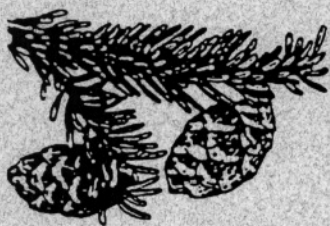

CANADIAN TREE IMPROVEMENT ASSOCIATION/
ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES



Tree Seed Working Group

NEWS BULLETIN

No. 31, May 2000

EDITORS NOTES

Here it is May 2000 already, and I am only now getting around to finishing off this NewsBulletin. The November Issue was conspicuous by its absence. While I did receive a couple of articles, there was not enough material, in my opinion, to publish a November issue. To those of you who have recently contributed material I want to give you my sincere thanks (as well as my apologies for those who submitted an article for the November issue). Several of our regular contributors once again came through: Bruce Downie (see the feature article in this issue), Dale Simpson, Dave Kolotelo, and Gaetan Daoust. Do these names seem familiar? They should!! How about the many anonymous members of our working group? We would like to hear from you!!

The first CTIA/ACAA of 'the new millenium?' will be held August 13 to 17 in Sault Ste Marie, Ontario. It has been three years since the last CTIA/ACAA meeting. This years' theme:

"Genetic Resource Management - Building Strategies for the new Millenium"

is one that will undoubtedly be informative, and promote great discussions. Dennis Joyce and crew have put together a dynamic slate of speakers - Please make a point to attend. See later in this NewsBulletin for more details and who to contact regarding the meeting.

There will also be a Pre-Conference meeting of the Tree Seed Working Group. The theme for the TSWG meeting is germane not only to that of the CTIA plenary session but to biodiversity and genetic resource conservation in general.

The theme for the workshop is:

"The Role of Ex-situ Germ Plasm Storage in Conservation"

There will be three speakers presenting different perspectives on germplasm storage:

- 1) **The role of the National Tree Seed Centre:**
Dale Simpson, Canadian Forest Service
- 2) **The role of the Provincial Tree Seed Centres:**
Dave Kolotelo, B.C. Ministry of Forests
- 3) **Long-term Storage of Recalcitrant Hardwoods:**
Henry Kock, University of Guelph Arboretum

Long-Term Pain for Short-term gain?

As we look around us, we seem to be inundated with new announcements of government cutbacks. While fiscal restraint is certainly a laudable goal, as I write this editorial I can't help but look at how this has impacted many in the forestry profession: seed science and tree improvement professionals are no exception. Recently, the Nova Scotia government placed the Tree Breeding Centre at Debert (and hence effectively the cooperative tree improvement program they manage) into a holding pattern. In addition to directly affecting our Working Group Chairman, Howard Frame (and of course his staff), there are some serious overtones. Once again, we see the mothballing of a long-term program. There are many historical examples from within Canada in which operational R & D is deemed to be 'a cost' to be minimized. Unfortunately, tree improvement often falls into this category. There is often the perception that when a program is up and running successfully, that all of the work associated with that program is 'routine' and self-perpetuating.

This is further compounded by the apparent bureaucratic myth that the continued success of these successful programs will not be compromised by 'short-term' breaks in program funding.

If such breaks are really short-term, then this may be true, but as many of us have seen, once cut, it is often very difficult to get a program rejuvenated. I think that it is critical for us to remind our political masters that long-term programs, if they are to be successful, **MUST** be funded appropriately (= consistently over the long-term and not punctuated with periods of being 'put on hold').

The Search for a New Working Group Chairman

We will be having a Working Group business meeting in conjunction with our workshop in August at which time we will be electing a New Chairman. Howard has had to tender his resignation due to his work commitments. Might it not be time to elect a Chair from 'Middle Canada'? Please contact Howard or myself if you would like to offer.

Once again, please make an effort to attend the CTIA/ACAA and our TSWG meeting and come prepared to participate.

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Comments, suggestions and contributions for the Newsletter are welcomed by the Chairman or editor.

Canadian Tree Improvement Association Meeting

August 14-17, 2000,
Sault Ste. Marie, Ontario

"Genetic Resource Management: Building Strategies for the New Millenium"

The following material, taken from the registration package provides an excellent description for this conference:

The millenium is a time for both reflection and speculation. In the tree improvement field there are many reasons for celebrating our progress across the country. The use of genetically improved stock continues to increase and many second generation breeding programs are underway. However, with the advent of international concerns and the resulting demands

for ecological sustainability of forest practices, the science of forest genetics is being asked to provide guidance in striking a balance between conservation and the use of genetic resources.

The conference will focus on the role that genetic resource management should strive for in achieving the goal of ecological sustainability of Canada's forests.

For more information please contact:

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IUFRO Seed Physiology and Technology Research Group

The following two tidbits were taken from the IUFRO Seed physiology and Technology Newsletter No. 53.

State-of-the-Knowledge Report

IUFRO President, Jeff Burley had issued a challenge to all IUFRO Divisions, Research Groups and other units to prepare a "State-of-the-Knowledge" report in time for the World Congress in 2000. George Edwards was pleased to report that the Seed Research Group rose to the challenge and has submitted a SKR report titled "Forest Tree Seeds at the end of the 20th Century".

In fact, George reported that RG 2.09.00 was the FIRST RG to submit a report.

[Ed note: I would encourage all TSWG readers to take a moment to visit the Research Group's home page at

<http://iufro.boku.ac.at/iufro/iufro.net/d2/hp20900.htm>

For more information on the research group, please contact the Chairman:

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National Tree Seed Centre

Centre staff were busy over the winter months processing seedlots that were donated by the provinces of Québec and Saskatchewan. These donations are invaluable in order for the Centre to expand the inventory of collections from within the natural ranges of species native to Canada.

Germination testing for a number of species is up to date and the results will allow us to evaluate germination trends over time in order to determine how frequent a species needs to be tested. Generally, as seedlots age, the germination decline increases requiring they be tested more frequently but for some species, such as black spruce (*Picea mariana*), germination remains very high for at least 25 years.

An undergraduate student completed his thesis evaluating the effect of several treatments on germination of white ash (*Fraxinus americana*) seed. Dormancy, commonly associated with ash, consists of two types: internal and external. Internal is caused by the embryo and external is attributed to the seed coat and pericarp.

