Dead and dying seeds will float by creating the precise density differential through dryback. Initially, metabolic activity within the seeds first needs to be mobilized before the sequence proceeds to its end.

Incubation
Represents a modified stratification and requires that seed be at a high moisture content (28% to 35%) to begin the process in alleviating seed dormancy. The seedlot is first given 24-hour aerated cold water soak, spun dry (removing surface moisture only) followed by three weeks in a customized refrigerated misting cabinet with temperatures of 2 °C to 5 °C and 100% humidity. Seeds are contained in a 6- by 28-inch acrylic tube fitted with a combination of nylon screening and gortex end covers held in place with friction rings constructed from nylon tubing that is fitted to the inside of the tube. The gortex end covering allows for free air exchange and moisture uptake by the seeds. The nylon screening provides a separation between the inner chamber and gortex, thus preventing the seed mass from blocking the air exchange across the membrane (Downie 1999). At this stage it is essential to allow for additional water and proper air exchange by the seeds to ensure that initial metabolic activity is not impeded and the seeds do not suffer anoxia. Rolling the tubes daily repositions the seed mass and ensures a close inspection of the seeds. The optimum seed mass per tube is 3.500 kg., but if necessary, the maximum seed mass per tube could be as much as 5.600 kg. Required humidification within the cabinet is provided using a Slat/Fin Electric Warm Mist Germ Free Humidifier®.

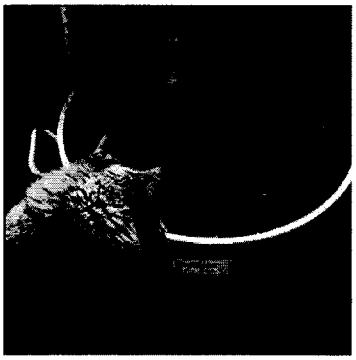


Figure 11. Soak pot c/w aeroators

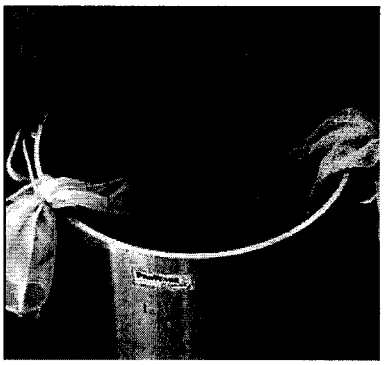


Figure 12. Soak pot c/w aerators

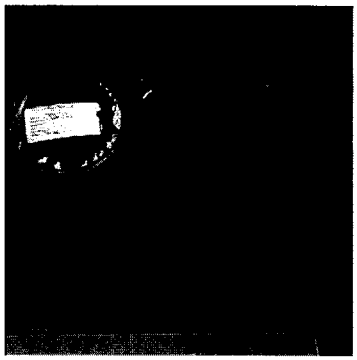


Figure 13. Tubes

Drying

Dryback is the procedure to effectively create a density differential and establish a sink/float ratio that matches the dead seed component from the most current germination test results. Marked sampling assessment over the drying period is used to establish when the separation point is attained. Prior to beginning the full drying phase, and this is the essential key to getting an optimum dryback period, the seeds are removed from their tube, placed into a nylon mesh bag, spray rinsed for part of the time while in the spin dryer and then put into the right side of our refrigeration unit at 2 °C to 5 °C where no humidity is added. We call this process Alternate Water Stablization (AWS). The seeds are uniformly spread within the bag on a screen shelf and left for a period of approximately 16 hours. This is a secret so don't tell anybody! Seedlots will lose some of their water over this time in varying degrees, from as little as 0.2% to almost 3% (2.73%), which is rather significant. When the seedlot exhibits a substantial moisture reduction it can indicate that dead/dying seeds are going to be removed from the seedlot quickly and that you just might expect an excellent germination increase. The example of 2.73% was from a black spruce seedlot that ended up with a germination capacity of 100%.

After completing the AWS step the seeds are weighed to determine their moisture content from the dry-weight calculation obtained after the pressure vacuum treatment or from initial moisture content information. The seedlot is now ready for the full drying phase. Depending how you feel about the drying times, intervals are very flexible: as little as 5 minutes to as much as 25 minutes, using a temperature range of 25 °C to 28 °C. The dryer used for our work is my own design and operates as a fluidized bed dryer giving the seeds the latitude to move within the tray. The dryer can deliver a maximum air volume of 1100 ft^3. A 4-speed fan gives effective control of air volume. Seldom has anything but the first selector position been used. An overhead hood and filtering mechanism provide dust removal over the drying phase. Seed drying can present a problem in this stage and rather than increase temperature it is much safer to increase the air volume. At the finish of each drying interval, 2 x 100-seed samples are collected to assess the very important sink/float ratio. What you are trying to represent in this ratio is the dead fraction of the seedlot; in other words, if 91 % was the initial germination, an average of nine seeds would be required as the floating fraction. To get to this average, successive drying intervals may be required, with varying time durations. You should need less time the closer you get to the marker point. As well as collecting the seedlot moisture content at each of these intervals, each 100-seed sample is weighed, to again note what has occurred and provide a tracking sequence.

