

TICtalk



Forest Genetics Council
of British Columbia

Information for and from the tree improvement
community of British Columbia

Vol. 6, No. 1 June 2005

Sixth Edition

Welcome to the sixth edition of *TICtalk*. This issue will be our first in electronic format. A sincere thank you to Roger Painter for the editorial, our authors and the editorial committee of: Jack Woods, John Russell, Andreas Hamann, Chris Walsh, Peter Forsythe, and Roger Painter.

Diane Douglas

Looking back to when we first started revitalizing tree improvement offers a very satisfactory view. Approximately 50 people met in a room at the Richmond Inn along with our former Chief Forester Larry Pedersen in October 1995. The meeting was a rough affair and there were more questions than answers and certainly no consensus on just about anything. Larry walked away from that meeting and initiated a review within the Ministry of Forests that eventually snowballed into a close hard look at the entire industry. From that beginning a number of people worked to develop a new model for Tree Improvement while convincing FRBC that we were the best thing since sliced bread and worth investing in. Part of that involved setting some goals for ourselves and recognizing that if we wanted to move forward, then we needed to work together province wide. Over 10 years later we are close to those lofty goals but what is really clear is that we have built a powerful engine to drive our program that covers almost all aspects of the industry. Many of the issues we faced have either gone away or become unimportant. We also seem to have built ways to deal with issues a lot better. What has also become apparent is that we have developed a business out of all the parts

of tree improvement in BC. And a business that is a co-operative. We have developed a number of specialized programs from breeding and operations to gene conservation and extension. Each one of these programs has set goals of their own. And it continues to grow. A perfect example is the inclusion of a stronger focus on Seed Orchard Pest Management in the last year. The results are the creation of a science based operational program that is second to none in the world.

Look back for a moment. We were looking at 6% gain, with a number of old orchards only getting one and a half the cost of wild collections, while producing very little in the way of seed for the largest species in the province. Financial resources were on the decline and government viewed us as a "nice to do" program with no direction; a sure candidate for the chopping block. Since that time we have doubled the gain, re-energized most of our orchards, retired others, initiated a program of expansion that will see us eventually meet 70% of the entire need of reforestation in the province and take on challenges like gene conservation.

Sometimes it pays to look over our shoulder and say, "well done everyone." So what's next?

Roger Painter

INSIDE

Chief Forester's Standards for Seed Use	2
Investigations of Douglas-fir Resistance to <i>Phellinus weirii</i>	3
Technical Advisory Committee for Coastal Hardwood Species Established	8
Hardwood Management on TFL 43	8
Germination: Definitions, Assumptions, Implications	9
Tree Improvement and Forest Genetics Client Survey	12
Events	13
Contributors	14
TICtalk Availability	14

Chief Forester's Standards for Seed Use

submitted by Brian Barber

In November 2004, Jim Snetsinger, Chief Forester (CF), introduced his *Chief Forester's Standards for Seed Use*. These standards came into effect on **April 1, 2005** and apply to persons who use seed in establishing a free growing stand under the *Forest and Range Practices Act* (FRPA).

These standards are those the CF considers necessary and appropriate for registering, storing, selecting, and transferring seed used for Crown land reforestation. Their purpose is to maintain the identity, adaptability, diversity and productivity of the provinces' tree gene resources. They are based on stewardship principles and over forty years of research in forest genetics and tree seed management.

These standards represent an updated consolidation of the seed use requirements that existed under the Forest Practices Code, which included regulations, a guidebook and ministry policies. As such, this document serves to provide "one-stop shopping" for seed collectors, seed orchard and nursery managers, forestry professionals, licensees and ministry staff alike.

These standards were developed in conjunction with *FRPA* and its regulations by ministry staff in consultation with forest sector representatives over the past two years. Input was provided by licensees, members of the Forest Genetics Council of BC and its technical advisory committees, BC Tree Seed Dealers Association, and the Forest Nursery Association of BC.

The *Chief Forester's Standards for Seed Use* can be viewed and downloaded from the Ministry of Forests' website at: <http://www.for.gov.bc.ca/code/cfstandards/>. This site also includes a link to Questions and Answers regarding the standards. Training for field staff will be offered in the fall of 2005.

For further information regarding these standards, please refer to the above website or contact Brian Barber, RPF, Technical Advisor, Tree Improvement Branch, Ministry of Forests at: (250) 356-0888 or Brian.Barber@gems4.gov.bc.ca.

The Chief Forester's Standards for Seed Use can be viewed and downloaded from the Ministry of Forests' website at: <http://www.for.gov.bc.ca/code/cfstandards/>.

Investigations of Douglas-fir resistance to *Phellinus weirii*

submitted by Rona Sturrock

Background

Laminated root rot (LRR), caused by the native fungus *Phellinus weirii*, is widespread in southern British Columbia (BC), Washington, Oregon, northern California, western Montana, and northern Idaho (Figure 1). In western North America there are two forms¹ of the fungus – a cedar form that causes butt rot in western redcedar and a Douglas-fir form that infects and kills Douglas-fir and several other conifer species (e.g., *Abies*, *Tsuga*, *Picea* and *Pinus*). All hardwoods are immune to infection by both forms of the fungus.

Laminated root rot begins in a stand when roots of susceptible hosts contact infected stumps or roots left from the previous stand (Figure 2). Fungal mycelia initially colonize root surfaces, eventually penetrating host bark and cambium to cause decay in wood tissues. Infected trees suffer increased susceptibility to windthrow and insect attack, growth loss, and mortality.

Figure 1. Distribution of laminated root rot (Douglas-fir and cedar forms) in western North America.