Seedlots in storage for 22 years at 4°C and -20°C and 1 year at -20°C were evaluated for viability by excising embryos, placing them in germination boxes on Kimpak and putting the boxes in a germinator for 21 days. Four seedlots from each storage temperature with the highest viability were chosen. Seed was subjected to 4

treatments: control, pericarp removed, soak in 3 % hydrogen peroxide (H₂O₂) and pericarp removed plus soak in H₂O₂. One hundred seed of each seedlot and treatment was placed in a germination box. The boxes were placed at room temperature for 2 months followed by 5 months in a cooler then moved to a germinator for 2 months.

Twenty-two-year-old seed stored under frozen conditions germinated better than seed that was not frozen. Removal of the pericarp dramatically improved germination for seed stored at both temperatures (Table 1).

Comparing germination of 1- and 22-year-old seed stored at -20°C, the older seed had higher germination and again germination was improved by removal of the pericarp (Table 2). The evidence suggests that dormancy is caused by the pericarp which when removed results in a substantial improvement in germination.

Table 1. Germination of 22-year-old white ash seed stored at 2 temperatures and subjected to 4 treatments

Treatment	4°C	- 20°C
Pericarp intact	9%	27%
Pericarp removed	59%	75%
Pericarp plus H ₂ O ₂	18%	35%
No pericarp plus H ₂ O ₂	64%	78%

Table 2. Germination of 1- and 22-year-old seed stored at -20°C and subjected to 4 treatments

Treatment	1-yr-old	22-yr-old
Pericarp intact	21%	27%
Pericarp removed	62%	75%
Pericarp plus H ₂ O ₂	15%	35%
No pericarp plus H ₂ O ₂	66%	78%

Various trials have been conducted to evaluate the effect of varying stratification times on germination of beech (*Fagus grandifolia*) and striped maple (*Acer pennsylvanicum*). As well, storage experiments have been initiated for red oak (*Quercus rubra*), pussy willow (*Salix discolor*), Bebb willow (*S. bebbiana*) and red-topped willow (*S. eriocephala*). Some preliminary results will be given in a future "News Bulletin."

All indications are that there will be a medium to heavy fruit and seed crop this year. We will be busy making collections throughout the Maritimes.

Reference

Knox, D.P. 2000. A comparison of four treatments on germination of white ash (*Fraxinus americana*) seed. Unpubl. BscF thesis, Fac. For. & Env. Manage., Univ. New Brunswick, 28 p.

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Publication of interest to seed researchers

The **Journal of New Seeds** published by Haworth/Food Products Press, Binghamton, New York will produce a special double-issue on "Seed Policy & Law". This special issue will cover emerging aspects of intellectual property rights, seed import and export, agro-economics, legal bio-technology, legal aspects of genetic engineering, etc.

I invite the interested colleagues to contribute to this volume. I am also looking for a "Guest Editor" for this special issue. Expression of interest or recommendations are solicited.

Furthermore, research papers/reviews for the "Journal of New Seeds", and completed book manuscripts or book proposals related to seed production, biotechnology, marketing, testing and regulation are invited for consideration of publication by Haworth's Food Products Press, New York. For further information visit the website at: <http://www.haworthpressinc.com>

Thank you,

Amarjit S. Basra

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The Seed Identification Manual

by Alexander C. Martin and William D. Barkley

This title was first published by the University of California Press deals with the long-standing need for a reference work dealing exclusively with seed identification.

The immediate aim of the manual is to help agriculturists, foresters, wildlife biologists and others interested in land-use programs to identify the seeds in their particular fields of interest. The authors have, in the main, restricted the content of the description to those characteristics useful for identification. The descriptions are, to the extent possible, nontechnical and therefore useful to a broad range of interests and skills.

This book has become a classic and will shortly be, once-again, available.

ISBN 1-930665-03-2

For more information on this title please point your browser to:

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Textbook of Pollen Analysis 4th. edition

by Knut Fægri, Johs. Iversen, Peter Emil
Kaland and Knut Krzywinski
(ISBN 1-930665-01-6)

This book is a reprint of the fourth edition of the Textbook of Pollen Analysis and is unique in its approach as it discusses both the practical and

theoretical aspects of palynology. It uses palynological techniques as tools for solving problems in quaternary geology, ecology and archeology.

This edition of this standard reference has the same objectives as the earlier ones but the objectives have been widened, particularly the archaeological. There are over 130 illustrations and the identification keys have been thoroughly revised and are now illustrated. Here are a few excerpts describing this book:

"...will certainly benefit all in understanding the principles of pollen analysis. All students, palynologists and libraries should have it as a text book for reference." *Marine Geology*

"... Classic and much-used text book... will remain an indispensable book for those interested in paleoecology and practicing pollen analysis." *The New Phycologist*.

"unsurpassed in its restriction to basic principles, breadth of coverage, clarity of expression and emphasis on ecology." *Review of Paleobotany and Palynology*.

Wet vs. Dry Pollination in White Spruce

To improve our pollination technique for white spruce (*Picea glauca* [Moench] Voss), a study was initiated in 1992 to compare wet and dry pollen application techniques. The study was carried out in collaboration with Stéphan Mercier from the Direction de la recherche forestière, Ministère des Ressources naturelles du Québec.

White spruce containerized grafts were induced to flower during the previous summer according to the operational method in place (GA_{4/7}, root pruning and heat stress) at the Canadian Forest Service, Laurentian Forestry Centre in Quebec. In mid-December 1992, the grafts were brought into a heated greenhouse and placed under conditions to initiate growth. One month later, flowers reached the stage of receptivity.

The treatments used were: (1) wet pollination applied on bagged flowers; (2) dry pollination on bagged flowers; and (3) wet pollination on unbagged flowers. From one to three ramets for eighteen different genotypes were used. The experimental design was a complete randomized

block (clone) design with some missing combinations. In many cases, where we could install more than three bags on each graft, treatments 1 and 2 were repeated. A mixture of pollen (polycross) was used as well as specific pollens from different genotypes coming from our white spruce breeding population. Treatments were applied when almost all the flowers were receptive and were repeated two or three days

later. A domestic sprayer with a 100 ml bottle was used for the wet pollination. The pollen was mixed with spring water at a concentration of 2.5 % and the mixture was kept at 4°C before repetition of the treatment. For each application, 25 ml of solution was used. Dry pollination was applied with a 10 ml syringe and a rubber bulb at a rate of 1-2 ml per bag. A summary of the experiment is presented in Table 1.

Table 1. Wet vs. dry pollination of white spruce. Summary of number of genotypes (NG), number of crosses (NCr), average number of cones per cross (ANCoCr), average number of seeds per cross (ANSCr) and average number of seeds per cone (ANSCo) per treatment.

