



IMPROVEMENT

December 2001

Seed and Seedling Extension Topics

Diane Gertzen - Editor

Spring is here and I can almost hear the precision sowing machines humming away. This time of year is especially gratifying in sharp contrast to the huge changes experienced this past winter in the forest industry. For the Ministry of Forests, the words of the day are: core review, downsizing, redundancies, facility sell off and substantial budget reductions. Industry continues to be shaken by the softwood lumber dispute and its ramifications for long-term stability. It is hard not to dwell on these issues but our industry is resourceful and innovative so we will evolve to meet the new agenda. For the moment the energy crisis of last winter has abated and the result has been a leaner more energy conscious industry.

Closer to home, successful conifer seed workshops were; held in Prince George, Summerland and Surrey, BC. For the first time, we had a large contingent of field reforestation specialists along with seed processing and nursery personnel. This interest in seed culminated in the release of a new "Seed Handling Guidebook" (see page 26). Congratulations to Dave Kolotelo and all contributors for a very well received addition to our conifer seed knowledge. On the larger front, forest genetics workshops were held in Williams Lake and Kamloops, BC. This now makes a total of 5 regional workshops that have given forest practitioners a real sense of the direction and gains to be derived from tree improvement programs in BC.

This year is the joint meeting of the Forest Nursery Association of BC and the Western Forest & Conservation Nursery Association which will be held at the beginning of August in Olympia, Washington. Tom Landis is the chief organiser and has promised to personally accept Canadian money at par..., just kidding Tom. It will be a brilliant forum for technical information and just plain chat so do not miss this one.

And last but not least, Dr. Sven Svensen at Oregon State University has started two discussion e-mail groups. The first is on retractable-roof greenhouses and the other concerns our number one nursery plague, liverwort. If you would like to be a member of these discussion groups: please email him at: **Sven.E.Svenson@orst.edu**.

Ah, spring..., I can feel the liverwort growing.

David Trotter Guest Editor

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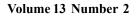
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GROWER'S NOTES

Summary of Summary of Samples sent to the Forest Health Clinic April 1, 2000 to March 31, 2001

Forest Health Clinic Summary

Overview:

The Forest Health Clinic (FHC) received 105 requests from various agencies relating to tree health and growth. Of these, 25 were from the British Columbia Ministry of Forests (MOF). There were 8 requests from foresters dealing with plantations and other tree establishment problems. Requests often contained more than one seedlot, container or type of seedling. In order for the levels of infection to be accurately determined a single sample often included a range of symptoms. Therefore the number of assays performed was much higher. Container grown seedlings represented over 65% of the requests. Sixteen percent of the requests were from field planted stock. Preventative assays represented 17% of the requests containing in excess of 65 samples composed mainly of seed or containers. Submissions of water and bareroot soil were also included in this category. These samples were assayed for potential pathogenic fungi and recommendations were made to nursery personnel on how to eliminate or minimize losses before they have a chance to occur. Over 10% of the samples were from Christmas tree growers who sent damaged plant tissues to the Christmas tree specialist at Timbers Reforestation Green Center for recommendations. These samples were forwarded to the FHC for identification of fungal species present and to determine if their role was causal or secondary. Douglasfir accounted for 34% of the samples by tree species with Abies and Picea species representing 17% and 16% respectively.

When age class was the criteria, 58% of the samples were 1+0 stock, 13% were over 2 years old and 12% were germinants. Over 35% of the samples had unspecified damage to the whole plant, damage to the roots represented 28% of the samples, while foliage and shoot damage combined represented 28%. Seed

represented 58% of the tissue type in preventative assays.

Fusarium diseases, either seed infection, damping-off, shoot blight or root rot, accounted for 40% of all samples sent. Grey mould (*Botrytis*) occurred on 18% and *Pythium* root rot on 15% of the samples. *Cylindrocarpon* was isolated in 11% of the samples.

The Clinic hosted two post-secondary institutions one from Malaspina the other from UBC, the focus was on demonstrating how the various assays provide information required for integrated pest management programs in the forest industry. Workshops on forest seedling disease recognition and control were given at Skimikin as part of the Simon Fraser University Masters of Pest Management program and at Surrey for the Forest Nursery Pesticide Applicators course.

Important occurrences

Fusarium continues to present a problem to growers. Used containers, foreign seed sources and in-house stratification were identified as contributing factors. A post stratification treatment with 3% hydrogen peroxide was shown to reduce *Fusarium* levels in one submission containing both pre and post treatment samples. A number of samples had Fusarium levels in excess of 10% of the seed after treating with hydrogen peroxide. However, no pretreatment samples were sent, so it was difficult to determine how effective the 3% hydrogen peroxide was. Clearly the variable results from post stratification treatment of seed with hydrogen peroxide needs to be examined and a more standardized approach adopted. Critical factors involved with the use of hydrogen peroxide include; age of stock solutions, the level of contamination present in a particular seedlot and the duration of the treatment.

Seeds, imported from outside Canada, were found to contain a number of insect pests. *Seed chalcids*





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(*Megastigmus sp.*), cone midges (*Contarinia sp.*) and seed worms (*Cydia* sp.) were identified in samples submitted for assessment. In addition over 25% of imported seedlots also tested positive for *Fusarium*.

The fungus *Volutella* sp. was found growing on spruce seedlings in such abundance that the lower foliage on the seedlings was lost. Species of *Volutella* are known to attack boxwood and Japanese spurge. Disease occurs when plants are grown in dense, crowded conditions and usually after physical or abiotic damage has predisposed the plants to infection. The mycelium on the spruce did not appear pathogenic but probably proliferated due to warm and moist conditions.

The fungus, *Cephalosporium*, was observed in several samples this year. In the past it has been isolated from roots and media on a routine basis. However, an isolate from one location produced the perfect stage *Ceratocystis*. Species of *Ceratocystis* have been associated with vascular wilts on many species of plants. This fungus should be monitored in the future in case new cultural or environmental conditions allow it to become pathogenic.

Abiotic stem lesions continued to occur in some species particularly, Douglas-fir. No pathogens were found despite several attempts to isolate from these lesions.

Environmental factors associated with rapid growth and fluctuations in vapor pressure appear to be the primary factors involved in this problem.

Forest seedling production is evolving on an annual basis; with improvements in media composition, containers, fertilization schedules and pest control. Changes in production methods can stimulate the occurrence of new problems. High energy costs have had an impact for a number of seedling producers. The Clinic received several samples in which germinants had developmental problems linked to lower greenhouse temperatures. The impact of changes in growing regimes can not be predicted but increased monitoring should be implemented to ensure early diagnosis of any problems that occur. *Botrytis* has become less prevalent primarily due to the reduction of seedling densities. Dead foliage in the lower canopy provides a substrate for the build up of inoculum and crops with damaged foliage should be monitored closely to avoid grey mould outbreaks.