Earlier, the separation flume had been filled with the appropriate water volume. To conduct the sink/float procedure, water is used from the flume only. This represents the exact water characteristics when the seed is ready for its separation stage. This is very important in order to ensure consistency between assessment and separation.

Figure 14. Transport sink/float containers.

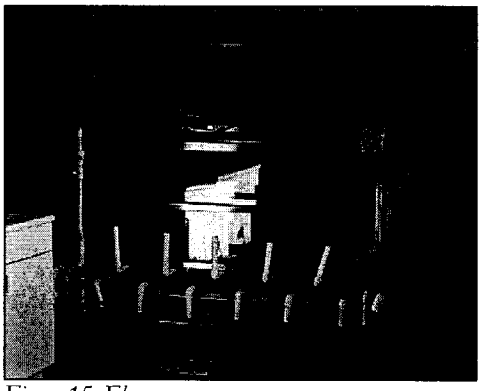


Figure 15. Flume.

The individual samples are dropped into separate transparent containers with water, stirred, and observed. Floaters are viewed by cut testing to look at anatomical structures present; in other words, empty seed, dead-filled and damaged tissues, and absent or immature embryos. As a general rule of thumb if the average floating seed number represents the dead fraction of the seedlot plus or minus one or two seeds, then it is ready for the separation stage.

When pressure vacuuming has been used in the treatment of jack pine (always) or lodgepole pine, new germination tests are set up using a 2-week stratification period (not a 3-week as in the incubation stage). This allows the upgraded PREVAC® germination results to be used in place of the initial germination information, establishing a float average using the most accurate germination value.

Separation

Separation occurs as dead and dying seed float while good seeds sink into sedimentation compartments along the bottom of the separation unit known as a separation flume. This particular flume has six bottom compartments into which the seed can settle.

Bottom fractions are termed as sinkers and identified as "B." These fractions sink and settle across the bottom of the Separation flume and are grouped as follows:

- B1 & B2-Highest and best fraction of living viable seed with the best germination/vigor. Appropriate for single seeding. Kept separate after separation.
- B3 to B6-Lower germination/vigor than found in the B1& B2 fractions. More appropriate for double seeding. Grouped as one after separation.

Top fraction seeds are termed as "floaters." This fraction floats and is regarded to be debris that contains the following:

- Weak, dead filled and empty seeds. There will be some germinates within this fraction, but germination capacity and vigor are low and a large number of abnormal germinates can be expected.

The seeds, now ready for separation, are poured into a nylon mesh bag. From the bag the seeds are introduced into the feed hopper of the flume. The seeds move along a vibratory channel at a slow, even feed rate into a water bath. By prewetting the seeds before being discharged into the separation tank, surface tension is released on the seedcoat. This limits air bubbles that might affix themselves to the seeds. Bubbles adhering to good seeds can cause undesirable events to occur such as a seed floating to the surface or extended travel in the water current, causing the seeds to end up in an alternate compartment with poorer quality seed. If bubbles adhering to seedcoats are a problem for you, devise a prewetting routine prior to separation.

Each seedlot will have its own sink characteristics with different fractional components. This means a seedlot could have a B1 and floaters only, while another could be made up of a B1, B2, B3-6, and floaters. This type of segmentation in certain cases is a judgment call and relies solely on the experience of the individual doing the treatment. Separated fractions can become too fine and can end up creating problems for a seeding system for which they were originally intended to provide enhancement. Don't get too fine, keep it operational!

Once the separation is completed, samples are removed from each fraction for moisture testing in order to calculate new dry weights and germination testing to validate that the separation work is completed. Seedlots have a moisture management routine, to ensure that the seeds are at a moisture level that will accommodate transportation even to far-ranging destinations and storage/handling prior to set seeding schedules.

CALCULATING MOISTURE CONTENT

Moisture charting calculations eliminate a dependence on electronic moisture meters (weighing accuracy is a prerequisite).