Pollination treatment	NG	NCr	ANCoCr (\pm se)	ANSCr (\pm se)	ANSCo (\pm se)
Wet-bagged	15	36	15 \pm 19	37 \pm 63	3.1 \pm 6.1
Dry-bagged	17	39	30 \pm 45	307 \pm 813	9.0 \pm 14.4
Wet-unbagged	14	18	27 \pm 44	5 \pm 16	0.1 \pm 0.6
All	18	93	24 \pm 37	144 \pm 543	5.0 \pm 11.2

The overall results for wet pollination were very disappointing when compared with dry pollination. One of our expectations, mainly based on the success obtained by breeders on *Pinus radiata*, was to obtain better results for this technique than dry pollination applied with a syringe. If we compare the treatments (wet vs. dry) on bagged cones, it is easy to identify which one was the best. Dry pollination produced three times more seeds per cone than wet pollination. However, the average of 9 seeds per cone for dry pollination applied inside represent roughly half the average obtained outside (16 seeds per cone) in the white spruce breeding program from 1988 to 1998.

The second expectation of the study was to use less pollen with the wet technique. This was confirmed during the study. Indeed, approximately one third of the pollen was used compared with dry pollination but this was not advantageous since we obtained only one third of the seed yield per cone (3.1 vs. 9.0). Webber (1991), for interior spruce, obtained seed yields from wet pollination (applied as an aerosol) that were less but comparable to dry pollination, an unexpected result considering the poor results obtained for Douglas-fir. However, considering the handling difficulties to handle and the inferior results in terms of seed yield, wet pollination was not recommended for interior spruce.

Microscopic observations, made by S. Mercier, revealed that pollen grains applied through wet pollination stick almost everywhere on the flowers (scales, bracts, axis) as with dry

pollination but they did not reach the micropyle, as we observed for the dry technique. On average, for wet and dry pollination, 1.1 and 13.7 pollen grains per micropyle were observed respectively. Wet pollination applied on un-bagged flowers did not give any interesting results (0.1 seed per cone) even if pollen was generously applied. On average, less than one pollen grain was observed on the micropyle.

Summary

In conclusion, dry pollination applied with a syringe remains the best technique and, as demonstrated by Webber (1991), direct application should be favoured (pollen propelled between the scales and down the central axis). The flower structure on spruce seems to be well adapted to wind pollination.

Literature Cited

Webber, J. E. 1991. Interior Spruce Pollen Management Manual. Land Management Report Number 70. British Columbia Ministry of Forests. 25 pp.

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Feature Article

Upgrading seed quality of conifer seed lots: The how and glimpses of the why:

By Bruce Downie

Abstract

Increased seed cost has led to a heightened desire to use single seed sowing to decrease the number of seeds utilized to put the next generation of trees in the ground. This necessitates that the seeds used in sowing have high germination capacity, and preferably be able to complete germination both quickly and uniformly. Several techniques are described that will eliminate seeds that, for various reasons, are not likely to complete germination or are of poor vigor. Additionally, techniques used to alleviate dormancy when present and/or enhance the speed of germination are presented. These techniques can be integrated into a protocol that minimizes the time required to complete them. Additionally, the conditions of one treatment can be altered to benefit the following treatment without compromising the biological effects of either treatment. Finally, some of the currently accepted reasons for why the various treatments result in improved seed germination percentage and/or speed are presented.

Introduction

With the advent of the desire for sustainable forestry in North America came a need for a reliable source of seeds of commercially important species. These seeds would be used to start the seedlings that would comprise the next generation of forest following harvest. Through plus-tree selection, seed orchards established to supply these seeds also held out the promise of superior wood production and quality, increasing the seeds' value. The care and maintenance of the orchard, fertigation, spraying with insecticide, mowing, subsoiling, pruning, progeny trials, and subsequent roguing, further increased seed value above that of stand produced seed. In some parts of North America, seed lots are comprised of seed from assemblages of clonal females and seedlings are raised from these seeds as a group. This practice has come about upon the realization that some plus-tree progeny typically establish more slowly than others and desirable

traits enhancing growth do not manifest themselves until later in the life of the saplings from these females. If planted with seeds from other clones in multi-seed sowing these individuals would suffer a competitive disadvantage leading to culling and the loss of the genotype from the population. Although segregation of seed lots by clone is a desirable practice, it does increase the value of the seed orchard seed still further. Obviously, it is in the best interest of the persons responsible for producing the seedlings from seed orchard seed to minimize the number of seeds used to produce a given number of seedlings. With the increasing emphasis on containerized seedling production in greenhouses the grower requires seeds that all germinate quickly and synchronously. Even with the best seed orchard produced seed, all seeds often do not complete germination, they do not complete germination immediately after sowing, and they do not all complete germination on the same day! Additionally, evidence exists that implicates culture practices in seed orchards for exacerbating some of the problems associated with germinating coniferous seeds (Barnett 1996). These include a greater propensity to incur mechanical damage due to larger seed size and possibly deeper dormancy. The results are; 1) containers with empty cavities where the seed placed in the cavity failed to complete germination; 2) a crop of seedlings that take a week longer in the heated greenhouse in April due to slow germination; 3) a crop of seedlings that will have a lot of oversized and undersized culls due to a drawn out germination process. How are such dire results to be avoided or at least mitigated?

The desire to mitigate the problems associated with growing coniferous stock (slow germination (Baron 1978), asynchronous germination (Mexal and Fisher 1987, Boyer et al. 1987), poor percentage germination (Grover 1962, El-Kassaby et al. 1992), immaturity at the time of cone collection (Caron et al. 1990, 1993) and susceptibility to mechanical damage (Huss 1950, 1956, Barnett 1996)) has prompted much empirical research resulting in many pre-sowing treatments designed to increase percentage, speed, and uniformity of germination (Simak 1976, Fleming and Lister 1984, Haridi 1985, Huang 1989, Huang and Zou 1989, Downie and Bergsten

1991, Downie et al. 1993) and alleviate dormancy when present (Edwards 1986). However, these treatments are usually prescribed without knowledge of the physiological events being altered to permit/accelerate germination, or the mechanism of alteration per se. There are two categories of activities that can ameliorate the problems of less than 100% germination, slow germination, and/or erratic germination. The first involves the identification and removal of those seeds that would probably not complete germination. The second involves bringing the remainder of the seeds in the seed lot to the brink of radicle protrusion without its culmination by treatments designed to enhance all aspects of physiological events beneficial to germination. Before embarking on descriptions of treatments utilized to remove unproductive seeds and to enhance the germination characteristics of others I emphasize that it is not sufficient to simply know how to perform these treatments but also why they work. Only by knowing what the treatments affect physiologically and genetically will we have information allowing insightful troubleshooting and the informed development of new and improved methods of enhancing the percentage germination, germination speed, and germination uniformity of conifer seeds.