Styroblock assays

Sanitation of growing containers remains one of the most important steps in disease prevention for forest seedling production. Clients should check containers for fungi to determine if pathogens are at a level that would require cleaning. They should also check on the efficacy of their sanitation methods by submitting treated containers for pathogen assays. This year 21 samples of both dirty and cleaned styroblocks were assayed for the presence of the water moulds Pythium and Phytophthora using the selective media PPA. Presence of these watermoulds occurred only on unsanitized blocks. This showed that current cleaning methods are effective for this group of organisms. A second selective media, Komada's, was used to assay for the presence of Fusarium, Cylindrocarpon and Phoma. Not all sanitation methods eliminated these pathogens. Most treatments reduced the numbers enough to be confident that seedling disease losses would not be significant. Controlled tests and the historical use of a sodium metabisulphite wash have proven to be the most effective procedure. Steam sterilization boxes are currently being used by some growers for cleaning the styroblocks and after proper calibration eliminated pathogens from all the samples tested.

Seed Assays

Seed can also be a source of pathogens whether borne inside the seed or present on the seed surface. Fungi often colonize seed during the stratification process and become visible as mould. This stimulated several requests for identification of the moulds present and their potential to cause damage to the germinant when sown. *Fusarium* was isolated from many of these stratified seed samples along with a number of saprophytic fungi that grew at the low stratification temperatures. Seedlot assays found several important seed-borne pathogens including the cold fungus *Geniculodendron pyriforme*.





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Water Assays

The use of a green pear as bait to determine if *Pythium* or *Phytophthora* were present in the water supply confirmed water as a source of these organisms. This helped several clients eliminate this source of pathogens by water treatment.

Germinant assays

Over 10% of this year's requests had disease development in new germinants. *Fusarium* either seed-borne or from containers was the major cause of germinant mortality. Actively growing vigorous seedlings appeared the most resistant to early attack from this fungus. Disease levels were increased because of the low greenhouse temperature regimes noted earlier.

Phoma also colonized numerous germinant samples but only after drought or heat stress predisposed seedlings grown in used, unsanitized styroblocks.

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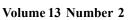
Seedling assays

General symptoms of tree decline expressed as needle die-back, mottled chlorosis, wilt or abnormal bud development can often be caused by root of shoot pathogens. To determine the actual cause of these symptoms foliar, root and growing media assays were required.

Roots that were obviously discolored and had the presence of decay stimulated the majority of assays for root pathogens. *Pythium, Fusarium* and *Cylindrocarpon* were responsible for the majority of root rot in container grown stock. Seedling assays at the time of extraction and after cold storage showed that late season root rot syndrome of Douglas-fir was present in a number of crops this year. Classic symptoms are small or empty buds, thick and often spongy lower stems and thick, dark roots. Once again, the most active fungal organism at this time was *Cylindrocarpon*. Crops grown for summer field planting, but held over winter for field planting the following spring had the highest incidence of this problem.

Gary Roke John Dennis Natural Resources Canada Canadian Forest Service Pacific Forestry Centre







Tabular Summary of Forest Health Clinic Reports For the period May 1, 2000 to April 9, 2001

Total requests BCMF requests

105 submissions 25 samples

Number of samples by type: Preventative assavs:

		assaysı	
Container -	112	Bareroot soil/Peat-	2
Compost-	1	Styroblocks-	21
Plantation -	17	Water -	5
Christmas tree -	12	Seed -	39

Number of samples by tree species:

Number of samples	by age:	Number of sample	es
ponderosa pine	2	yellow cedar	2
lodgepole pine	17	western white pine	4
loblolly pine	1	western red cedar	8
interior Douglas-fir	9	western larch	4
coastal Douglas-fir	41	western hemlock	5
black spruce	1	spruce (Sx)	15
alder	2	spruce spp.	8
Abies spp.	25	sequoia	3

		by damage	
0.5+0.5 -	12	whole plant -	56
1+0 -	78	foliage-	29
1+1-	5	roots-	41
1p+1-	7	shoots-	13
2+0-	5	seed-	6
3+	12	germ	16

Number of samples with the following diseases:

Botrytis	11	Phaeocryptopus	2
Cylindrocarpon	39	Phoma	25
Dermea	1	Phomopsis	1
Fusarium	89	Pythium	26
Geniculodendron	3	Septoria	1
Kethia	1	Sirococcus	1
Meria	1	Volutella	1
Milesina	2	Pestalotiopsis	3

Insects:

Adelges cooleyi	1	<i>Lygus</i> bug	1
Adelges picea	2	<i>Cydia</i> sp.	2
Megastigmus sp.	2	Contarinia sp.	2
Fungus gnat 3			

Samples sent in by Month:

April	8
May	7
June	10
July	16
August	6
September	19
October	3
November	20
December	5
January	5
February	6
March	8
April	2



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UPDATES

TREE

Revised 2001 BCMOF Sowing Guidelines

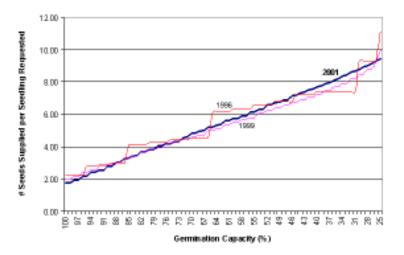
The BC Ministry of Forests sowing **guidelines** have been revised for this coming (2002) sowing season. The **guidelines** calculate grams of seed required to fulfill a sowing request of x number of seedlings based on the germination capacity (GC) and seeds per gram of an individual seedlot. The **guidelines** also calculate the potential seedlings for a quantity of seed and for a seedlot, presents the potential trees per gram. A full outline of the revised sowing **guidelines** can be found at:

http://www.for.gov.bc.ca/TIP/publications/updates/vol5no2.pdf

The previous (1999) sowing **guidelines**, including instructions on adjusting grams and fractional sowing can be found at:

http://www.for.gov.bc.ca/TIP/publications/updates/ vol3no4.pdf

The new sowing **guidelines** allocate less seed to seedlots with a GC > 88% and more seed to seedlots with a GC < 74% as illustrated below.



A variety of issues were considered in the development of the new sowing **guidelines**. It was decided that unique sowing **guidelines** would not be developed based on species, genetic class, genetic worth, growing environment or stock type. Client feedback indicated that seed allocation should be a function of seed quality (namely germination capacity. An important factor in formulating these guidelines was the large amount of variability found between nurseries in seed-use.

The first step to gains in seed efficiency is an understanding of how the sowing **guidelines** work. This will allow informed discussions (or negotiations) between the seed owner and nursery. In some situations large seed-use efficiency gains can be made (i.e. single-seed sowing of lodgepole pine), but not all nurseries will have the appropriate combination of geographic location, type of growing environment, stock type, equipment available, labour issues and individual nursery policy to meet every need. These new sowing **guidelines** are considered the best 'average', but efficiency gains can be made by going beyond average performance considering each nurseries unique situation.

> **Dave Kolotelo** Tree Improvement Branch Ministry of Forests



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TECH TALK

2001 White Pine Quality Assurance Monitoring

Large differences in germination capacity have been documented between dormancy breaking protocols (stratification) performed in operational seed preparation [OSP] and in the lab [LAB] over the last several sowing seasons. We try and minimize procedural differences, but some attributes such as seed volume cannot be made equivalent between the two areas. The current methodology for western white pine (Pw) includes a 14-day running water soak followed by 98 days of cold stratification (germination test type=G55).