Since the late 1950s BC has made significant investments in a breeding program for coastal Douglas-fir (Fdc). Highly productive seed sources are now available but little is known about the resistance of these sources to pathogens like *P. weirii*. Cooperative work by the Canadian Forest Service (CFS) at the Pacific Forestry Centre (PFC) and the BC Ministry of Forests (BCMof), which began in the early 1990s, has yielded evidence of genetically-based resistance by Fdc to *P. weirii*. Related, ongoing work by the CFS and researchers at the University of British Columbia (UBC) and forest professionals in Industry, is investigating aspects of the *P. weirii* pathosystem including host defense responses and variation in the genetics and virulence of the fungus.

Screening Full-sib Fdc Families

An initial screening in the mid-1990s of 97 Fdc full-sib families representing a broad cross-section of the breeding program saw several thousand seedlings inoculated with two isolates of *P. weirii*. No single family showed outright resistance (i.e., all families had some mortality) but statistical analyses showed significant differences in mortality between some families and between the two isolates used. A repeat screening trial with a narrowed base of 15 of the original 97 Fdc families (five families each per high-, mid-,

Figure 2. Crown of one of many Douglas-firs in this plantation affected by laminated root rot. Note the chlorotic and stunted foliage.



Laminated root rot, caused by the native fungus *Phellinus weirii*, is widespread in southern British Columbia, Washington, Oregon, northern California, western Montana, and northern Idaho

1 There is agreement among taxonomists that the two forms are actually two closely related species, but the correct names for them have not yet been agreed upon. The species on Douglas-fir is proposed to be called *P. sulphurascens*; the species on western redcedar, *P. weirii*.

Defense mechanisms in plants consist of preformed barriers such as a waxy cuticle and inducible defense systems.

and low-resistance ranking) is still underway. Initial results reveal six Fdc families with the same resistance ranking that they demonstrated in the first screening. Ongoing cooperative trials with two industrial partners (TimberWest Forest Company and Western Forest Products) are assessing the performance of 1) seedlings from the original 15 families outplanted in a stand naturally infected with *P. weirii* and 2) older siblings of the same 15 families inoculated with the fungus in a coastal stand on Vancouver Island. These trees were aged 15 years at the time of inoculation.

Tree-Pathogen Interactions

Host Proteins

Defense mechanisms in plants consist of preformed barriers such as a waxy cuticle and inducible defense systems. Pathogen-induced defenses in conifers include cell-wall degrading enzymes (e.g., chitinase and glucanase) and other pathogenesis-related (PR) proteins. Chitinases are frequently associated with plant defense responses against fungal attack because they hydrolyse chitin, a structural component of fungal cell walls. The CFS, principally in the laboratory of Dr. Abul Ekramoddoullah, has identified two PR proteins that are up-regulated in *P. weirii*-infected roots. The first, an endochitinase protein (ECP) has also been found in the apoplastic fluid of Fdc needles, some distance away from where the fungus is actually present and causing infection on roots (Figure 3). This systemic response to fungal infection is interesting and has potential implications for our ability to detect *P. weirii* without using laborious root excavations. This ECP also demonstrates some antifreeze activity in Fdc and has been found to be up-regulated in the roots of

interior Douglas-fir infected with the root pathogen *Armillaria ostoyae*. The CFS has developed an antibody to the protein and has used it as a probe for the detection of ECP and to measure its expression in host tissues.

The second PR protein found to be up-regulated in *P. weirii*-infected Fdc roots is a thaumatin-like protein (TLP). Thaumatin-like proteins have demonstrated antifungal activity *in vitro*, though the precise mechanism of action is not clear. Like the ECP found in Fdc tissues, the TLP found in Fdc tissues by CFS researchers also can be isolated from needles and *A. ostoyae*-infected roots. The induction of both of these proteins at sites of root infection and their presence in needle tissues suggests that they play a general role in adaptation to stress and may be part of an integrated defense response initiated by hosts to impede further pathogen spread.

A third protein, an 18.5 kDa stress-related protein (PSE m I) associated with overwintering in Douglas-fir has also been shown to be up-regulated in roots and foliage of *P. weirii*-infected fir. There appears to be a correlation between tolerance ranking and PSE m I but more measurements need to be made to confirm this.

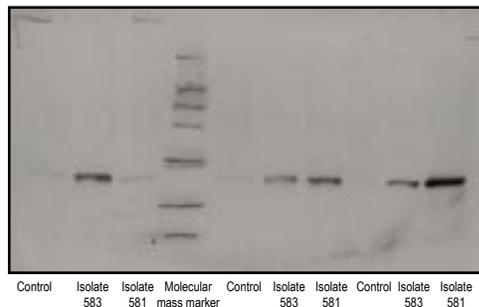
Brief molecular primer

DNA is of course the carrier of genetic information in all cells. It usually consists of two polynucleotide chains arranged in a double helix. Genes are those nucleotide sequences of the DNA molecule that eventually code for specific proteins. Before protein synthesis begins one strand of the DNA double helix is used as a template to synthesize a messenger RNA (mRNA) molecule. This mRNA migrates from the nucleus to the cytoplasm where it becomes bound to the ribosome to be translated into polypeptide sequences/proteins.

Complementary DNA or cDNA is the double-stranded DNA complement of a mRNA sequence. cDNA has two advantages over chromosomal (genomic) DNA: first, it has no introns (the non-coding sequences of genes), making it an easier source from which to identify and characterize genes, and second, cDNA represents only those genes that are actively used by the cell. Preparation of cDNA is often the first step in cloning DNA sequences of interest. A cDNA library is a collection of cDNA clones that are generated *in vitro* from the mRNA sequences isolated from an organism or a specific tissue or cell type or population of an organism.

Pathogen-induced defenses in conifers include cell-wall degrading enzymes (e.g., chitinase and glucanase) and other pathogenesis-related (PR) proteins.