The first three treatments described are designed to remove filled seeds from a seed lot that, due to death, damage, or disease, will not complete germination. These treatments are designed to follow routine seed lot enhancing treatments such as gravity separation to remove empty seeds. They include, PreVac for the removal of mechanically damaged seeds, incubation, desiccation, separation (IDS) in a floatation chamber for removal of dead and low vigor seeds from a seed lot, and flume or sedimentation IDS for the classification of a seed lot into various fractions based on seed vigor. Previously, these latter two techniques met with considerable opposition in North America based on the fear that seed lots treated in this manner would have reduced genetic diversity if particular genotypes were more subject to death during storage than others. To my knowledge, the validity of this concern has never been tested experimentally. However, if the decision to use a seed lot that has decreased in percentage germination has been made, then it is a moot point as to whether the discarded

fraction is comprised of a few genotypes or not. Obviously, since they are dead, the seeds eliminated by these techniques will not be contributing to the next generation whether removed or not. The treatments described following those used to remove seeds from the seed lot are designed to enhance the percentage, speed and/or uniformity of germination. These treatments are also designed to follow routine seed lot enhancing treatments.

Treatments to remove unproductive seeds from a seed lot

PreVac: PreVac is a treatment developed in Sweden to remove seeds that have mechanically damaged testas (Bergsten and Wiklund, 1987). Cracked testas were a common problem previously, accumulating during cone kilning in older kilns, and during mechanical seed dewinging. Barnett (1996) cautions that modern dewinging practices may yet result in mechanical damage if not conducted carefully and with proper regard to differences among species in the tenacity of wing retention and the propensity of the testa to incur damage. Additionally, Barnett (1996) states that seeds produced by seed orchards, due to their larger size, are more prone to mechanical damage.

In modern kilns the seeds released from cones drop through the rotating wire mesh cage immediately and are transported by conveyor belt outside the kiln environment. Hence, they are not subject to impact from tumbling cones or to the deleterious temperature inside the kiln. Modern wet dewinging methods also minimize seed mechanical damage. However, some seed lots may still be mechanically damaged each year (Barnett 1996). Mechanical damage can severely decrease seed germination depending on the species. It is particularly deleterious in *Abies* (Gunia and Simak 1968), but also in Norway spruce (*Picea abies*) (Huss 1950, 1956), white spruce (*Picea glauca*) (Downie and Bewley 1996), black spruce (*Picea mariana*) (Lantheaume and Trembley 1993), and Douglas fir (*Pseudotsuga menziesii*) (Allen 1958).

The PreVac technique consists of placing dry seeds of the suspect seed lot in water in a container and placing a screen over them so that they are held just under the surface of the water, but not on the bottom of the container. By repeatedly introducing a vacuum in the closed container, water is drawn in through any testa cracks and under the testa of mechanically damaged seeds in the seed lot. This water displaces air residing between the testa and nucellus, decreasing the buoyancy of the mechanically damaged seed until they sink to the bottom of the container. At this point the cover is removed from the container, the screen lifted and the undamaged seed skimmed from the surface of the water. The seed on the bottom of the container can be discarded. This technique depends upon the density of the dry seeds being low enough to allow them to float in water.

Imbibition, desiccation, separation (IDS):

Developed in Sweden by Simak (1973, 1984), the IDS technique has been a routine treatment in Sweden and Finland for Norway spruce, Scots pine (*Pinus sylvestris*), and lodgepole pine (*Pinus contorta*) seed destined for nurseries since the 1980's. It takes advantage of the fact that both dead and live seed imbibe water at the same rate, but live seed quickly increase their affinity for water while dead seed do not. The result is that, upon uniform desiccation in an air wash from below, live seed retain the water they have imbibed tenaciously, while dead seed release imbibed water relatively easily (Bergsten 1987, Downie and Bergsten 1991). Like PreVac, this results in a density difference between live and dead or nonvigorous seed such that they can be separated. Preferably, this separation can occur in water but, depending on the innate density of the seed in its dry and fully imbibed state, other liquids may be more beneficial. For example, a mixture of hexane and water or alcohol and water has been used on some *Abies* species seeds that were too light to sink in water even when fully imbibed.

Regardless of the liquid used for separation, the theory is that desiccation in the air wash continues until sub-samples of seeds taken from the wash have the same percentage of seeds floating as the percentage of seeds that do NOT complete germination in preliminary germination tests. At

this point the whole seed lot is introduced into a large separation column, stirred well to remove any bubbles from the testa which affects buoyancy, and the bottom fraction removed through an outlet in the bottom of the tank. The top fraction is then discarded. In tests with Scots pine, Norway spruce, white spruce, eastern white pine (*Pinus strobus*), lodgepole pine, and jack pine (*Pinus banksiana*) statistically significant improvements were obtained using water as the separation medium (Bergsten 1987, Bergsten 1988, Downie and Bergsten 1991, Downie and Wang 1992).

Despite the obvious advantages of being able to separate the live seeds from dead or weak seeds in a seed lot, IDS has not been widely adopted in North America. Part of the reason is that, even within a species, the efficacy of the technique varies considerably among seed lots. For some seed lots, the top fraction contained many seeds that were capable of completing germination. This may be due to innate variation in the rate of water imbibition among individual seeds (Tillman-Sutela 1997). However, many of these seeds have been shown to be of inferior vigor relative to those seeds comprising the bottom fraction (Downie and Wang 1992).

Variations on a theme-Flume or sedimentation

IDS: In order to separate seeds that have a density when dry greater than water or a density when wet less than water, various densities of liquids can be employed to perform traditional IDS fractionation. Often, however, the liquid mixtures necessary to obtain a suitable density for separation contain chemicals noxious to the seeds. Bergsten (1988) developed the IDS sedimentation desiccated seeds regardless of their density relative to water (Fig. 1).