A total of twenty western white pine (Pw) 2001 sowing requests (SRQ) had 25 grams added to allow for a direct comparison in germination capacity (GC) (effectiveness in breaking dormancy) between the OSP and LAB methods. This allowed for a direct comparison between OSP and LAB methods:

For the samples obtained from OSP requests moisture content will be estimated i) after draining, just prior to surface drying; ii) after surface drying; iii) after 7 weeks or the half-way point in stratification and iv) at end of stratification = 14 weeks (98 days). The LAB samples will also have similar moisture content estimates performed, but since surface drying does not occur, only i, iii and iv will occur for lab sampling. The results will quantify variability in moisture content after draining and determine whether lab moisture content in stratification is closer to moisture content before or after surface drying in OSP. All moisture content estimates were based on targetting procedures.

Results

Germination

Even with our efforts to mimic the LAB procedures in OSP the results indicate that our OSP techniques are inferior in breaking dormancy to lab procedures. The germination capacity (GC) of the OSP requests averaged 58%, while the LAB and previous SPAR¹ GC both averaged 91% based on 19 samples². The falldown in germination ranged from 6 % to 60% indicating that the individual seedlot may have a large impact on the way white pine responds to OSP techniques.

Extended Stratification

For three sowing requests, the nursery requested that we hold the seed for an additional two weeks of stratification. Sampling and germination testing were performed after 14 weeks stratification (pretreatment completion) and after 16 weeks stratification. The additional two weeks stratification resulted in a 5% average increase in GC (71 to 76%). An additional request was tested after 15 and 16 weeks stratification with a 3% gain in GC.

Following the testing of operational sowing requests cutting tests were performed on ungerminated seed. Most seed appeared to be viable exhibiting a firm, white megagametophyte and a mature apparently healthy embryo. Nurseries that performed cutting tests on white pine requests also confirmed the apparent viability of ungerminated seeds. These results indicate that stratification may not have been of a sufficient duration (or moisture content – *covered later*) to totally overcome dormancy.





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Stratification Unit Size

For white pine, sowing requests are divided into stratification units of 1000 grams or less (i.e. a 3200 g request is divided into 4 units of 800 grams each). All other species use a 3000 g minimum stratification unit size. It has been suggested that smaller 'units' may have higher moisture contents and this may increase germination in Pw. The coefficient of determination (r^2) between bag size and the moisture content i) after draining and ii) after surface drying both were 0.60. The moisture contents during stratification were not as well correlated with bag size [≈ 0.10 at 7 and 14 weeks] indicating that bag size has much less influence on stratification moisture content (at least after 7 weeks). The relationship between bag size and nursery germination falldown (Lab minus Nursery GC) was weak ($r^2 = 0.25$), but it did indicate that smaller bag sizes are experiencing smaller falldowns in GC (Figure 1

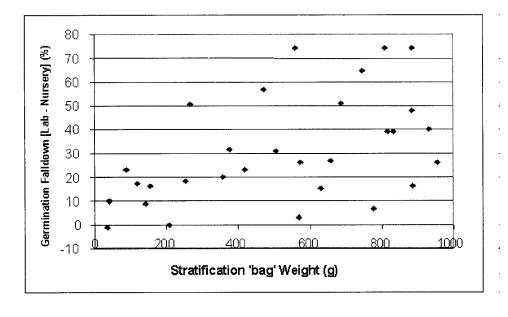


Figure 1. The relationship between sowing request bag size and germination falldown.

Moisture Content

For OSP the average moisture of the seed following a 14-day soak and drainage of excess moisture was 40.6%. After surface drying the moisture content was reduced to 35.5% indicating that surface drying removes 5.1% moisture from the seed. This was fairly consistent across seedlots and the r-squared value between drained and surface dry moisture content was 0.89. After surface drying the OSP samples have a moisture content 1.7% less than LAB samples.

The OSP samples appear to lose moisture during stratification in comparison to lab samples (Figure 2). This appears to be a significant difference in the two treatments : lab samples are maintained in stratification at a moisture content approximately 3.4% greater than in OSP! The lab samples appear to maintain fairly consistent moisture content from draining through to shipping (0.9% drop). The lab samples are not surface dried as the small samples (100 seeds) dry very quickly and could result in insufficient moisture within the seeds. The lab samples are also stratified in a closed unit that does not permit air exchange.





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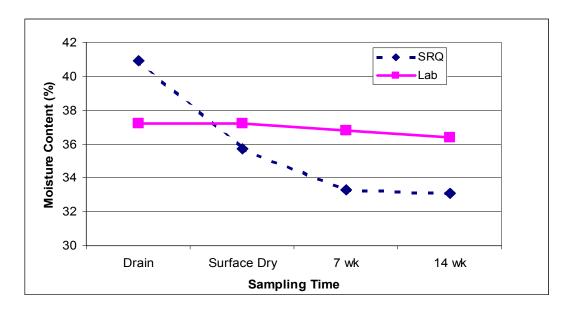


Figure 2. Comparison between moisture content after Drain, Surface Drying and 7 & 14 weeks of stratification for lab samples and operational sowing requests (SRQ) [n=19].

Discussion

The results clearly show that the conditions provided to pretreat LAB samples is not being replicated for OSP. The LAB prepared samples had an average germination 33% greater (91% vs. 58%) than that achieved with OSP. This difference is considered accurate as differences in sample time, location and methodology were virtually eliminated.

The most significant pretreatment difference is the size of the sample pretreated. In the Lab 100 seeds are used per replicate and up to a maximum of 1000 grams (approximately 50 000 seeds) are used in OSP. In the lab, seeds are not touching during stratification and there is a large air: seed ratio. The container is closed during stratification in the lab. In OSP adjacent seeds are touching (from all directions) within the one Kilogram seed mass and the air:seed ratio and the kimpack:seed ratio is reduced relative to the lab. To provide an equivalent air: seed ratio in OSP one would need an air volume of 60 to 75 litres within the stratification unit for one Kg of seed [totally impractical]. The most obvious physiological difference between the Lab and OSP is the moisture content of the seeds during stratification. The average OSP moisture content of 33.1% appears insufficient to efficiently overcome dormancy compared to the 36.5% level experienced in the Lab. This relatively high moisture requirement is considered to be critical and will be the focus for improvements in OSP. For other species (i.e. Pli and Sx) the optimal moisture content for stratification is 30%.

The strategy adopted has been to try and mimic the tray system used successfully in the lab. This may not be appropriate as a container to precisely mimic this environment is probably too large to be practical. The tray system is also problematic as it occupies a large area and makes it difficult to monitor and 'manipulate' seeds. The tray system in OSP has not provided improved germination in the two years in which it was implemented (2000 and 2001.



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The most frustrating part of working with Pw has been the failure of the lab-tested procedures to meet our expectations when conducted on operational quantities of seed (up to 1 Kg). It is possible that there is no one pretreatment that will produce equivalent results with small (100 seeds) and large (up to 500 000 seeds) quantities of seed! Feedback from some nurseries stratifying their own requests indicates that our problems are not unique, but some growers are quite successful at stratifying Pw. They emphasize that the seeds must be kept "moist" during stratification and that seed "manipulation" or the movement of seed within the stratification unit is required and is performed by some on a daily basis.