Figure 3. Western immunoblot showing accumulation of an endochitinase protein (ECP). Proteins were extracted from one-year-old needles of clonal Douglas-fir (TimberWest's Line 4) control trees or those inoculated with either *P. weirii* isolate PFC-Pw 581 (virulent) or PFC-Pw583 (less virulent).



cDNA Libraries

The CFS has completed two cDNA libraries from *P. weirii*-infected Fdc; one from roots and a second one from foliage of infected trees. Processing of the latter library eventually yielded partial sequences for four genes that showed homology for encoding known proteins. Full-length sequencing of these genes is needed to determine what their role might be in the interaction between Fdc and *P. weirii*.

Fungal Proteins/Enzymes

Fungi produce a remarkable array of enzymes and can degrade almost any organic substance. As a necrotrophic, wood-decaying pathogen, *P. weirii* secretes a wide range of extracellular enzymes including cellulases and ligninases. The CFS has completed a cDNA library for *P. weirii* isolate PFC-Pw581. This is an isolate that has demonstrated significant and consistent virulence in host inoculation studies (see section below on diversity of *P. weirii*). Processing of this library yielded two genes of potential interest. The first gene codes for a 22kDa protein the CFS has named *Phellinus weirii 1 (Phe w 1)*. The identity and function of this protein remain to be found. The second gene encodes a hydrophobin protein about 10kDa in size. Hydrophobins are small proteins thought to be ubiquitous in filamentous fungi. There is also evidence that hydrophobins play a role in fungal pathogenesis. The CFS has developed antibodies to both of these proteins and is continuing investigation of their roles in the development and pathogenicity of *P. weirii*.

Terpenoid Defenses

Conifers are known to have evolved complex oleoresin-based defense strategies both as preformed defenses and as inducible defenses against insect pests and pathogens. Oleoresin consists largely of a mixture of terpenoids of various structural types, including monoterpenes (C₁₀), sesquiterpenes (C₁₅) and diterpene resin acids (C₂₀). Enzymes called terpenoid synthases (TPSs) generate the enormous diversity of carbon skeletons characteristic of terpenoids. In conifers, oleoresin terpenoids are formed in specialized cells and secreted into extra-cellular resin ducts. Although the phenomenon of defense-related development of traumatic resin ducts (TDs) has been well described in some species of spruce, it is less characterized in Douglas-fir.

Research conducted by the CFS in cooperation with Dr. Jörg Bohlmann and colleagues at the Biotechnology Laboratory at UBC during 2001–2003 looked at terpenoid defenses in coastal Douglas-fir. Funding for this research came from Forest Renewal BC and Forestry Innovation Investment.

In the first component of this research the creation of a cDNA library for 'healthy' Fdc led to the identification of five TPS genes that contribute to Fdc defensive resin composition or other non-resin antimicrobial defense systems. A second component of the terpenoid research was investigation of the ability of methyl jasmonate (MeJA) to induce defense responses in Douglas-fir. Treatment of plant tissues with chemically defined elicitors such as MeJA provides useful means for highly reproducible, non-destructive induction of defense responses and allows for their detailed histological, biochemical and molecular characterization. It has been demonstrated that MeJA induces the development of TDs and other terpenoid defenses in conifers. MeJA treatment has been shown to protect Norway spruce seedlings against the fungal pathogen *Pythium ultimum*.

We applied a 0.01% solution of MeJA to the soil of potted full-sib Fdc seedlings and found that it induced the formation of TDs in roots and stems. A total of 35 terpenoids were observed in the MeJA-treated Fdcs. These included 22 monoterpenoids, eight diterpenoids, and five sesquiterpenoids. This study provides the first description of the effect of MeJA applied to roots through the soil on the anatomy and terpene chemistry of a gymnosperm. This information will be beneficial to the identification of terpenoid-related resistance traits. Another experiment investigating the potential for MeJA applications to protect Fdc seedlings from infection by *P. weirii* is currently underway at the PFC.

Diversity of *P. weirii* – Inoculation trials with clonal Fdc

It is well known that plant pathogenic fungi vary in their pathogenicity. A significant outcome from the CFSs first screening trial was evidence that isolates of *P. weirii* vary in their virulence (the number of trees they kill) and aggressiveness (the rate at which they kill). To better understand this variation, the CFS in cooperation with the BCMoF and TimberWest Forest Company, initiated inoculation experiments with two sources

Conifers are known to have evolved complex oleoresin-based defense strategies both as preformed defenses and as inducible defenses against insect pests and pathogens.

A significant outcome from the CFSs first screening trial was evidence that isolates of *P. weirii* vary in their virulence (the number of trees they kill) and aggressiveness (the rate at which they kill).

of clonal Fdc. Clonal host material, with its much-reduced genetic variability, is best used for measuring virulence and aggressiveness factors for different isolates of a single fungus. This information is essential to the successful development and deployment of disease-tolerant trees.

CFS–BCMOF Study

Rooted cuttings (stecklings), representing 23 clones created from nine full-sib families of Fdc, were produced by staff at the Cowichan Lake Research Station. In the fall of 1999, the stecklings were inoculated with *P. weirii* isolates PFC-Pw581 and -Pw583. Disease development and mortality were monitored for four years. By the end of the experiment the fungus had killed about 85 and 65% of stecklings inoculated with isolates 581 and 583, respectively. While results provided further evidence of the virulence and aggressiveness differences between isolates, all Fdc clones appeared equally susceptible to the fungus.