The principle of IDS sedimentation is the same as that for IDS separation, seeds are first imbibed and then subjected to uniform drying in an air wash. At some point in the process, the whole seed lot is introduced into the seed hopper attached to the sedimentation tank (Seed inlet, Fig. 1). Water is run continuously through the tank using the continuous water inlet (Fig. 1) to maintain the height of water in the tank at the

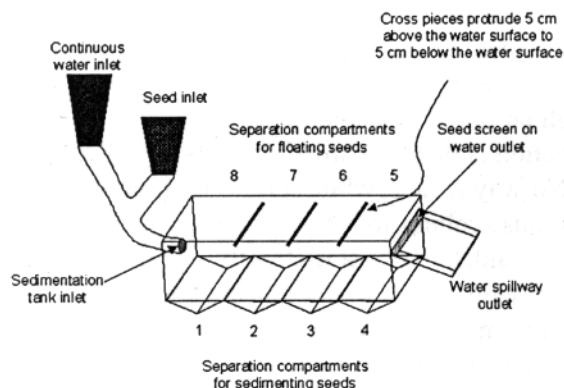


Figure 1. Basic design of the IDS sedimentation flume for use in separating conifer seeds into various classes based on buoyant density after imbibition and partial desiccation. 1-4) Seed hoppers used to fractionate seeds more dense than water into 4 classes. All hoppers have outlets permitting the removal of the seeds collected in them. 5-8) Surface compartments defined by the horizontal crosspieces that extend both above and below the water level in the flume as determined by the height of the water spillway outlet. Seeds with a density less than that of water are fractionated in these compartments.

desired level and to minimize disturbances in the water flow in the tank upon introducing the seed sample. The seeds are flushed into the tank at the same rate of water flow. The introduced seeds exit the sedimentation tank inlet beneath the water level in the tank. The seeds retaining the most water (theoretically the most vigorous) now do one of two things depending on whether the buoyant density of partially desiccated seeds is greater or less than that of water. Let us first examine their fate if their buoyancy is greater than water. The heaviest seeds will partition away from the incoming seed/water stream and sink into compartments 1 and/or 2. The next heaviest will sediment into compartment 3, the rest in compartment 4. Those seeds collected in compartment 4 are the least vigorous seeds of the lot. Alternatively, if the density of the seeds is less than that of water, the heaviest seeds will travel furthest in the inlet water flow and not surface until they reach section 5 on the surface. The fraction comprising seeds with slightly less retained water will surface in compartment 6, etc. with the poorest seeds of the lot partitioning to compartment 8. Naturally, it is possible with some species' seeds to have both seeds that sediment into compartments 1-4 and seeds that surface in compartments 5-8 resulting in fine separation of the seed lot into vigor classes.

Treatments to enhance the percentage, speed and/or uniformity of germination

Moist chilling: Alleviation of dormancy and enhanced speed of germination: Moist chilling alleviates dormancy in seeds that display this trait and speeds the completion of germination in seeds from both dormant and non-dormant seed lots. To date, there have been no instances in which the uniformity of germination has also been consistently enhanced by moist chilling. The germination curve is moved to the left by moist chilling but typically, is not compressed (Fig. 2). Moist chilling is the most widely applied treatment used to enhance the seed germination of conifers prior to sowing.

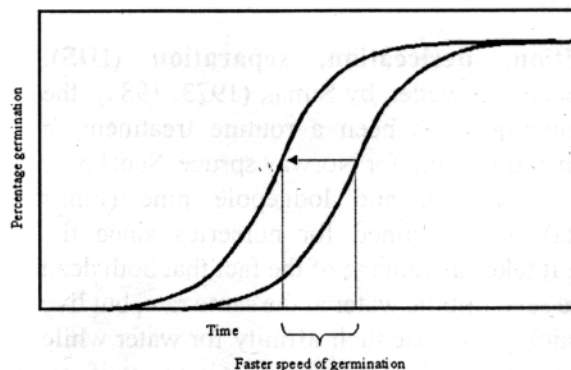


Figure 2. Moist chilling is effective in increasing the speed of germination of seeds from both dormant and non-dormant seed lots. However, the uniformity of germination is seldom, if ever, affected.

In a study of moist chilling techniques, there were no significant differences among treatments chilling white spruce seeds at controlled moisture contents above 20% moisture content fresh weight (MCFW) and routine nursery treatment. The nursery treatment examined was the so-called soak-and-go technique where seeds were first soaked in running tap water overnight prior to being placed in 4 mil thick plastic bags. Subsequently, the bags, with a headspace equal to the volume of the seed mass inside them, were sealed and placed at 5°C. The soak-and-go treatment resulted in a final seed MCFW of approximately 25%.

Striking results were obtained whenever seed MCFW was at or below 20%. In these seeds, dormancy was not alleviated during moist chilling, even after 12 weeks treatment at 5(C (Downie et al. 1998). It is therefore, imperative that seed being moist chilled have attained a MCFW of at least 25%.

Barnett (1996) states that orchard-grown seed is larger than stand grown seed. He then affirms that larger seed complete germination faster than smaller seed. This he suggests is due to a decrease in dormancy. However, speed of germination is not the true measure of dormancy, failure to complete germination at optimal temperature is. Barnett (1996) mentions that fewer large, seed orchard derived seed complete germination than stand grown seed. Tree spacing and nutrient supply to the mother tree did not affect seed dormancy (Caron et al. 1990, 1993) in managed white spruce (*Picea glauca* [Moench.] Voss.) stands. However, at least one nursery manager feels that seeds from plus trees of sugar pine (*Pinus lambertiana*) in seed orchards are more dormant than their wild type counterparts (Lori Lippitt, pers. Comm. 1997, Davis, CA) and has increased the duration of moist chilling to remedy this.

Greater dormancy in seed orchard produced seed may explain controversies arising in the literature between studies on the same species performed on seeds from open-pollinated trees from seed collection zones which had few dormant seeds or none at all, and those performed on seeds from cultured, orchard-grown trees which contained many dormant seeds (compare Heit 1958 with Chaisurisri et al. 1992). How seed orchard cultural practices affect the mechanism of seed dormancy is hitherto uninvestigated in the Pinaceae but is an important aspect of the increasing shift to orchard grown seeds to provide seedlings for reforestation efforts.

Membrane tube invigoration: Gore Tex membrane tube invigoration was developed by Bergsten (1987) and used to increase the speed of germination of Scots pine and Norway spruce seeds. It has subsequently been shown to be effective in increasing the speed of germination of eastern white pine (Downie and Bergsten 1991), white spruce,

black spruce, and jack pine (Downie et al. 1993). It is extremely versatile allowing seeds to be moist chilled at a controlled moisture content and subsequently invigorated at 15(C without removing them from the container. It is more effective in increasing the rate of the completion of germination than either moist chilling or priming on solutions of polyethylene glycol (Downie et al. 1993).

