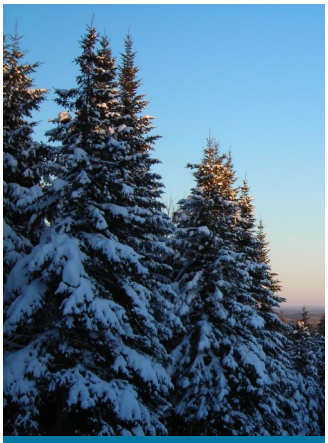

SMarTForests Newsletter

Edition 2 November 2013

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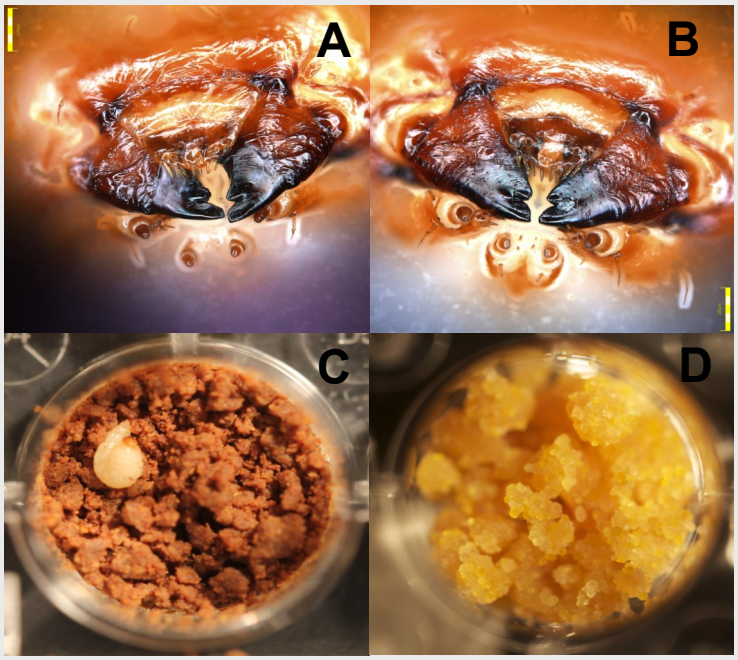
Chewing on Gravel: Stone Cells and Spruce Defense against the White Pine Weevil

The white pine weevil (*Pissodes strobi*) is a major pest of Sitka spruce (*Picea sitchensis*) and interior spruce (*P. glauca x engelmannii*) in British Columbia and Norway spruce (*P. abies*) plantations in eastern Canada (King and Alfaro 2009; Daoust and Mottet 2006). Weevil damage to the apical leaders of young trees results in top kill. Repeated attack results in deformed stems and stunted trees lacking apical shoot growth.

Early provenance trials established by the B.C. forest service in conjunction with IUFRO (International Union of Forest Research Organizations) identified several resistant Sitka spruce populations from eastern Vancouver Island and the lower Fraser Valley, British Columbia (King and Alfaro 2009). Resistance of Sitka spruce to white pine weevil attack is thought to be the result of combined effects of chemical (i.e. terpenoids) and physical defense mechanisms (i.e. resin canals and stone cells) that affect both the adult and larval life stages of the insect. Pre-formed (constitutive) and induced traumatic resin canals (formed after the weevil attacks) are filled with terpene-rich oleoresin and their role in defense against the white pine weevil has been documented (Hall et al. 2011; Robert et al. 2010).



Recently, stone cell abundance was identified as a key trait of Sitka spruce resistance against the white pine weevil (King et al. 2011). However, information about mechanisms by which stone cells affect weevils, genes and enzymes regulating stone cell formation, and biochemical composition of stone cells is not currently available for this important anatomical defense trait.

Figure 1. Mandibles of white pine weevil larvae fed on a semi-artificial diet containing no stone cells (A and C) and a diet consisting completely of stone cells (B and D). Stone cells have no measureable effect on mandible wear of white pine weevil larvae.