Recommendations

Several changes are being recommended for 2002 sowing of western white pine. The testing of recommendations is problematic due to the need to have operational quantities of seed available for trial purposes and the time required to obtain results with Pw pretreatments (approximately 5 months). These recommendations are directed specifically at OSP and no changes are being recommended for the lab testing of Pw.

- 1. Operational stratification of **Pw requests should revert to being performed in polyethylene bags**. Smaller sized stratification units (500 g) will be tested on a limited scale in 2002 to evaluate potential benefits.
- 2. The moisture content of Pw requests stratified in OSP should be increased to better correspond to the moisture content experienced in the lab. The increased moisture content can be accomplished by instituting the following:
 - a) Eliminate surface drying performed on Pw sowing requests.

- b) Monitor moisture content during stratification. All sowing requests stratified at the TSC should have fresh weights recorded following draining and after one month of stratification. This will allow for an adjustment in moisture content.
- c) I am recommending that the target moisture content range of Pw should be between 35 and 38% during stratification. Moisture content adjustment should occur if the moisture content is less than 34% or greater than 40%.
- 3. All **Pw sowing requests should be monitored and 'handling' during stratification every Monday, Wednesday and Friday**. This involves a visual inspection for fungal growth and moisture status (excessively wet or dry), and handling of the seed within the bag to break up any fungal colonies and redistribute moisture within the stratification unit.
- 4. The TSC should volunteer to extend stratification to our clients up to a maximum of 120 days. All OSP results indicate that increased stratification will increase GC and 120 days is the standard duration used in the US to stratify Pw. The large amount of viable seeds following standard stratification suggests that the duration may not be long enough. All nurseries growing Pw have agreed to this extension, but it may not always be possible due to the late entry of sowing requests.
- 5. To improve the reliability of germination estimates for Pw there should be at least two germination tests performed on a seedlot prior to use for sowing. This will be impossible for new seedlots, but these seedlots will be retested again prior to the next sowing season. A thorough review of retesting frequencies will occur in the winter of 2002 and will likely result in more frequent Pw testing





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- 6. All returned select white pine sowing requests should be tested on arrival at the Tree Seed Centre. This allows for an evaluation of seed quality to determine the fate of the seed (destroy *vs.* dryback, store and retest in 6 months). It can also provide valuable information on whether dormancy is reintroduced following drying and storage at -18°C.
- 7. Increase the extension and communication of issues and findings related to operational stratification of western white pine.

This is an abbreviated version of a full ten page report that can be obtained by contacting me directly. Please forward comments to Dave Kolotelo [Dave.Kolotelo@gems7.gov.bc.ca] or call me at (604) 541-1683 extension 228. Thank you!

> **Dave Kolotelo** Tree Improvement Branch Ministry of Forests

¹ SPAR – Seed Planning and Registry System – these GC test results indicate the previous test result on which sowing request sizes are based on (with seeds per gram).

² One sowing request was shipped to the nursery prior to testing and no SRQ germination or moisture content during stratification data was obtained.

Genetic Improvement and Somatic Embryogenesis

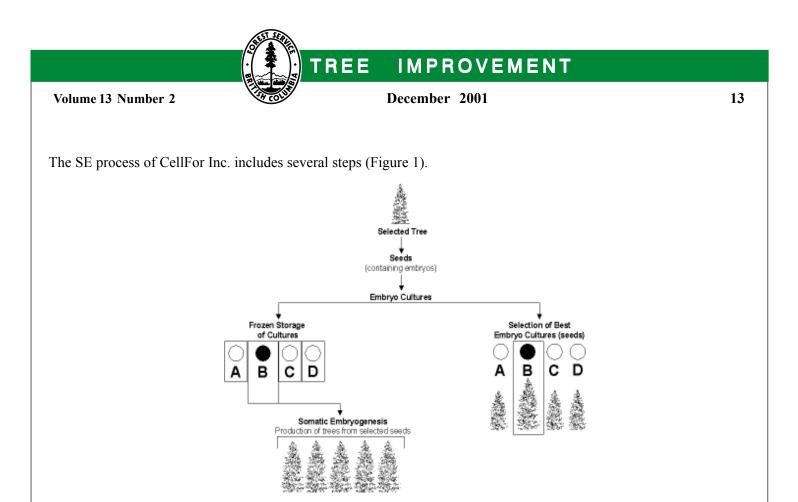
The ultimate goal of any tree improvement program is to maximize the genetic gain per unit time/area. Traditionally, seed orchards acted as the factory where the genetic gain is being packaged and delivered to nurseries for the production of genetically improved seedlings needed for reforestation programs. It is not a secret that, in most cases, wind-pollinated seed orchards have fallen short of expectations and several organization/countries adopted the path of making elite crosses followed by vegetative propagation for the production of material with high genetic gain. This approach made family forestry feasible, and the time and expense required for serial propagation and the rooted cutting production were certainly justified by the additional gain. It is also important to point out that in spite of the additional gain attained by family forestry, this gain represents only the average of the parents used in the elite crosses. Thus, foresters are deprived from exploiting the within family selection that is associated with sexual reproduction. In simple terms, within family variation could not be assessed or evaluated by family forestry due to the fact that the time required for within family comparison will affect the age and hence the juvenility of the donor plants. Here is where Somatic Embryogenesis (SE) comes to the picture because it provides a tool that overcomes the

limitations of both the classical delivery systems through seed orchards and family forestry and providing the most viable option for practicing clonal forestry.

The Technology

Vegetative propagation of conifers can be achieved through one of three approaches: macropropagation (e.g., rooted cuttings), micropropagation (e.g., organogenesis) gametic or somatic embryogenesis. and Macropropagation is widely used in a variety of species, such as spruces, radiata pine and yellow cedar but it is dependent on the availability of juvenile material. Micropropagation requires treating explants with growth regulators to induce bud or shoot formation, however, the ability to obtain a plant with both fully developed root and shoot has proven difficult for some species. Also, explants can only be obtained from young-juvenile materials such as embryos, cotyledons and hypocotyl segments. Thus, juvenility plays an important role. Somatic embryogenesis, a non-sexual developmental process that is capable of producing embryos from somatic tissues under in vitro conditions. The technique was independently reported in conifers by Chalupa (1985), Hakman et. al. (1985) and Nagmani and Bonga (1985).





These are induction, cryopreservation, liquid culture multiplication, somatic embryo maturation, desiccation, germination and transplanting of embryos to seedling containers for subsequent growth in the nursery. Most of the information pertaining to induction is published and available in the public domain (see the above-mentioned references). However, several of the following steps are proprietary and many of them are either patented or are in the process of being patented. These are described, as follows:

Induction: involves placing mature or immature embryos on a sterile culture medium and incubation to allow somatic tissues to develop. The tissue developed from each individual mature or immature embryo consists of a mass of immature embryos with one single genetic identity that is capable of continuous proliferation.

Cryopreservation: is the storage of developed somatic tissue produced during the induction in a frozen condition in liquid nitrogen. This step is important for maintaining the somatic tissue for a long period of time. It could be considered as a clone bank or gene conservation bank of unique genotypes, and provides tree breeders and foresters the time required to conduct clonal testing (clonal testing will be explained below).