Invigoration takes place in a plexiglas tube with Gore-Tex end walls (Fig. 3). A screen separates the inner tube chamber from the Gore-Tex fabric to prevent the seed mass from blocking air exchange across the membrane. The seeds are introduced into the tube through a hole in the middle of the tube. The weight of water necessary to hydrate the seeds to the desired MCFW is calculated (Bergsten 1987, Downie and Bergsten 1991) and water is sprayed onto the seeds with a mister while the seeds are occasionally stirred around inside the tube. Upon attaining the desired weight of seeds and water, the hole is plugged with a rubber bung and the tube placed inside a glass tank on top of stands to keep the tube out of the water condensing on the bottom of the tank. An atomizing humidifier is used to maintain a constant fog of water vapor in the chamber. Condensing water exits through a drain in the bottom of the tank. The apparatus can be placed in 5(C for moist chilling, or 15(C for invigoration. For the first few days, the tube is surface dried and reweighed to maintain the correct weight of water and seeds to attain the desired MCFW. The seeds are also moved around inside the tube to ensure uniform water uptake. The single advantage Gore Tex membrane tube invigoration has over moist chilling or invigoration in 4 mil plastic bags is that gaseous exchange occurs uninhibited across the membrane without the loss or gain of moisture. Gaseous exchange in the plastic bag is limited to the headspace of the bag.

Partial redrying: This treatment has been extensively used to moist chill *Abies* species seeds (Edwards 1982, 1986, Leadem 1986). *Abies* seeds are especially prone to completion of germination at low temperature (Stein 1951; Franklin and Kreuger, 1968) if they have recourse to excess water. In this treatment, seeds are first

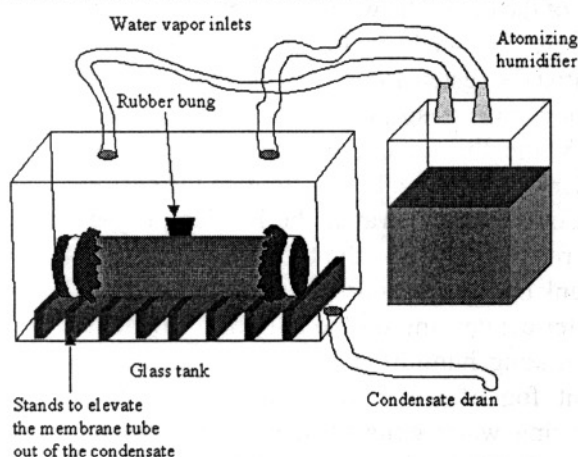
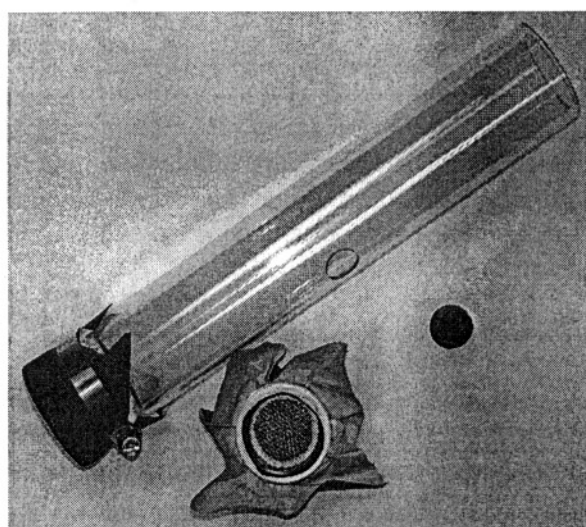


Figure 3. Membrane tube invigoration components. A) An invigoration tube with one of the Gore-Tex end walls removed to show the screen separating the tube body from the membrane. The rubber bung has been removed from the hole in the center of the tube through which the seeds are added or removed. B) Apparatus for membrane tube invigoration

imbibed to MCFW in excess of 30% and subsequently redried to 30% MCFW. The seeds are then placed in 4 mil thick plastic bags with a headspace, the bags tied off, and the whole placed at moist chilling temperatures 2-5(C. At 30% MCFW the seeds have sufficient water to alleviate dormancy but insufficient water to complete germination in treatment. Upon transfer to excess water or moist soil at higher temperatures radicle protrusion is faster and more seeds complete germination than if the seeds were untreated. This concept has been extended to permit the complete redrying and storage of moist chilled seeds

without the loss of the beneficial effects of moist chilling and/or invigoration (Bergsten 1987, Jones and Gosling 1990). This permits moist chilling to be scheduled during less hectic periods of the year and the seeds stored ready for shipment and/or planting during the peak sowing season.

Integration of processes designed to enhance conifer seed germination parameters

Below is a flow chart of the treatments discussed above and how they can be integrated into a process that routinely imparts to seed lots the ability to complete germination quickly and to the best of their biological ability (Fig. 4). Some of the treatments are optional, i.e. there is no need to treat mechanically sound seed by PreVac or seed germinating to a high percentage with IDS unless a fractionation based on vigor is required (Downie and Wang 1993). Others are beneficial regardless of whether the seed lot is comprised of seeds from a dormant seed lot or not, fast or slow germinating seed, or seed of high or low vigor (moist chilling, membrane tube invigoration).

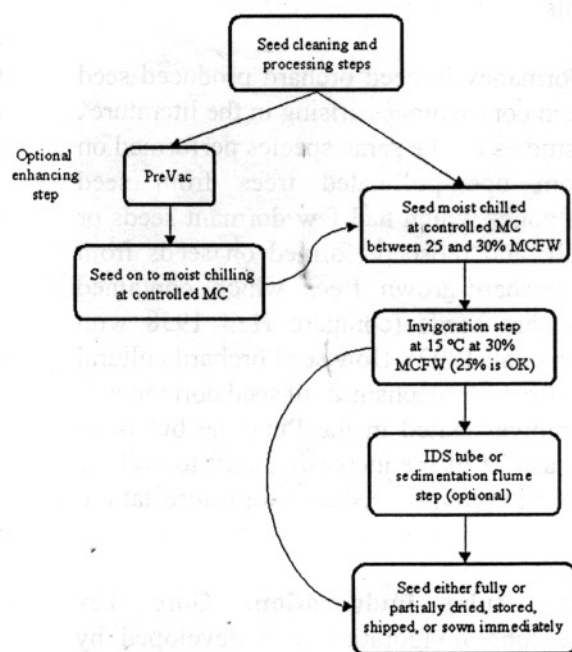


Figure 4. A flow chart of integrated treatments used to enhance the percentage and speed of germination of conifer seeds.