CFS–TimberWest Study

Another trial with clonal seedlings was established in 2001 in cooperation with TimberWest at their Mt. Newton facility on the Saanich Peninsula. These seedlings (i.e., emblings) were produced by somatic embryogenesis (SE). Emblings were inoculated with *P. weirii* isolates PFC-Pw581 and -Pw583 and disease development and mortality were monitored for three years. Similar to results from the rooted cutting study (above), all four lines of SE seedlings appeared equally susceptible to *P. weirii* and isolate PFC-Pw581 again demonstrated greater virulence and aggressiveness than isolate PFC-Pw583 (Figure 3). The need to understand more about *P. weirii* variation is clear.

Genetic Diversity of *P. weirii*

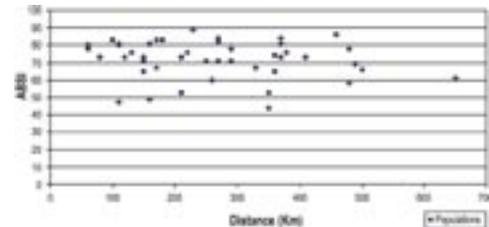
Dr. Isabel Leal's laboratory at the PFC, in cooperation with Dr. Colette Breuil and Dr. Young Woon Lim (post-doc) in the Department of Wood Science at UBC, is investigating the genetic diversity of *P. weirii* using several molecular techniques and tools. Our results to date support the proposal that the fungus causing root rot of Douglas-fir and other coniferous species be called *Phellinus*

sulphurascens and the fungus causing root and butt rot of western redcedar be called *P. weirii*. We also have established evidence that the *Phellinus* species affecting Douglas-fir in BC is a genetically homogeneous one (Figure 4). More details on the genetic diversity of *P. weirii* and *P. sulphurascens* will appear in a future edition of TICtalk.

Acknowledgements

The author thanks the following individuals

Figure 4. Degree of genetic similarity between pairs of populations of the *Phellinus* species affecting Douglas-fir in BC compared to their geographic distance. Maintenance of relatively high ABSI (average band sharing index) values with increasing distance indicates genetic homogeneity for this species.



and their organizations for their cooperation and assistance in this research: CFS staff at the PFC – Kevin Pellow, Dan Hall, Arezoo Zamani, Abul Ekramoddoullah, Isabel Leal, X. (Bill) Yu, and J-J. Liu; Don Carson, John Ogg, Keith Bird and other staff at the BCMOF's Cowichan Lake Research Station; Jack Woods (now with SelectSeed Co. Ltd.), Michael Stoehr, and Alvin Yanchuk, Research Branch, BCMOF; Tim Crowder and staff at TimberWest Forest Company's Mount Newton Seed Orchard; Doug Stables of Western Forest Products Ltd.; Jörg Bohlmann, Dezene Huber, Ryan Phillipe and other colleagues and technical staff at the Biotechnology Laboratory at UBC; and Colette Breuil, Young Woon Lim and other colleagues and technical staff at the Department of Wood Science at UBC.

Past and current funding for this research comes from a variety of sources including the CFS' research budget, the CFS' Genomics Research Initiative 2002–2005, Forest Renewal BC, and Forestry Innovation Investment.

Our results to date support the proposal that the fungus causing root rot of Douglas-fir and other coniferous species be called *Phellinus sulphurascens* and the fungus causing root and butt rot of western redcedar be called *P. weirii*.

Literature

- Ekramoddoullah, A. K. M., X. Yu, R. Sturrock, A. Zamani, and D. Taylor. 2000. Detection and seasonal expression pattern of a pathogenesis-related protein (PR-10) in Douglas-fir (*Pseudotsuga menziesii*) tissues. *Physiologia Plantarum* 110: 240–247.
- Lim, Y.W., Y.C.A. Yeung, R.N. Sturrock, I. Leal, and C. Breuil. 2005. Differentiating the two closely related species, *Phellinus weirii* and *P. sulphurascens*. *Forest Pathology* (accepted).
- Huber, D.P.W., R.N. Phillippe, L. Madilao, R.N. Sturrock, and J. Bohlmann. 2005. Changes in anatomy and terpene chemistry in Douglas-fir (*Pseudotsuga menziesii*) seedlings following treatment of roots with methyl jasmonate. *Tree Physiology* 25: in press.
- Robinson, Richard M., Rona N. Sturrock, Joanne J. Davidson, Abul K.M. Ekramoddoullah, and Duncan J. Morrison. 2000. Detection of a chitinase-like protein in the roots of Douglas-fir trees infected with *Armillaria ostoyae* and *Phellinus weirii*. *Tree Physiology* 20: 493–502.
- Sturrock, R.N. and G. Reynolds. 1998. A new technique for inoculation of conifer seedling roots with the laminated root rot pathogen *Phellinus weirii*. *Can. J. Plant Pathology* 20:324–330.
- Zamani, A., R.N. Sturrock, A.K.M. Ekramoddoullah, J.J. Liu, and X. Yu. 2004. Gene cloning and tissue expression analysis of a PR-5 thaumatin-like protein in *Phellinus weirii*-infected Douglas-fir. *Phytopathology* 94: 1235–1243.
- Zamani, A., R. Sturrock, A.K.M. Ekramoddoullah, S.B. Wiseman, & M. Griffith. 2003. Endochitinase activity in the apoplastic fluid of *Phellinus weirii*-infected Douglas-fir and its association with over wintering and antifreeze activity. *Forest Pathology* 33(5):299–316.

Technical Advisory Committee for Coastal Hardwood Species Established

submitted by Chang-Yi Xie

Hardwoods form an important component of forest resources in coastal British Columbia.

Hardwoods form an important component of forest resources in coastal British Columbia. A variety of high-value wood products can be produced from those species and their market opportunities are rapidly expanding.