Liquid culture multiplication: represents the true cloning step in the process (commonly known as bulking up). During this step, the induced tissues are exponentially multiplied. The multiplied tissues are undifferentiated and are used for the actual production of embryos.





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Somatic embryo maturation: treats and separates the undifferentiated tissues produced during the cloning step into well-differentiated embryos. CellFor uses a propri-

etary method using bioreactors to produce these mature, synchronized well-differentiated embryos in high numbers (tens of thousands to millions) (Figure 2).

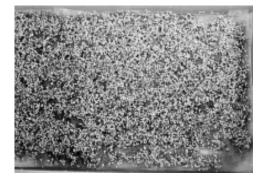


Figure 2. Thousands of synchronized somatic embryos

Desiccation: this step is unique to CellFor's proprietary technology. It mimics natural seed development and provides a technology that allows CellFor to produce embryos at any time of the year, without the restrictions imposed by the production of embryos with high moisture content that can not be stored. This makes it possible to produce millions of embryos that are ready for planting during the narrow biological windows of seedlings production.

Sowing and Germination: are similar in principle to the sowing germination of zygotic seed in a greenhouse

environment. However, there are special challenges because the somatic embryo lacks a megagametophyte (endosperm) and seed coat. Accordingly this step has been the subject of intensive research and development activities. Various research groups are considering different approaches such as the production of synthetic seeds (i.e., encapsulation) or the used off naked embryos. CellFor's proprietary technology uses the latter methods in which treated embryos are mechanically sown into mini-plugs (Figure 3) that can be transplanted into either container or bare-root nurseries for seedling production.

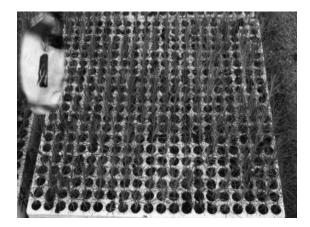


Figure 3. CellFor mini-plug product. Insert showing root development of indivicual mini plugs.





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Importance of clonal testing:

Genetic segregation and recombination among genes are fundamental to sexual reproduction. They represent the mechanism responsible for the genetic similarities and differences among individuals within a family. That is why brothers and sisters differ in their attributes. Thus, when controlled crosses are conducted among elite parents in a tree improvement program, the offspring are expected to exhibit variation, which geneticists call "within family variation". When clonal forestry is practiced, the cloning process acts as an amplifier. The amplification power can be harnessed and exploited to provide the foresters with the maximum genetic gain that can be attained. Thus, before large-scale production of a particular clone is commenced, it is recommended that clonal tests be conducted. When a cross is made between elite parents, several seeds are produced. The SE technology produces several clones (a clone is a representative of a single seed) for each cross (Figure 4). For example, if 50 clones were produced for one cross, few (50 to 100) copies of each clone should be produced and utilized in a clonal test to compare the performance of these clones. Results from the clonal testing phase will identify the desirable clones. These clones represent the ones that go through the amplification process and thousands or 100s of thousands copies are produced for reforestation. It is important to note that the number of clones within each cross are important factors to consider during the implementation of clonal forestry program, so the level and distribution of genetic variation within plantations are optimized.

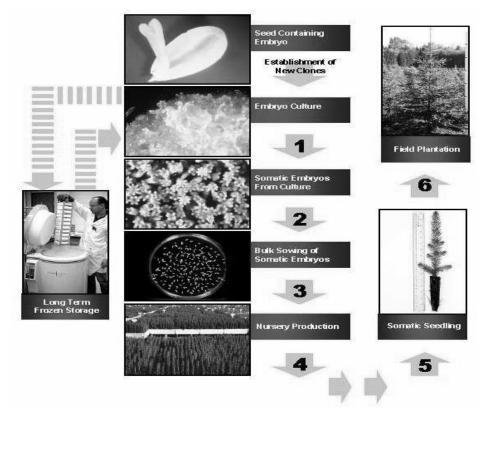


Figure 4. Schematic showing the concurrent clonal testing and clonal cryo-storage.





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Where and when SE should be implemented?

It is important to state that SE should be used to augment the classical tree improvement delivery systems and not to be viewed as a replacement. High genetic gain clones should be considered for high productivity sites, thus allowing the forester to get more wood from less land. Clones with high resistance level to pests can effectively be produced through SE as a specialty product. These specialty products can not be produced through sexual reproduction, or even rooted cuttings after sexual reproduction, due to the limitations explained above. Additionally, SE could be utilized in the testing phase of breeding programs in order to provide the breeder with progeny with high genetic uniformity.

Cost vs. value:

The common dogma is that the development costs of SE are high and thus its place in forestry is very limited. This view reflects the philosophical difference between the cost oriented forester and the value-oriented executive. Any new technology should be considered based on the value that it will generate. Economic analyses that consider the costs of SE vs. conventional forestry methods have proven that the value of SE far exceeds conventional methods when it is being applied to good

and high quality sites. Therefore, the objective way of evaluation is to consider the value that can be attained from SE net of the costs. It is not rational to focus on comparing the costs without calculating the value. SE allows foresters to get more wood from less land, this, of course, provides broader societal values when issues like conservation are also considered.

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Y**ousry A. El-Kassaby** CellFor Inc.





December 2001

Introduction

Commercial nurseries in BC that grow reforestation stock receive advertising from companies selling ectomycorrhizal inoculum. Since nurseries are always interested in improving the value of their stock while minimizing costs, there are often questions about the value of inoculation with ectomycorrhizal fungi. While it is indisputable that conifers form mycorrhizae in BC forests and benefit from these associations when nutrients and water are limiting, the evidence for benefits of inoculation with ectomycorrhizal fungi in the nursery is not so clear.

In 1996, we initiated a study of the inoculation of conifer seedlings with ectomycorrhizal inoculants with funding from Forest Renewal BC. There were two main parts of the study: to determine mycorrhizal status of standard commercial stock and the effects of inoculation in the nursery; and to determine the effects of nursery inoculation on subsequent field performance on cutblocks, roads and landings. We report here on the results of part one; part two is in the ground and will be monitored for 5 years.

Hunt (1991, 1992), Chapman (1992), Roth and Berch (1992), and Dangerfield and Dennis (1978) have examined the mycorrhizal status and results of inoculation of select nursery stock in BC, but there has never been a systematic assessment of stock from a large number of BC nurseries. Over 3 years, 22 different BC commercial nurseries participated in this study, supplying stock to us for assessment of mycorrhizal status, and two companies supplied us with commercially available ectomycorrhizal inoculum.

Materials and Methods

Commercial nursery stock mycorrhizal status

For 3 years, commercial nurseries in BC donated uninoculated Douglas-fir, lodgepole pine, and interior spruce stock for the assessment of mycorrhizal status. All of the stock had been grown in styroblocks using standard nursery practices. For each species, each nursery sent us 10 - 20 seedlings.Mycorrhizal status was assessed after roots were cleared and stained since this is the only practical means by which minimally developed mycorrhizae can be detected (Roth and Berch 1992). Determination of percent mycorrhizal colonization was based on the gridline intercept method (Giovanetti and Mosse 1980).