1) Standard oven test with 1- or 2 5-g sample(s) to obtain moisture content (MC) of seeds.
2) Weigh seeds and record at this point; this becomes the fresh weight (FW).
3) After MC has been determined, calculate dry weight (DW) for the bulk lot.
4) Calculate the target fresh weight (TFW) and (if desired) calculate and construct a moisture chart for the desired target moisture content range (TMC).

a) MC = FW - DW/FW x 100 (oven test 17 hours at 103 °C)
= 5.000 - 4.254/5.000 x 100
= 0.746/5.000 x 100
= 0.1492 x 100
= 14.92%

b) DW = [1-(MC/100)] x FW
= [1-(0.1492/100)] x 240 g.
= [1-0.1492] x 240 g.
= 0.8508 x 240 g.
= 204.192 g.

DW100 = [1-(MC/100)] x FW100
= [1-(0.1492/100)] x 0.657 g.
= [1-0.1492] x 0.657 g.
= 0.8508 x 0.657 gm.
= 0.559 g.

c) TFW = DW/[1-(TMC/100)]
=204.192/[1-(14/100)]
= 204.192/[1-0.14]
= 204.192/0.86
= 237.432 gm.

TFW100 = DW100/[1-(TMC/100)]
= 0.559/[1-(14/100)]
= 0.559/[1-0.14]
= 0.559/0.86
= 0.650 gm.

Moisture Chart

A short cut to all of this goes like this; moisture test and calculate dry weight(s) as indicated. Determine your target moisture content desired and record the weight to which to dry the seed back. Seedlot (seed) moisture content can be obtained at any time by dividing the present weight of the seed being dried into the calculated dry weight and then subtracting that value from 100. The difference is the current moisture content for the seedlot (seed).

Moisture %	Weight in Grams	100 Seed Wt. in Grams
14.92	240	0.657
14	237.432	0.650
13	234.703	0.642
12	232.036	0.635
11	229.429	0.628
10	226.888	.0621
9	224.386	0.614
8	221.947	0.608
7	219.561	0.601
6	217.225	0.595

Prepared by Mishtu Banerjee; Scientificals Consulting/ Kim Creasey; Nature's Common Elements

RESULTS BY EXAMPLE: SUCCESSFUL & NOT

It is a fact that most of the species and the corresponding seedlots treated through these upgrading procedures and protocols have very positive results and enable nurseries to utilize single sow applications. A small percentage of the seedlots treated for many and some undetermined reasons respond negatively to the processes. The species that tends to return the most variable response is white spruce and more specifically originating from NW Ontario and parts of NE Ontario. It is very interesting to observe the varying characteristics and to try to hypothesize the question of "WHY." Many thoughts can come to mind depending on individual seedlot information and circumstances.

Questions to name a few;

- degree of dormancy versus modified stratification period-enough or too much
- correctness of initial germination information supplied
- pathogenic problems and corrective measures
- adequate moisture levels-too much or not enough
- inhibitory effects attributed to decreased water permeability (Baron 1978; Downie 1999)
- decreased oxygen permeability (Koslowski and Gentile 1959) (Downie 1999)
- restriction of expansion of the megagametophyte and embryo (Asakawa 1956; Downie 1999)

As difficult as it may be, the best one can do is "Read The Seed" to the best of your ability and to gain an intimate understanding of what is taking place biologically. Certain species tend to be very open to interpretation while others are very discrete and subtle.

SUMMARY

This presentation was designed as a practical and operational approach with the intent of sharing some handy tricks and tips that you may find useful in your own program. Equipment used on our operation has evolved through a combination of imagination/necessity, the mother of all invention. Reading the seed and understanding what is in front of you is your key to unlocking a multitude of secrets. "Follow and Observe Nature and You'll find the Common Elements." Treat the upgrading task as a challenge and have fun with it.

REFERENCES

Downie, B. 1999. Upgrading seed quality of conifer seedlots: The how and glimpses of the why. Proc. of the 19th Annual meeting, Forest Nursery Association of British Columbia.

Asakawa, S. 1956. On the water absorption by *Pines koraiensis* seeds. J. Jap. For. Soc. 38:125129.

Baron, F j. 1978. Moisture and temperature in (elation to seed structure and germination of sugar pine *(Pious lambertiana* Dougl.). Am. J. Bot. 65:804-810.

Downie, B. 1999. Upgrading seed quality of conifer seedlots: The how and glimpses of the why. Proc. Of the 19th annual meeting, Forest Nursery Association of British Columbia.

Kozlowski, T.T., and Gentile, A.C. 1959. Influence of the seed coat on germination, water absorption, and oxygen uptake of eastern white pine seed. For. Sci. 5:389-395.