Red alder, once the target species of eradication now fetches the price equivalent to coastal Douglas-fir. Big leaf maple logs are in increasing demand for flooring and furniture. The outstanding yield (MAI > 30 m³/year/ha) of hybrid poplar (black cottonwood × eastern cottonwood) has generated enormous interest in British Columbia and its tissue products is among the highest value-added wood products in the province.

Realizing the rising significance of hardwoods in coastal BC, the Forest Genetics Council (FGC) of British Columbia initiated a Technical Advisory Committee (TAC) for coastal hardwood species in October, 2004. This committee is chaired by Dr. Chang-Yi Xie of Research Branch, Ministry of Forests and has members from the forestry industry, UBC and Ministry of Forests. The role of the committee is to provide technical and policy information to the Forest Genetics Council and contribute to the development of FGC business plan and budget. The committee will gladly address any concerns and appreciate any suggestions regarding all aspects of forest gene resource management of coastal hardwood species in BC.

Hardwood Management on TFL 43

submitted by Dan Carson

Background

In 1985 Scott Paper Limited was granted TFL number 43. To this date it is still the only hardwood TFL in the province. The licence area is made up of 10 000 ha of alluvial floodplains along the southern coast with a mean elevation of 20 m. Included in the license are 500 ha of highly productive private land in the Fraser Valley. Approximately 50% of the total area is productive with the remainder being non-productive water or wetlands. The majority of stands are a mixture with hardwood leading (cottonwood, alder) and a minor coniferous component (Sitka spruce, western hemlock and redcedar). All of the mature stands in the license have naturally regenerated following railway harvesting early in the 20th century. Collectively these are some of the most productive sites in the country.

Site Conditions

The license is divided into three operation areas along major rivers: Kingcome, Fraser and Homathko. The biogeoclimatic zone is the CWH with the following sub zones ds1, dm and vm. The yearly rainfall ranges from 99 cm in the CWH ds1 to 330 cm in the CWH

vm1. The ds1 does experience a growing season moisture deficit severe enough to affect plantation establishment on coarse-textured gravel soils. By far the most common site series encountered in all areas is mid bend floodplain with very limited amounts of low and high bench. The soil texture is mostly fSL with no coarse fragments except where there are deposits of gravel (100% CFC). Because of the rapid decomposition of leaf litter there is only a thin forest floor (0–5 cm) with a Ah horizon generally absent. The understory vegetation is usually a fully developed mixture of salmon berry, thimble berry and devils club. The resulting brush hazard on these sites is extreme which makes conifer management an impractical option.

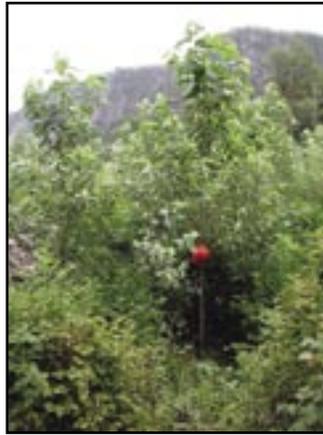
Forest Management

Without exception all of the sites within the license on Crown land are managed for the production of large-diameter cottonwood/hybrid poplar pulp logs. The management target for the extensively managed stands is an average stump diameter of 45 cm over a 25-year rotation. On the best sites a unit volume of 375 cubic metres per hectare is expected which translates into a MAI of

Without exception all of the sites within the license on Crown land are managed for the production of large-diameter cottonwood/hybrid poplar pulp logs.

15 cubic metres. Because stands of clonal material do not self-thin, all stands are established at densities near that of the final crop density of 450 sph.

Hybrid poplar is the species of choice for reforestation activities on coastal floodplains. Currently the average genetic gain of all operational varieties is a substantial G+54. Scott Paper Limited has an ongoing tree breeding program that aims to increase the gains over natural cottonwood and increase pest resistance. All of the 13 varieties of hybrid poplar presently deployed operationally have gone through exhaustive pest and pathogen testing. The advantage of clonal forestry is that it allows for the selection of the most productive individual from a population. The downside is an increase risk of damage from pest and pathogens. To minimize the risk of plantation damage each variety that is planted is limited to an area of about 5 ha within a given cutblock. The planting stock is typically 1+0 1.8 m Ax unrooted whips. The tall stock type allows the tree to be above the



non-crop vegetation during the first growing season. First year survival rates seldom are less than 95 percent provided that planting stock is transported and handled correctly. Unlike conifer seedlings, hybrid poplar whips must be transported and planted frozen. Hybrid poplar is the most shade

intolerant of the commercial tree species so partial cutting is not a management option. Before planting, all sites are mechanically site prepared (spot cultivated or mounded) to reduce below ground competition and reduce the soil bulk density to enhance rooting. Site preparation also significantly reduces the level of damage from deer browsing. Following planting, the trees are spot-fertilized at an application rate of 75 kg/ha to promote early diameter

and height growth. No further treatments to the stand are required once initial treatments are completed. The target leader growth rate is 2.0 m per growing season. By the end of the second growing season the stands are freegrowing and have surpassed the minimum heights required for green-up.

Hybrid poplar is the species of choice for reforestation activities on coastal floodplains.

Germination: Definitions, Assumptions, Implications

submitted by Dave Kolotelo

Germination is an important seedlot attribute that can impact seed pricing, seed use, and the success of your crop. Germination is intuitively understood, but I would like to provide a condensed review on the Definitions, Assumptions, and Implications behind the word. The discussion will focus on conifers, but some of the quantitative information is equally applicable to angiosperms (any discussion regarding dormancy mechanisms may not be!). It certainly isn't the last word on the subject, but I think it is a worthwhile review and if it initiates some lively, pro-active discussions that would be a bonus.