Inoculation with commercially available ectomycorrhizal inoculants

Seeds of interior Douglas-fir, interior lodgepole pine, and interior spruce were stratified in January and February at the Tree Seed Centre, Tree Improvement Branch, and sown at Extension Services, Surrey, in April. After germination, fertilizer was applied following standard nursery procedure.

At approximately 8 weeks of age, Douglas-fir inoculated with mycelial slurry of Laccaria laccata and interior spruce and lodgepole pine with Hebeloma longicaudatum supplied by Mikro-tek following procedures described and demonstrated by Mikro-tek in Timmins, ON. The mycelial slurry was fragmented using a blender, diluted in tap water, and applied to seedlings in styroblocks using a watering can. At the same time, Douglas-fir was inoculated with Rhizopogon parksii, interior spruce with Rhizopogon subcaerulescens, and lodgepole pine with Rhizopogon rubescens. The Rhizopogon inoculum was supplied by Mycorrhizal Applications, Grants Pass OR. In 1999, we also inoculated Douglas-fir and lodgepole pine with Rhizopogon parksii collected near Williams Lake BC by Bill Chapman, Cariboo Forest Region.

Plants were lifted in November. At lifting, 40 seedlings from each species and treatment were selected systematically with equal number of samples taken from each styroblock; no samples were taken from the edge rows. They were placed in cold storage until examined and mycorrhizal status assessed using the method described above.



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Results and Discussion Standard Nursery Stock

In all 3 years, lodgepole pine (Pl) seedlings showed the highest spontaneous mycorrhizal colonization rates in commercial nurseries (Table 1), followed by hybrid spruce (Sx), and finally interior Douglas-fir (Fd). In all 3 years, Fd was virtually nonmycorrhizal at time of lifting regardless of which nursery raised it.

The lack of colonization in Douglas-fir is of real interest since there are many anecdotal reports of poor early performance of Douglas-fir seedlings on hot, dry interior sites. Any improvements in root and mycorrhiza development for Douglas-fir in the nursery that translated into improved early growth in the field would be of real value because improved early growth could directly affect time to free-growing and green-up.

Table 1.	Mycorrhizal colonization of uninoculated conifer seedlings raised in container
	nurseries in 1997 – 1999 in British Columbia.

	Sx		Pl		Fd	
Year	Mean	Range	Mean	Range	Mean	Range
1997	45	4 - 83	59	12 - 93	9	0 - 30
1998	64	22 - 89	68	13 - 96	2	0 - 8
1999	42	15 - 71	75	41 - 85	1	0 - 8

For all 3 conifer species, the range of mean colonization for individual nurseries varied tremendously. For instance, in 1997 the mean colonization for Sx ranged from 4 - 83% which means that Sx from one of the nurseries had only 4% of the fine roots tips colonized by mycorrhizal fungi and another had 83%. This variation can be explained by many factors from genetic differences (seedlot) to cultural differences.

For this part of the study, we conclude that lodgepole pine container stock in BC nurseries is highly mycorrhizal without inoculation and that interior spruce is just slightly less so. This does not mean that the mycorrhizal fungi occupying the roots will give the seedling an advantage when outplanted because indigenous fungi often replace nursery fungi. But, given the occupation of roots by ectomycorrhizal fungi, such as *Thelephora terrestris*, that are adapted to nursery conditions, it may be difficult for inoculated fungi to compete. The situation is different for Douglas-fir because this species is so poorly colonized by nursery fungi that there is the potential for an inoculated fungus to succeed.

Inoculated Nursery Stock

Response of mycorrhizal status to inoculation in the nursery at Extension Services varied tremendously from year to year (Table 2). In 1998, inoculation of lodgepole pine with *Hebeloma longicaudum* from Microtek did not improve colonization. In 1999, inoculation with *H. longicaudum* or *Rhizopogon parksii* from Oregon or BC improved colonization. In 2000, seedlings inoculated with *Rhizopogon rubescens* had the same level of colonization as the uninoculated controls but seedlings inoculated with *H. longicaudum* had reduced colonization compared to the others.

For some reason, in 2000, virtually all inoculated seedlings of all 3 conifer species had lower colonization than the uninoculated control seedlings. The results from 1998 and 1999 had encouraged us to think that colonization of Douglas-fir could be improved with





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inoculation. However, in the 2000 inoculation neither ectomycorrhizal fungus improved colonization. This variability of response to inoculation from year to year is a problem for anyone thinking about buying inoculum.

From this component of the study, we conclude that mycorrhizal inoculation in the container nursery with the inoculants tested does not reliably improve percent ectomycorrhizal colonization of Douglas-fir, lodgepole pine or interior spruce. We don't yet know whether inoculation improves field performance, despite the lack of difference in colonization, because our field trials are still being monitored. We also conclude that there is real potential for inoculation of Douglas-fir if procedures, such as altering the form or rate of application of fertilizers, can be standardized to the point that an improvement in colonization can be assured.

Table 2.Mycorrhizal status of conifer seedlings raised in container nursery at Extension Services and inoculated
with two commercial and one experimental ectomycorrhizal fungus inoculant

× 8		<	Mycorrhizal A	Applications	8	Mikrotek		Experimental
Year	Conifer species	Uninoculated control	Rhizopogon parksii	Rhizopogon rubescens	Rhizopogon subcaerulescens	Hebeloma Iongicaudum	Laccaria laccata	Rhizopogon parksii
1998	PI	92 a	-	-	-	93 a	-	-
8	Sx	61 a	-	-	-	75 a	-	-
2	Fd	Ob	-	-	-	-	7 a	-
1999	PI	76 b	97 a	-	-	98 a	-	97 a
	Fd	1 b	38 a	-	-	-	38 a	33 a
2000	PI	88 a	-	90 a	4	75 b	2	-
	Sx	61 a	<u>_</u>	-	47 b	51 b	5	-
	Fd	5a	16	-	4	-	Ob	1

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Comparison of Western Hemlock A-class and B-class Seedlots in a Nursery

Introduction

Are genetically improved seedlots of western hemlock different from wild-stand seedlots in the nursery? We grew seven 'A'-lots, fifteen 'B'-lots, and three elite fullsibling family lots together in one greenhouse, with both single- and double-sowing, to find out.

Objectives

The objectives were to

- quantify the amount of morphological variation within several western hemlock seedlots throughout one growing season,
- examine the effects of thinning on morphological variability,
- compare fall frost hardiness among A-class and B-class seedlings.

Methods

Twenty-five seedlots, comprising 15 B-class, seven Aclass, and three full-sibling elite lots, were sown in February 2000 at the Ministry of Forests Nursery Extension Services nursery in Surrey. The 'B' lots were selected to represent a range of latitude and elevation. The 'A' lots represented a range of genetic worth and came from three different seed orchards. One half of each seedlot was single-sown, while the other half was double-sown and thinned. Three half-styroblocks were sown for each seedlot and thinning treatment combination. Thinning was done using the operational procedure of removing either the smallest germinant or the one closest to the edge of the cavity.

Height of a random sample of seedlings from each seedlot and sowing treatment was measured monthly

throughout the growing season. Dormancy was induced with drought stress – no black-out was applied. While the effect of black-out on 'A' lots and 'B' lots is of interest, it was decided that at first it would be better to focus on the more natural growth patterns before adding in an artificial black-out treatment.