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Mission and Project Goals

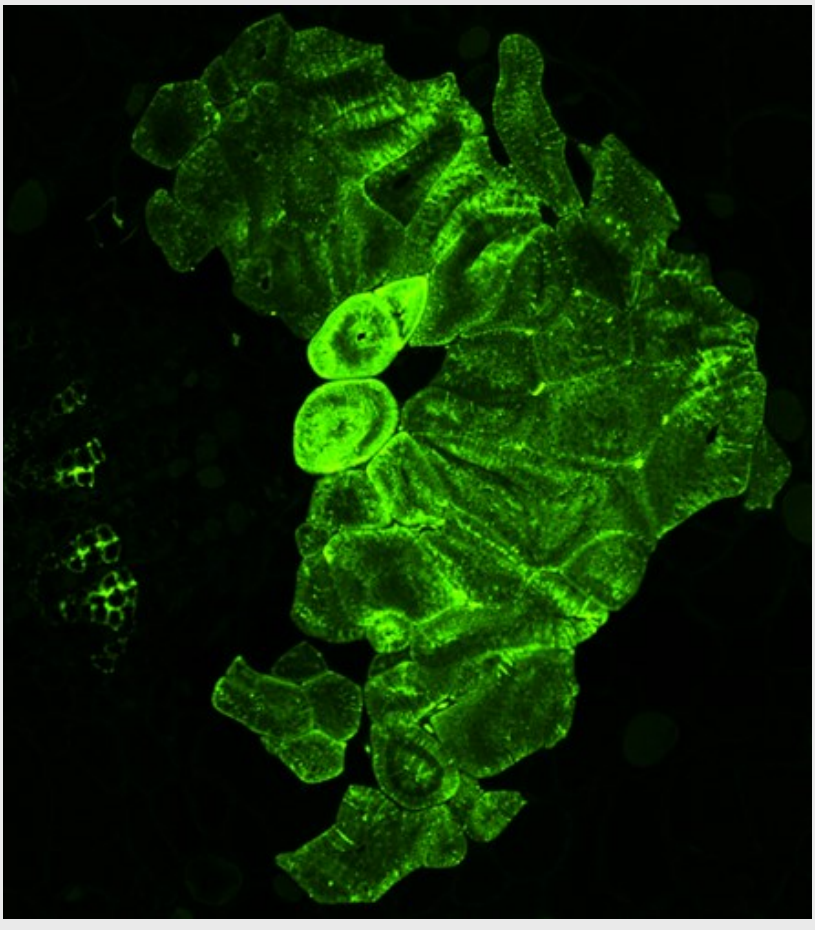
The SMarTForests project builds on a decade of research discoveries in spruce genomics by previous projects: Arborea (Université Laval) and Treenomix (University of British Columbia). The new team combines the strengths of the two previous projects, building on their extensive background knowledge and experiences. Our mission is to break new ground in spruce genome sequencing and strongly represent Canada in international conifer genome initiatives, and to achieve efficient translation of results toward end-users from across Canada.

The SMarTForests project has three major goals:

1. Develop marker systems to aid in MAS.
2. Sequence the white spruce genome.
3. Analyze impacts of forest genome on economics and society.

Some of our research in the SMarTForests Project has focused on elucidating aspects of stone cells in Sitka spruce resistance using experiments with weevil larvae *in planta* and *in vitro* coupled with cutting edge genomic, biochemical, and microscopic techniques to study stone cell composition and development as well as the effects of stone cells on weevil biology.

Stone cells are a specialized cell type found scattered throughout the phloem and bark of Sitka spruce trees. Stone cells are a constitutive defense thought to physically interfere with insect feeding. Pioneering studies by David Wainhouse and colleagues focusing on Sitka and Norway spruce found that trees with increased quantities of stone cells were resistant to the spruce bark beetle, *Dendroctonus micans* (Wainhouse et al. 1990). The resistance exerted by stone cells on bark beetle larvae was hypothesized to be from increased wear on the insect mandibles (chewing mouthparts) combined with an anti-nutritive effect. Our initial results indicate that stone cells do not impact white pine weevil mandibles (Figure 1), but at high density in living trees do significantly impact larvae growth and development. A semi-artificial diet for white pine weevil larvae, recently developed in our laboratory, was used to test the effect of stone cells *in vitro* and supports our findings *in planta* (Figure 1, page 1).