Definitions

Germination is generally associated with emergence of the radicle through the seed coat. The International Seed Testing

Association (ISTA 2004) defines germination as "the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether it is able to develop further into a satisfactory plant under favourable conditions." This broad definition is intended to allow for a variety of testing methods used through the world to be acceptable, but a certain level of subjectivity is involved in judging whether a germinant is able to develop into a satisfactory plant. Questions arise regarding what is a satisfactory plant? with implications to seedling specifications, but that is beyond the scope of this review.

In BC, the criterion for counting a normal and healthy germinant is the ratio of the radicle length to the seed coat. For all species (except Ba and Bn) a seed is classified as germinated

Germination is an important seedlot attribute that can impact seed pricing, seed use, and the success of your crop.

A germination test is composed of four replicates of 100 seeds that have been randomly selected and are representative of the seedlot being tested.

For information on species specific treatments refer to the *Seed Handling Guidebook* (Kolotelo et al. 2001).

once the radicle is 4× the length of the seed coat. For Ba and Bn the criteria is 2× the length of the seed coat due to the larger size of these seeds. In some parts of Canada, germination vigor classes are used in the classification of germinating seeds. This method categorizes germinants into various classes based on how far seedling development has progressed. It will not be discussed further here, but if interested the following paper is worth reading (Wang 1973).

The Germination Test

A germination test is composed of four replicates of 100 seeds that have been randomly selected and are representative of the seedlot being tested. Proper sampling cannot be over-emphasized – the test result is only as good as the sample taken! The seeds are pre-treated with a soak (except Cw and some hardwoods), and generally a period of stratification. For information on species specific treatments refer to the *Seed Handling Guidebook* (Kolotelo et al. 2001). Following treatment, each 100-seed replicate is transferred to a germination dish containing a piece of 22-ply wadding paper, 50 ml of water and filter paper on which the seeds are placed. Each dish is labelled with the seedlot number, test type, test identification number and replicate number.

On Monday, Wednesday and Friday, germination counts are performed in order to calculate the Germination Capacity (GC) and the Peak Value (PV) of the seedlot (Figure 1). The germination capacity is the percentage of seeds that have germinated during the test (21 or 28 days depending on species) based on the average of all four replicates. The PV is an estimate of germination rate and the point whose tangent has the steepest slope on

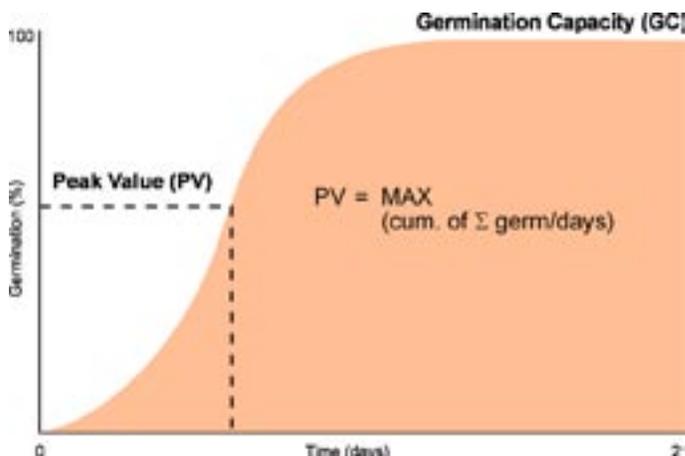
the germination curve. The PV is presented as the peak germination percent/peak count day (e.g., 89/10 indicates that the peak value corresponds to 89% germination in 10 days). For statistical analysis or use in formulas the integer (i.e., 8.9 in above example) should be used for evaluating differences in germination rate. Both the GC and the PV are available on SPAR and on sowing request labels. The GV was formerly used, but this variable combines both GC and PV together in one variable making it difficult to assess each independently. A more thorough example of the calculations can be found in the *Seed Handling Guidebook* (Kolotelo et al 2001).

Germinants categorized as ‘abnormal’ are not included in the GC or PV of a seedlot and are tallied separately by abnormal class. The most common abnormal germinants are where the cotyledons emerge first (reversed = easy to recognize) and germinants with stunted radicles (a more subjective classification). Reversed germinants generally are only a fraction of a percent of the seed in a seedlot, but the percent of stunted radicles can be much higher for some seedlots. Stunted radicles appear to be associated with seedlot age and species. In general, *Abies* spp. tend to have higher proportions of stunted radicles even on new seedlots, but older seedlots of Cw, Hw, and Lw also have higher levels of these abnormalities. No study has clearly determined the cause of these abnormalities, but it appears related to ageing and possibly damage to the root cap protecting the root apical meristem. Other less common abnormal classes are: stunted hypocotyl, thickened hypocotyl, thickened radicle, twin; rotten, weak and megagametophyte collar.

Germination Test Accuracy and Precision

The germination test is composed of four replicates and ISTA provide tolerances for the maximum tolerated range between the four replicates (two-way test at 2.5% significance level). If one of these replicates is outside the tolerances then the test is repeated. Formerly the Association of Official Seed Analysts (AOSA) allowed calculation of germination parameters based on the remaining three replicates if one replicate was out-of-tolerance. This is no longer the case and both ISTA and AOSA indicate that tests should be repeated if a single replicate is out of tolerance. This is a method improvement that ensures all tests have the same sample size involved in the calculation of germination parameters.

Figure 1. The germination curve illustrating the Germination Capacity (GC) and Peak Value (PV).