Moist chilling seeds in the presence of excess water has decreased considerably in practice, seeds are typically moist chilled at some controlled moisture content whether by design (invigoration, partial-redrying) or inadvertently (soak-and-go). The MCFW at which the seeds are moist chilled is above 20% but no more than 30% (Edwards 1982, 1986, Leadem 1986, Downie and Bergsten 1991). So long as the moisture content is above 20% then dormancy, if present, can be alleviated (Downie et al. 1998) and so long as it is 30% or below radicle protrusion cannot occur. If the range of MCFW used in moist chilling is between 25 and 30% it is also appropriate for the invigoration procedure conducted at 15(C. Although 30% MCFW seems to be close to optimal, 25% also allows the seeds to be invigorated, albeit, more slowly (Bergsten 1987, Downie and Bergsten 1991). Invigoration has been shown to enhance the benefits of moist chilling in all species tested to date (Downie and Bergsten 1991, Downie et al 1993). So, following moist chilling, the whole apparatus in Fig. 3 can be wheeled out of the cold room into 15(C, or the temperature in the room increased to 15(C, thereby linking moist chilling with an invigoration step. Invigoration in plastic bags has not been tested to my knowledge and may present an easy method of invigorating moist-chilled seeds by simply moving the bags from 2-5(C to 15(C if the limited availability of oxygen and buildup of carbon dioxide can be overcome. Membrane tube invigoration permits almost limitless free gaseous exchange across the membrane in an atmosphere of 100% relative humidity. This safeguards the seeds respiratory requirements at 15(C while ensuring they do not desiccate. However, seeds invigorated at 15(C enclosed in 4 mil plastic bags may suffer anoxia since the availability of oxygen and capacity to disperse carbon dioxide is limited by the available headspace in the bag. Whether limited gaseous exchange is a serious problem when invigorating in closed plastic bags or not remains to be determined. In Sweden, Bergsten (1987) concluded that the invigoration step could be linked to an IDS step if that was deemed necessary.

Glimpses of the WHY?

In practical situations, the important issue is THAT a treatment is available to enhance seed performance, be that increased percentage germination, faster speed of germination, or greater germination uniformity. WHY the treatment works is immaterial. However, to perpetuate the

improvement of seed treatments it is necessary to know what processes must occur in order for germination to proceed to completion both quickly and uniformly. Without this knowledge there will be no further advances in seed treatments to improve the percentage, speed, or uniformity of germination beyond what they are today. In addition to the benefits such knowledge brings to practical aspects of reforestation, understanding the various requirements for the completion of germination is important in and of itself. Below are some of the underlying causes of poor and slow germination in conifer seeds.

Treatments to remove unproductive seeds from a seed lot

The physical process by which mechanically damaged seeds are removed from seed lots is straightforward, unlike the processes involved in the other two techniques discussed. It is also apparent how physical damage to the testa can occur. However, what is not so apparent is why mechanically damaged testas usually decreases the percentage germination of a seed lot. In *Abies* species, apparently the copious amounts of resin in the testa contains compounds inhibitory to seed germination (Gunia and Simak 1968). But many other conifer seeds that do not contain large reserves of resin in the testa are also adversely affected by testa damage (Huss 1950, 1956, Downie and Bewley 1996, Allen 1958).

For white spruce, removing the testa at room temperature with tweezers resulted in a drastic decline in seed germination regardless of the care with which this process was performed (Downie and Bewley 1996). However, removal of the testa in liquid nitrogen resulted in better completion of germination than for seeds with intact testas. Presumably, even the small amount of resin present in the testas of white spruce is sufficient to inhibit germination. The nature of this inhibitor presumably present in the resin is completely unknown.

In the IDS technique, why is it that live, vigorous seeds retain the water they imbibe more tenaciously than dead or dying seeds? Understanding the mechanisms underlying greater water retention would permit imbibition treatments to be designed that would allow the maximum difference in density among vigorous

and dead and dying seeds to be attained. This would result in a better separation between live and dead seeds. Additionally, a marked variation in the speed with which individual Scots pine seeds imbibed water was responsible for a large number of live seeds being included in the surface fraction after IDS (Tillman-Sutela 1997). This is another reason for placing eventual IDS treatments at the end of integrated seed upgrading treatments thereby allowing all seeds sufficient time to imbibe water (Fig. 4). Unfortunately, little is known about how a live seed imbibes water or how it retains the water it does imbibe. The hydrolysis of large molecular weight stored reserves into smaller osmotically active molecules (Stone and Gifford 1997, King and Gifford 1997, Downie and Bewley in press) is one manner of making seed osmotic potential more negative, but this could be expected to occur in dead seeds as well since the cells autohydrolyse upon hydration. One difference would be that the cellular membranes in the live seed would be quickly reconstituted and create a minor barrier to water exit into the apoplast and eventually, out of the seed. The plasmamembrane of cells in the dead seed would be only poorly reconstituted into a discontinuous barrier to water movement. A detailed examination of why dead seeds release imbibed water more readily than live seeds would certainly benefit those interested in fine tuning the IDS techniques to permit more consistent separation of "the quick and the dead".

Treatments to enhance the percentage, speed and/or uniformity of germination

Moist chilling - Alleviation of dormancy and enhanced speed of germination: Pertaining to the genetic control of conifer seed dormancy and germination the only male influence is in the embryo, the megagametophyte being haploid and the surrounding structures diploid originating from the female parent (Edwards and El-Kassaby 1995). If completion of germination occurred solely due to the radicle forcing its way through the intervening tissues, one would expect a paternal contribution to germination rate and capacity (which includes dormancy). However, germination rate and capacity have been shown to be under strict maternal control (Bramlett et al. 1983, Caron et al. 1993, Sorensen and Cress 1994, Edwards and El-Kassaby 1995). Therefore, since no paternal control of germination

capacity or rate was found, it would seem that the radicle does not simply force its way through the intervening tissues but that they must first weaken and that these tissues play a crucial role in determining when and if the radicle protrudes. What are these intervening tissues? The tissues that the radicle must force its way through include, listed from the outside in, the testa, the nucellus, the megagametophyte, and, oddly enough, probably the embryo root cap itself (Downie et al. 1997a).