Some previous studies suggested stone cells to be completely lignified (Wainhouse et al. 1990), and recent work in Norway spruce identified the presence of minute quantities of two low-molecular weight phenylpropanoids, specifically the stilbene astringin and the dihydroflavonol dihydroxyquercetin 3'–*O*–β-D–glucopyranoside (Li et al. 2007). Stone cells in other systems, such as pear (*Pyrus* spp.), are primarily comprised of cellulose and lignin (Tao et al. 2009). Using histo-chemical and histo-biochemical imaging approaches to study stone cell composition we identified cellulose, hemicelluloses, and lignin as major components of the secondary cell-wall of stone cells (Figure 2).

Figure 2. Strong hemicellulose immunolabeling of the secondary cell-wall of a mature stone cell cluster (right) in Sitka spruce cortex. Xylem vessels (left) from nearby needles are also labeled, highlighting the similarity in secondary cell-wall composition in different cell types and organs.

Sitka spruce stone cell composition appears to be similar to that of xylem cells – another highly lignified cell type. These findings provide support to the hypothesis that stone cell formation is regulated through genes that effect secondary cell wall formation. Genes involved in stone cell biosynthesis in spruce are now being identified. We have discovered a critical point in time in the annual growth cycle when stone cells are first emerging in the apical shoot (Figure 3). We are using laser capture micro-dissection (Abbott et al., 2010) to isolate developing individual stone cells and cell clusters for transcriptome sequencing.

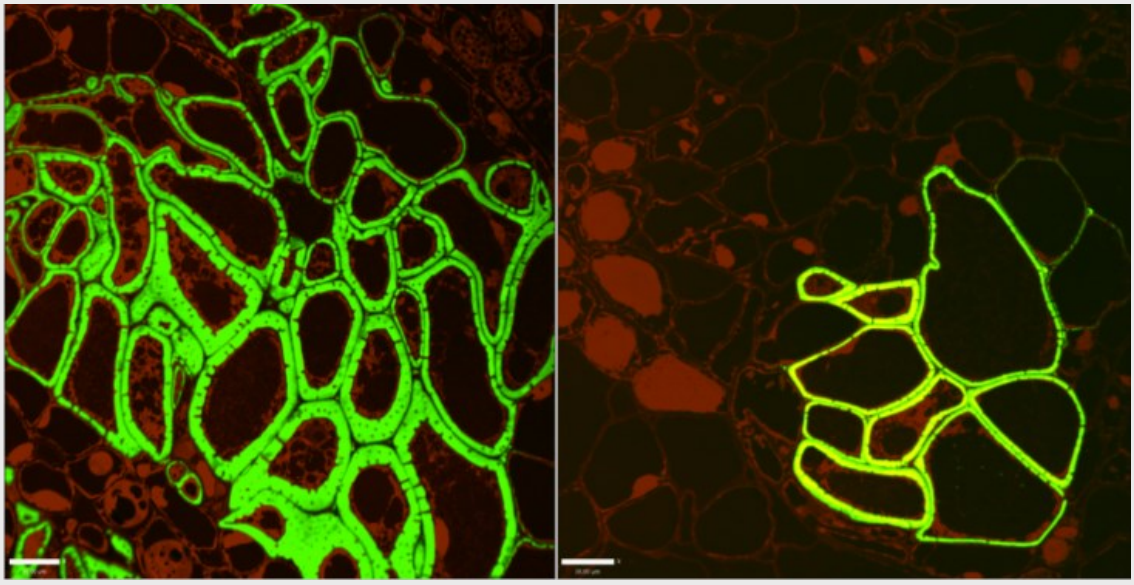


Figure 3. Strong hemicellulose immunolabeling of developing secondary cell-walls of stone cell clusters in resistant H898 (left) and susceptible Q903 (right) Sitka spruce cortex.

Identification of genes, metabolites, and molecular processes involved in the ecological function and formation of stone cells will lead to a better understanding of natural resistance mechanisms against the white pine weevil, and can support breeding programs for resistance. Our mechanistic and functional genomics work on the stone cell trait is in close interaction with other work in the SMarTForests Project that is looking at population genomics of stone cell traits.