The germination test also allows one to compare the precision of the test result based on the variability between the four replicates (see Kolotelo 2002 for more details). Based on the four replicates one can calculate the standard error and any confidence interval for a germination test. An example is two seedlots both with a GC of 85%, but seedlot A has replicate data of 84, 84, 88 and 83%, while seedlot B has replicate data of 87, 77, 88, and 87. Seedlot A would have a 95% confidence limit of germination between 82.4% to 87.2%, but seedlot B would have a much wider confidence limit of 76.6% to 93.0% indicating less precision in the GC estimate. This is not currently reported and although some nurseries have shown interest in this data there is no clear indication from nurseries that this would be operationally useful. I suggest the standard error is the best statistic to report allowing nurseries to calculate confidence intervals to their specific significance level of interest.

Species Average Germination

The species average (SA) germination is used when a seedlot is required for sowing during the same production year as it was collected and there is insufficient time to complete processing and testing. Each species has a SA germination on SPAR, but this germination is not specific to the genetic class and GC can vary by genetic class for some species (i.e., Yc, Pw or Lw). When additional information is available, such as historic performance from a specific orchard, then the owner can request an estimated germination (SE) value be used as the GC to calculate grams required for sowing requests if there is insufficient time to complete processing and testing.

Assumptions

The primary assumption of a germination test is that it is performed on a representative sample of the seedlot. A thorough sampling design that is outlined by ISTA (ISTA 2004) is used to sample seedlots for testing. Depending on seedlot size, the rules indicate the number of boxes to sample and the number of samples per box. Is the estimate perfect? No, conifers are extremely variable and we are using 400 seeds to estimate the GC of a seedlot that may be many kilograms (hundreds in some cases) in size. I don't foresee us increasing the number of replicates we use in testing and I think the way to deal with this is to provide the variation between the replicates as an estimate of how variable the result is. Everyone wants to be dealing with a uniform product, but I would feel

much less comfortable with our reforestation program if there was no variability present in the products we are putting on the landscape for most of the next century!

Another assumption of the germination test is that it is conducted under optimal conditions. In reality, the conditions we use in germination tests (temperature, light levels, and moisture levels) are international standards, applied to a variety of species. Are they optimal for everything – probably not, but imagine the job of arriving at the optimal combinations of these factors for all seedlots in storage. We have adjusted conditions when there is good evidence that it is justified. Good examples are the use of a constant 20° C germination temperature for Hw germination (Bientjes 1954) and the use of 25:15 temperatures for *Abies* spp. (Leadem 1989). I think the most important factor is that the testing method and procedures are consistently applied over time. Individual nurseries need to determine how well the standardized germination tests compare in their unique set of nursery conditions.

Operational Implications

How well do germination tests in a laboratory relate to germination in the nursery? The results are surprisingly good and I thank all nurseries that have provided feedback on actual nursery germination. The last summary I forwarded to nurseries summarized nursery falldowns from 1999 to 2003. Our big four reforestation species had the following average operational falldowns in germination: Pli = 1%; Sx = 0%; Fd = 3% and Cw = 3%. There is still room for improvement in Bl, Dr, SxS and Pw, but I am surprised at how close nursery results are to test results. Nurseries can take a great deal of this credit as they realise the importance of starting a crop off quickly and uniformly and they have improved their facilities to make sure this happens. Updated germination falldowns will be forwarded to nurseries and placed on our website prior to 2006 sowing request entry.

An area that still requires some improvement is recognition of the need to be highly efficient in seed use when we have a deficit situation for orchard produced seed. This refers primarily to Pli, but BC-produced Fdc is also in a deficit situation. Lodgepole pine has the best germination characteristics (capacity and rate) of any species in BC. Nurseries should have good data of the amount of seed required to produce Pli crops to virtually eliminate excess seed. This

The species average (SA) germination is used when a seedlot is required for sowing during the same production year as it was collected and there is insufficient time to complete processing and testing.

How well do germination tests in a laboratory relate to germination in the nursery? The results are surprisingly good and I thank all nurseries that have provided feedback on actual nursery germination.

Several forest companies are also restricting the amount of seed used for Pli sowing and we appreciate their efforts in ensuring that orchard produced Pli seed is used as efficiently as possible.

is preferred to having to hardwire more stringent sowing guidelines for orchard-produced Pli into SPAR. This past year a few nurseries that have reduced grams in the past have not. Everyone should be aware that seed is never the same once it has been soaked and stratified. It is much better to reduce grams up front. I'd like to identify several nurseries who have made efforts to reduce Pli gram for 2005 sowing: PRT Armstrong, PRT Vernon; Eaglerock, Silva Gro and Juniper Beach. Several forest companies are also restricting the amount of seed used for Pli sowing and we appreciate their efforts in ensuring that orchard produced Pli seed is used as efficiently as possible. Thank you.

If you have additional comments concerning the topic of germination I would appreciate your feedback. I can be reached at (604) 541-1683 extension 228 or at Dave. Kolotelo@gems7.gov.bc.ca.

References

- Bientjes, W. 1954. The effects of temperature, seed moisture and stratification on the germination behaviour of western hemlock seed. Res. Note 11. Univ. B.C. Forest Club.
- International Rules for Seed Testing. 2004. Published by the International Seed Testing Association.
- Kolotelo, D. 2002. Germination tests: how precise are they? CTIA Tree Seed Working Group Newsbulletin # 36. 2 pp.
- Kolotelo, D., E. Van Steenis, M. Peterson, R. Bennett, D. Trotter, and J. Dennis. 2001. Seed handling guidebook. BC Ministry of Forests Tree Improvement Branch publication. 106 pp. {contact me if you do not have a copy}
- Leadem, C.L. 1989. Stratification and quality assessment of *Abies lasiocarpa* seeds. FRDA Report 095.18 pp.
- Wang, B.S.P. 1973. Laboratory germination criteria for red pine (*Pinus resinosa* Ait.) seed. Proc. Association of Official Seed analysts. 63:94-101.