Frost hardiness was measured in mid-November, using chlorophyll fluorescence, at the Ministry of Forests Glyn Road Research Station in Victoria. Ten samples per seedlot were each tested at temperatures of +5C (control), -10C, -15C, and -20C.

Final measurements of height, caliper, root and shoot dry weights, and a visual assessment of frost damage were done in late November around the time of lifting.

Results and Discussion

a) Germination

Nursery germination percentages were similar to lab results from the Tree Seed Centre. Germination rate was not calculated in this trial. The Seed Centre found no differences in germination capacity or germination rate for 'A' and 'B' class western hemlock seedlots when all collections over the last 11–13 years were averaged (Kolotelo, 2000).

b) Growth curve

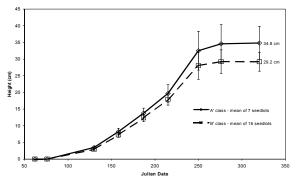
The 'A' lots were taller than the 'B' lots early in the growing season, with the difference increasing throughout the year (Figure 1). Of the full-sib lots, one (687x238) germinated more slowly than the others and was significantly shorter than the other two at the end of the season (data not shown).

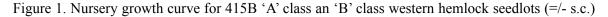


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Figure 1. Nursery growth curve for 415B 'A' class and 'B' class western hemlock seedlots (+/- s.e.)

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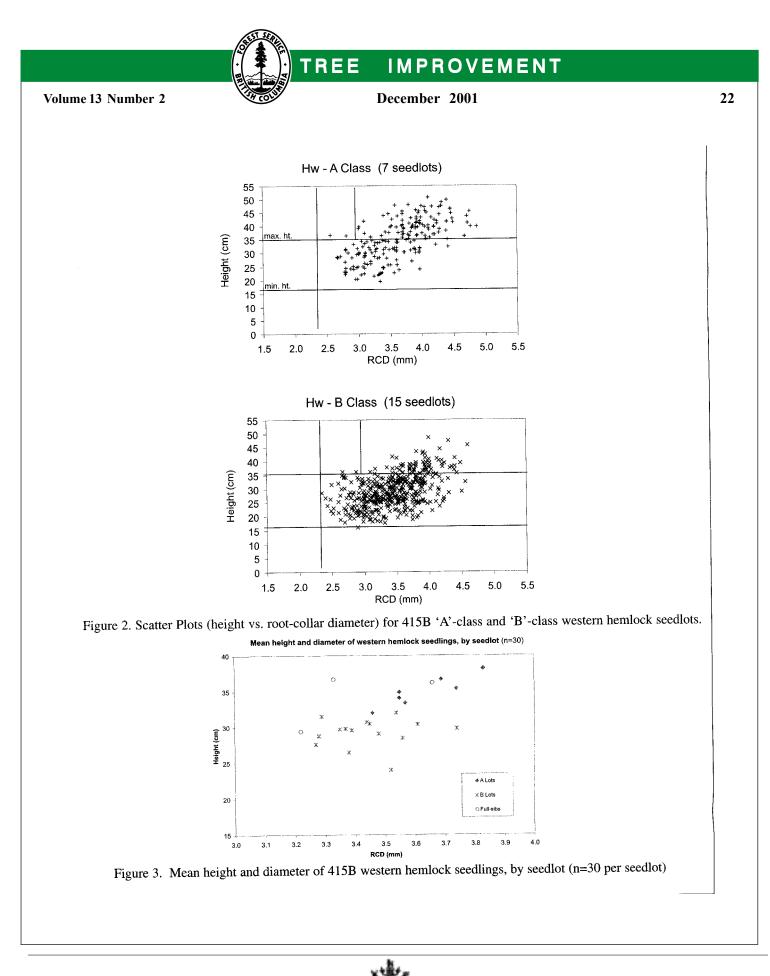


It does not appear that the 'A' lots grew longer into the fall than the 'B' lots, at least in terms of crop averages. Because we measured a random sample of seedlings rather than the same seedlings each time, however, we can not be sure if some individual trees did not carry on growing longer.

c) End-of-season height and caliper

At the end of the growing season, the 'A' lots were taller and had larger caliper than the 'B' lots. As can be seen from the scatter plots (Figure 2), the 'A' seedlots, without the benefit of blackout, could be considered overly tall, although the MoF target caliper for overheight seedlings was usually attained. The seedlot averages for height and caliper are shown in Figure 3. From here it can be seen that there is virtually no height overlap between the two types of seedlots – the shortest 'A' lot is about the same height as the tallest 'B' lot.





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Other morphological parameters such as branch number and length were investigated in a preliminary sample, but as it did not appear that there were any differences between genetic types for these parameters, they were not included in the final assessment.

d) End-of-season root and shoot mass

At the end of the growing season, the 'B' lots had significantly higher root:shoot ratio than the 'A' lots (Figure 4). The 'A' lots had a greater shoot weight, without having a greater root weight (the 'B' lots actually had a greater root weight than the 'A' lots, but this difference was not statistically significant). The 'B' lots *did* have significantly greater root weight than the elite full-sibs. The seedlot means for shoot and root dry weight are plotted in Figure 5. From this it can be seen that three of the 'B' lots have much greater root weight than the rest. If these outliers were removed, there would be no difference in root weight between 'A' lots and 'B' lots.

e) Within-seedlot variability

The percent coefficient of variation (CV) is a measure of variability that is adjusted for differences in means. There was no significant difference in CV between 'A' lots and 'B' lots, for any of the measured parameters. In other words, even though the 'A' lots were taller, they did not have more within-seedlot variability.

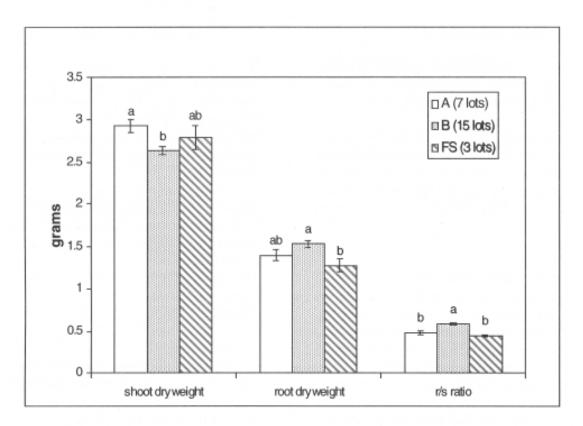
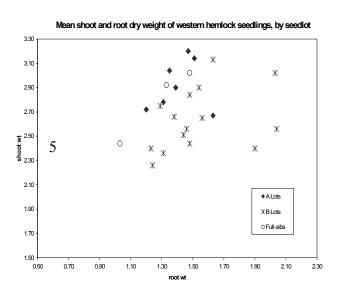


Figure 4. Mean dry weights of 415B western hemlock seedlings (+/- s.e.)



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The full-sibling families displayed a higher CV than either 'A' or 'B' lots in one parameter, root collar diameter, otherwise there were no differences.