REFERENCES

- Abbott, E., D. Hall, B. Hamberger, and J. Bohlmann. 2010. Laser microdissection of conifer stem tissues: isolation and analysis of high quality RNA, terpene synthase enzyme activity and terpenoid metabolites from resin ducts and cambial zone tissue of white spruce (*Picea glauca*). *BMC Plant Biol.* 10:106.
- Daoust, G. and M. Mottet. 2006. Impact of the white pine weevil (*Pissodes strobi* Peck) on Norway spruce plantations (*Picea abies* [L.] Karst.) Part 1: Productivity and lumber quality. *The Forestry Chronicle.* 82: 745–756.
- Hall, D.E., J.A. Robert, C. I. Keeling, D. Domanski, A. L. Qesada, S. Jancsik, M. Kuzyk,, B. Hamberger, C. H. Borchers, and J. Bohlmann. 2011. An integrated genomic, proteomic, and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce (*Picea sitchensis*) genotypes which are resistant or susceptible to white pine weevil. *Plant J*. 65:936–948.
- King, J.N. and R.I. Alfaro. 2009. Developing Sitka spruce populations for resistance to the white pine weevil: Summary of research and breeding program. B.C. Min. For. Range, Res. Br., Victoria, B.C. Technical Report. 050.
- King, J.N., R. I. Alfaro, M. Grau Lopez, and L. VanAkker. 2011. Resistance of Sitka spruce (*Picea sitchensis*) to white pine weevil (*Pissodes strobi*): characterizing the bark defence mechanisms of resistant populations. *Forestry*. 84: 83–91.
- Li, S.H., B. Schneider, and J. Gershenzon. 2007. Microchemical analysis of laser-microdissected stone cells of Norway spruce by cryogenic nuclear magnetic resonance spectroscopy. *Planta*. 225:771–779.
- Robert J.A., L.L. Madilao, R. White, A. Yanchuk, J. King, J. Bohlmann. 2010. Terpenoid metabolite profiling in Sitka spruce identifies association of dehydroabietic acid, (+)-3-carene, and terpinolene with resistance against white pine weevil. *Botany* 2010, 88:810-820
- Tao, S. T., H. Zhang, and S. L. Zhang. 2009. Anatomy, ultrastructure and lignin distribution of stone cells in two *Pyrus* species. *Plant Sci.*, 176: 413–419.
- Wainhouse, D., D. J. Cross, and R. S. Howell. 1990. The Role of Lignin as a Defense against the Spruce Bark Beetle *Dendroctonus micans* - Effect on Larvae and Adults. *Oecologia* 85: 257-265.

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Genome of a White Spruce

White spruce (*Picea glauca*) is a dominant conifer of the boreal forests of North America, and providing genomics resources for this commercially valuable tree will help improve forest management and conservation efforts. Sequencing and assembling the large and highly repetitive spruce genome though pushes the boundaries of the current technology. Assembling a large genome such as a conifer, is a daunting task. Estimated to be 20Gb of sequences, which is 7X larger than the human genome, it has proven to be difficult to assemble. In our recent publication (Birol et al. 2013), we describe a whole-genome shotgun sequencing strategy for a white spruce (PG29) using two Illumina sequencing platforms and an assembly approach using the ABySS software. We report a 20.8 gigabase pairs draft genome in 4.9 million scaffolds. The N50 size of scaffolds is 20,356 base pairs, which means that 50% of the genome is in scaffolds of 20,356 base pairs or more. We demonstrate how recent improvements in the sequencing technology, especially increasing read lengths and paired end reads from longer fragments have a major impact on the assembly contiguity. We also note that scalable bioinformatics tools are instrumental in providing rapid draft assemblies.

Reference:

Birol, I., A. Raymond, S.D. Jackman, S. Pleasance, R. Coope, G.A. Taylor, M. Man Saint Yuen, C.I. Keeling, D. Brand, B.P. Vandervalk, H. Kirk, P. Pandoh, R.A. Moore, Y. Zhao, A.J. Mungall, B. Jaquish, A. Yanchuk, C. Ritland, B. Boyle, J. Bousquet, K. Ritland, J. MacKay, J. Bohlmann, and S.J.M. Jones. 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* 29(12):1492-1497.