The amount of resistance to radicle protrusion that each of the structures exerts depicted as the percentage of the seeds in a dormant and non-dormant seed lot that are prevented from completing germination due to the testa, nucellus and megagametophyte, and embryo (presumably the root cap) reveal that dormancy in white spruce is imposed solely by the testa (Tables 1 and 2). The testa and/or nucellus has been implicated as responsible for imposing dormancy in almost every conifer species investigated (Stone 1957, Stearns and Olson 1958, Fowler 1959, Goo 1965, Barnett 1972, 1976, Taylor and Wareing 1979, Baron 1978, Hoff 1987). The inhibitory effect has been attributed to decreased water permeability (Baron 1978), decreased oxygen permeability (Koslowski and Gentile 1959), the presence of water-soluble germination inhibitors in the testa (Martinez-Honduvilla and Santos-Ruiz 1978, Gunia and Simak 1968), and the restriction of expansion of the megagametophyte and embryo (Asakawa 1956).

Table 1. Description of the germination of white spruce seedlots. Percentage germination without moist chilling percentage (w/o); Germination after moist chilling (w/s) and percentage germination of excised embryos on Potato Dextrose Agar (PDA) media (e/e).

Seedlot	Germination (%)		
	w/o	w/s	e/e
Dormant	47	71	98
Non-dormant	70	70	100

If the percentage germination of white spruce is examined as the different covers are removed, it is not only apparent that the testa imposes dormancy on the embryo, but that the nucellus

and megagametophyte also inhibit the embryo from protruding. The magnitude of inhibition by the megagametophyte and nucellus is equivalent to the inhibition of protrusion by the testa in dormant

Table 2. Contribution of different seed components to seed germination in white spruce.

Seed structure	Germination (%)	
	w/o	e/e
Testa	44% (51)	0% (30)
Nucellus and Megagametophyte	40% (51)	83% (30)
Embryo	16% (51)	17% (30)

NOTE: Values in brackets after the percentage contribution of the seed component is the difference in percentage germination between unchilled seeds and excised embryos on PDA. The percentage contribution of the seed component to the prevention of germination is for the difference between the unchilled seed and excised embryos. (Data reworked from Downie and Bewley 1996).

seeds (40 and 44% of the inhibition of radicle protrusion, respectively Table 1). Additionally, the nucellus and megagametophyte are the primary inhibitors of radicle protrusion in non-dormant seeds (87% of the inhibition). The nucellus and megagametophyte weaken during germination, prior to radicle protrusion (Downie and Bewley 1996). The force necessary to push through the nucellus and megagametophyte also declines considerably during moist chilling (Downie et al. 1997b). Since the testa splits at the suture prior to radicle protrusion the nucellus and megagametophyte are the only tissues opposing radicle protrusion. Either the nucellus, megagametophyte, or both weaken due to partial disassembly of the cell walls comprising them by the action of cell wall hydrolases (Downie et al. 1997b). What is important for practical purposes as well as exciting from a basic research perspective is that dormancy alleviation, probably by the weakening of the megagametophyte and nucellus, occurs only upon attaining or exceeding a discrete MCFW. This MCFW is between 20% where dormancy is not alleviated and 25% where it is. An analysis of moisture sorption/desorption isotherms in white and sitka spruce have shown this range of MCFW to define the transition from water binding region 3 and 4 (Downie et al. 1998). In

water binding region 4, metabolism can proceed uninhibited (Vertucci and Farrant 1995) and results in dormancy alleviation. In water binding region 3, regardless of the duration of exposure to 5 (C, dormancy was not alleviated! It is therefore, imperative that those practicing the soak-and-go method of moist chilling ensure that the seeds are imbibed at or above 25% MCFW.

How does the testa split allowing the radicle to protrude through it? One theory is that the testa suture is split by the embryo as it commences growth inside the seed. The conifer embryo is unique in the kingdom *Planta* in that the root cap of the embryo comprises between 25 and 40% of the total length of the embryo! Following the completion of germination, the cells of the root cap form a sheath of tissue enveloping the protruding radicle and some of these cells have been accreted onto the megagametophyte (Downie et al. 1997a). For this to happen, the radicle must grow through the root cap, forcing it to the sides of the megagametophyte like a wedge. This wedge exerts sufficient outward force on the megagametophyte, nucellus and ultimately, the testa, to split the testa along the suture from the inside, thereby liberating the radicle. The force generated is sufficient to accrete root cap cells onto the megagametophyte (Downie et al. 1997a).

A current model of conifer seed germination requires that the radicle grow into the ample embryo root cap forcing it to the side like a wedge into the megagametophyte and exerting outward pressure on the megagametophyte, nucellus, and testa. If the megagametophyte and/or nucellus have been weakened sufficiently by partial cell wall disassembly by cell wall hydrolases, the full force exerted by the root cap wedge is transferred to the testa, splitting it at the suture and eliminating it as a barrier to radicle protrusion. The radicle now pushes through the weakened megagametophyte and nucellus, completing germination. If the seed is dormant, the megagametophyte, nucellus, and possibly the root cap itself do not weaken sufficiently to permit the full force of the root cap wedge to be borne by the testa. The testa suture is strong enough to resist splitting if the megagametophyte and nucellus are bearing part of the force from the root cap wedge and the seed does not complete germination. Since the testa, nucellus and megagametophyte

are all maternal tissues this hypothesis is consistent with the observation that both germination rate and capacity are under strict maternal control (Bramlett et al. 1983, Caron et al. 1993, Sorensen and Cress 1994, Edwards and El-Kassaby 1995). Additionally, removal of the testa eliminates much of the opposition to radicle protrusion and enhances seed percentage germination. Therefore, treatments designed to enhance seed percentage germination, rate, and/or uniformity must facilitate the partial cell wall hydrolysis of the megagametophyte/nucellus to weaken them as barriers to radicle protrusion. Additionally, any treatment enhancing the wedge-like action of the root cap will result in splitting the testa sooner and in turn, faster radicle protrusion. The identification of the enzyme(s) responsible for weakening will permit directed intervention to enhance its/their action and enhanced conifer seed germination percentage, rate, and uniformity.

Summary

It is possible to remove filled-dead seeds that are mechanically damaged, dead or of low vigor from commercially valuable conifer seed lots. Additionally, techniques exist to alleviate seed dormancy when present, and enhance the speed of germination of conifer seeds. However, no treatment yet devised is capable of significantly and consistently enhancing the uniformity of germination. It is possible to integrate the techniques used to enhance seed germination parameters so that the conditions of one treatment are beneficial for use in the following treatment. This has resulted in a sophisticated seed germination enhancing protocol in Sweden and Finland. Still, far too little is known about how conifer seeds alleviate dormancy and how they complete germination. Without such basic knowledge, insightful engineering of future seed germination enhancing treatments is a matter of luck, not design.

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