Tree Improvement and Forest Genetics Client Survey

submitted by Kathie Swift and Diane Douglas

The survey was delivered in February, 2005 to a client contact list of 316 persons which included seed users and seed producers. Eighty-four responses were returned for a response rate of 27%.

The Forest Genetics Council of BC (Extension Technical Advisory Committee) and FORREX teamed up to assess whether current provincial extension programs for tree improvement and forest genetics information are meeting clients' needs by conducting a web based "Zoomerang" client survey. The survey consisted of 22 questions including an audience profiling section.

The goals of the survey were to:

- To gather feedback on whether existing forest genetics and tree improvement extension programs and products are meeting client needs
- Identify gaps in current information, and the best ways to fill those gaps.
- Determine which delivery methods are most effective for getting relevant information to those who need it.
- Evaluate peoples' awareness and use of available resources and contacts.

The results of this survey will be used to establish a baseline for future extension activities and materials as well as their evaluation.

The development committee consisted of Peter Forsythe, Lauchlan Glen, Hilary Graham, Steve Jenvey, Shawn Morford, Don Summers, Kathie Swift, Diane Douglas, Jodie Krakowski, Alan Wiensczyk, and Jack Woods.

The survey was delivered in February, 2005 to a client contact list of 316 persons which included seed users and seed producers. Eighty-four responses were returned for a response rate of 27%. The majority of the responses were from the major forest licensees (34% – silviculture foresters), followed by the provincial government (15%). We will keep you posted on accessing the final results of this survey.

The next step in the process will be an extension planning workshop, where details of the survey will be discussed and a five year extension plan for ETAC and FGC developed.

Events

British Columbia Seed Orchard Association (BCSOA)

2005 Biennial Meeting
Sidney, British Columbia
July 11–13, 2005

For more information contact:
Annette van Niejenhuis
250.652.4023
avanniejenhuis@westernforest.com

WFGA – 50th Anniversary “Looking back – Looking ahead”

Joint Meeting of the:
Western Forest Genetics Association (WFGA)
Northwest Seed Orchard Managers
Association (NSOMA)
North American Quantitative Forest Genetics
Group (NAQFGG)
Corvallis, Oregon
19–21 July 2005

For more information contact:
Randy Johnson
541.750.7290
randyjohanson@fs.fed.us
<http://www.westernforestry.org/wfga/wfga.htm>

Poplar Council of Canada Annual Meeting 2005

Prince Albert, Saskatchewan
August 21–25, 2005
<http://www.poplar.ca/home.htm>

Organized in conjunction with the national
annual meeting of the Canadian Institute
of Forestry/Institute forestier du Canada
http://www.cif-ifc.org/rebellion/cif_home_eng.htm

2005 Forest Nursery Association of BC (FNABC)

Prince George, BC
September 19–21, 2005

For more information contact:
Mike.Thehitz@prtgroup.com

Dendrome – News and Events

http://dendrome.ucdavis.edu/News_Page.htm

CSC Coastal Silviculture Committee summer workshop

June 15–16, 2005
Volume and Value in Managed Stands
Duncan and Shawnigan Lake area
<http://www.mala.ca/forestex/>

SISCO Southern Interior Silviculture Committee summer field tour

end of August, 2005
Focus: Acceleration of Lodgepole Pine
Silviculture Through Time: Integration of
Timber and Non-Timber Resources
Okanagan near Kelowna

For more information contact:
April Anderson at aaa@mail.netidea.com
<http://www.siscobc.com>

Contributors

Brian Barber, RPF
Technical Advisor
BCMoF Tree Improvement Br.
2nd Fl. 722 Johnson St.
Victoria BC V8W 1N1
250.356.0888
Brian.Barber@gems4.gov.bc.ca

Dan Carson, RPF
Scott Paper Limited
1625-5th Avenue
New Westminster, BC V3M 1Z7
604.520.9284
Dan_Carson@scottpaper.ca

Diane Douglas, P. Ag.
Extensions and Communications
BCMoF Tree Improvement Br.
2nd Fl. 722 Johnson St.
Victoria BC V8W 1N1
250.356.6721
Diane.Douglas@gems9.gov.
bc.ca

Dave Kolotelo, RPF
Cone and Seed
Improvement Officer
BCMoF Tree Seed Centre
Tree Improvement Branch
18793 32nd Ave.
Surrey BC V4P 1M5
604.541.1683 extension 228
Dave.Kolotelo@gems7.gov.bc.ca

Roger Painter
Tree Improvement Co-ordinator
BCMoF Tree Improvement Br.
2nd Fl. 722 Johnson St.
Victoria BC V8W 1N1
250.356.9276
Roger.Painter@gems8.gov.bc.ca

Rona Sturrock
Research Scientist
Government of Canada
Natural Resources Canada
Canadian Forest Service
Pacific Forestry Centre
506 West Burnside Road
Victoria BC V8Z 1M5
250.363.0789
rsturrock@pfc.cfs.nrcan.gc.ca

Kathie Swift
Extension Specialist,
Early Stand Dynamics,
FORREX
c/o 360 – 1855 Kirschner Rd.
Kelowna, BC V1Y 4N7
250.860.9663
Kathie.Swift@forrex.org

Chang-Yi Xie
BCMoF Research Br.
1st Fl. 722 Johnson St.
Victoria BC V8W 1N1
250.387.8911
Chang-Yi.Xie@gems7.gov.bc.ca

TICtalk Availability

TICtalk is available in electronic format at <http://www.fgcouncil.bc.ca/new-tict.html>.

If you wish to receive TICtalk by post, please send your request to:

Diane Douglas, MOF Tree Improvement Branch
Tel: (250) 356.6721 Fax: (250) 356.8124
E-mail: Diane.Douglas@gems9.gov.bc.ca