Data Release

White Spruce (PG29) genome first draft assembly

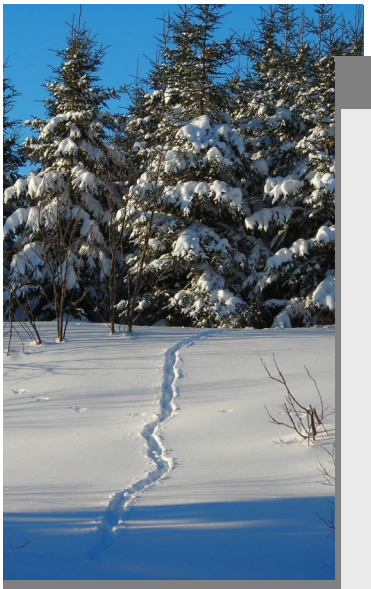
The SMarTForests Project has released a first assembly of the white spruce genome (*Picea glauca*) through the National Center for Biotechnology Information (NCBI): [Bioproject PRJNA83435, Accession ALWZ0000000000]. In addition to the NCBI portal, we have also provided a separate portal for the first draft assembly [ftp://ftp.bcgsc.ca/public/Picea_Glauca/]. This initial assembly of the white spruce genome sequence of an individual, diploid tree was based on shotgun sequencing using a high performance sequencing platform (HiSeq2000). We will continue to produce improved sequence resources and to up-date the white spruce genome assembly. The current white spruce genome assembly data release aims to support research and development in the scientific community according to the principles outlined in the Toronto workshop on pre-publication sharing of genomic data. [/Portals/0/1st%20assembly%20release%20notice.pdf] To cite this resource, please use the following article: **Birol et al. 2013 Bioinformatics 29(12):1492-1497**.

SNP data

The 13,461 *Picea glauca* single nucleotide polymorphisms (SNPs) described in two articles by Pavy and collaborators in 2013 (*BMC Biology* 10:184. (27p.) and *Molecular Ecology Resources* 13(2): 324 -336) are now available in the Single Nucleotide Polymorphism Database of NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/). SNP detection methods and genotyping results are described in the publications.

An atlas of 212,765 high-confidence SNPs

SNPs were detected among the sequences of several thousands of expressed gene sequences using Varscan software. Next-generation sequences were aligned on white spruce gene catalogue (GCAT) using Mosaik software. SNPs with minor allele frequency MAF < 0.01 were automatically discarded. The detection accuracy was verified with large genotyping datasets and indicated an overall high-confidence of the SNPs reported. These SNPs can also be accessed and retrieved from the Single Nucleotide Polymorphism Database of NCBI (http://www.ncbi.nlm.nih.gov/projects/SNP/).



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News and events

- Christmas Tree DNA: Complex Conifer Genome Begins to Yield to Research Efforts. *Associated Press*, with international coverage Dec 13 2012
- Pavy et al. A spruce gene map indicates ancient plant genome reshuffling and subsequent slow evolution in the gymnosperm lineage leading to extant conifers. *BMC Genomics* University Laval press release, with national and international coverage Dec 13 2012
- Namroud et al. Scanning SNPs from a large set of expressed genes to assess the impact of artificial selection on the diversity of white spruce. *Evol. Appl.* University Laval weekly newspaper Oct 18 2012
- Birol et al. News in Brief: Giant genomes felled by DNA sequencing advances. *Science News*. May 22 2013

For a complete list of news and events, please visit: **www.smartforests.ca**

Recent publications

- Birol I. et al 2013 Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* 29(12):1492-1497.
- Pavy N. et al 2013 Development of high-density SNP genotyping arrays for white spruce (*Picea glauca*) and transferability to subtropical and Nordic congeners. Molecular Ecology Resources 13(2): 324 -336.
- Verta J. P. et al 2013 Are long-lived trees poised for evolutionary change? Single locus effects in the evolution of gene expression networks in spruce. Molecular Ecology 22(9): 2369-79.
- Pavy N., et al 2013 The landscape of nucleotide polymorphism among 13,500 genes of the conifer *Picea glauca*, relationships with functions and comparison with *Medicago truncatula*. *Genome Biology and Evolution* 5. doi:10.1093/gbe/evt143 (16p.).

For a complete list of publications, please visit: **www.smartforests.ca**

Upcoming events

The Canadian Forest Institute e-lecture series on Genomics, tree breeding and economics is presented in November 2013. For more information: **http://cif-ifc.org/site/electure**

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