Interior spruce is the only BC conifer for which differences in seedlot variability in the nursery have been studied. In that case, the variability of A-class seedlots in the nursery was high but within the range of variability found in B-class seedlots (Hawkins and Krasowski, 1993).

f) Effects of thinning

One reason for the perception that seed orchard seed is more variable in the nursery may be that it is more often single-sown, and therefore not subject to thinning. Thinning is a process that presumably removes smaller and weaker specimens and thus results in a more uniform crop (E. van Steenis, MoF Nursery Extension Services, pers. comm.). Silva-Gro Nursery in Quesnel experienced increased variability and subsequent crop management and grading costs when switching from multiple to single sowing in high germination capacity spruce seedlots (Norm Livingstone, pers. Comm.) In this trial, there was no difference in final height mean, or in height variability, between single-sown and doublesown treatments (Table 1). The difference in root-collar diameter is very small, yet it is statistically significant at the .05 level. It is conceivable that the stems of the double-sown seedlings were disturbed during thinning process.

Table 1. End-of-season morphological measurements, with coefficients of variation, for single-sown and double-sown western hemlock seedlings, 25 seedlots combined.

Sow Type	Ht (cm)	CV	Rcd (mm)	CV
Single	31.3	22.1	3.5	13.2
Double	31.5	23.0	3.4	14.2

g) Frost hardiness

In a field study on Vancouver Island, high-gain western hemlock families had lower spring frost hardiness than wild-stand seedlots, although fall frost hardiness was not affected (Hannerz et al., 1999).

In this trial, chlorophyll fluorescence testing was done at the Ministry of Forests Glyn Road Research Station, because of their ability to test a large number of samples at a number of different temperatures. Unfortunately, the seedlings were hit by frost in October, before the



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testing took place. While only seedlings with no visible damage were selected for the test, the quantum yields of the controls were mostly below 65, indicating that there was already some tissue damage prior to testing.

However, significant results were still obtained from the trial. Quantum yields decreased with decreasing temperature treatment. By analyzing the difference in quantum yield between each test temperature and the control temperature, rather than the quantum yield value itself, we were able to get a measure of the tissue damage done at each test temperature.

There were no significant differences at $-10 \,^{\circ}$ C or $-15 \,^{\circ}$ C, but at $-20 \,^{\circ}$ C the 'A' lots had a significantly greater decrease in quantum yield than either the 'B' lots or the full-sibs. In other words, 'A' lots were less hardy than the 'B' lots at $-20 \,^{\circ}$ C.

As well as the above test, a visible frost damage assessment was done at lift. A total of 750 seedlings from all three reps were scored simply as either having frost damage or not. Using chi-square analysis, there were no significant differences among genetic types in the frequency of frost damage. Within rep one, however, which had the most frost damage, the 'A'-lots were more frequently damaged. Because it is only one rep, and bench position could be a factor, this cannot be considered significant.

Conclusions

Without blackout, orchard seedlots of western hemlock are taller, have greater shoot weight, and a lower root/ shoot ratio than wild stand seedlots. With experience, nurseries may be able to control these crop parameters quite satisfactorily using appropriate blackout treatments. It is perhaps unfortunate, however, that the nurseries are left to their own devices to learn how to grow 'A'-class seedlots, which, after all, are required by law to be used whenever possible. With the goal of the Forest Genetics Council of BC to increase the use of select seed to 75% of total annual provincial sowing requests by year 2007, is it time to consider having systematic testing and publication of seedlot growth characteristics?

Acknowledgements

Staff at the MoF Nursery Extension Services in Surrey did a great job of growing the seedlings for this trial. Sylvia L'Hirondelle and Wolfgang Binder provided their lab and their valuable expertise for the frost hardiness testing. Eric van Steenis and Rob Scagel provided advice throughout the trial.

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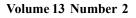
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Bevin Wigmore

Charlie Cartwright Research Branch Ministry of Forests







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PUBLICATIONS

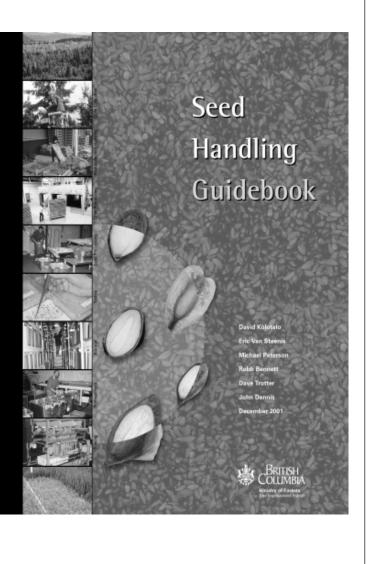
Seed Handling Guidebook

The Seed Handling Guidebook has been completed. The authors of the Seed Handling Guidebook are: David Kolotelo, Eric Van Steenis, Michael Peterson, Robb Bennett, Dave Trotter, and John Dennis.

The guidebook is 106 pages in length with 101 colour figures and 14 tables. It covers the introductory topics of seed condition, cone and seed insects and seed fungi and then fully covers the seed handling system. This spans all activities from cone collection to the sowing of seed in the nursery.

An additional section on the germination microenvironment and its manipulation is also included. A distribution list for the guidebook is being put together.

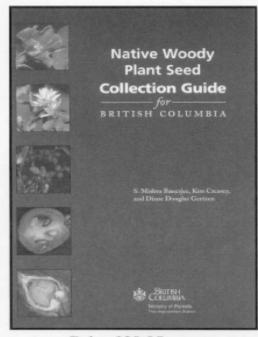
If you have not received a guidebook by mid-January, please contact Dave Kolotelo for information on obtaining a copy (1-604-541-1683 ext. 228) [Dave.Kolotelo@gems7.gov.bc.ca]. Enjoy.







Native Woody Plant Seed Collection Guide for British Columbia



Native plants provide beauty and utility in the context of maintaining natural and managed ecosystems. As people continue to utilize native plants, there is a need to develop standard practices for seed collection, vegetative propagation, and nursery culture of these species.

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Introducing the 2002 Edition of the

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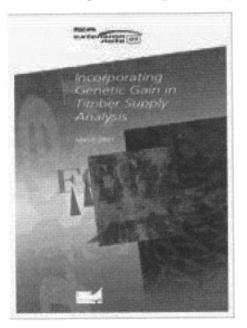


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Extension notes are produces on pertinent topics relating to Tree Improvement.



Extension Note 1

Incorporating Genetic Gain in Timber Supply Analysis, March 2001This extension note explains how the volume gains attributed to using select seed are modelled in stand yield projections and accounted for in TSR timber supply analyses. This note should interest timber supply analysts and people making decisions about seed orchard investments.

Forests Genetics Council of BC

fgc@cortex.ca

Extension Note 2

Biotechnology: Potential Applications in Tree Improvement, March 2001

This extension note describes types of biotechnology that are being used or have potential applications in tree breeding and production of planting stock. It notes some of the concerns about the use of genetic engineering in tree improvement, and how B.C. is responding to these issues.





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EVENTS

Joint Annual Meeting Forest Nursery Association of British Columbia Western Forestry and Conservation Nursery Association August 5 - 8, 2002 WestCoast Olympia Hotel Olympia, Washington

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Can West Hort Show September 18 - 19, 2002 Vancouver Trade & Convention Centre Vancouver BC Canada

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TREE IMPROVEMENT

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