Forests, Genetic Diversity, and Climate Change

Forest Biology Symposium and Western Regional Meeting of the Canadian Society of Plant Physiologists

Monday, May 10, 2010
9:00 am to 5:30 pm
David Strong Bldg. Room C103

Program and Abstracts

Forests, Genetic Diversity, and Climate Change

9:00 - 9:20 Coffee and Muffins
9:20 - 9:30 Welcome and Introduction – C. Peter Constabel, Director, Centre for Forest Biology

9:30 -10:20 **Symposium Speaker:** Tom Whitham - *A community genetics approach to ecosystem services, climate change, and restoration of forests*

10:25 -12:10 **Contributed papers** (15 min presentations – including questions)
Pia Smets *Climatic response of pine and spruce populations evaluated in controlled climate chambers.*
Laura Gray *Assisted migration to address climate change: recommendations for aspen reforestation in western Canada.*
Alvin Yanchuk *Implementing assisted migration of populations to mitigate the impacts of climate change: The assisted migration and adaptation trial (AMAT).*
Dawn Hall *An integrated genomic, proteomic and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce genotypes that are resistant or susceptible to white pine weevil.*
Andreas Gesell *MYB transcription factor in proanthocyanidin biosynthesis in poplar and apple.*
Kim SungSoo *Analysis of the Arabidopsis 4CL-like ACYL-CoA SYNTHETASE5 gene and co-expressed genes reveals an ancient biochemical pathway required for pollen development and sporopollenin biosynthesis.*
Scott DiGuisitini *A genomic resource for* Grosmannia clavigera *and insights into fungal tolerance towards tree defences.*

12:10 - 1:30 **Lunch and Poster Session** (lobby and C113)
BC Essential Oils Workshop – concurrent (David Strong Bldg Room C108) - 12:30-5:30

1:30 - 2:10 **Symposium Speaker:** Werner Kurz - *Forests and carbon: positive feedback to climate change or opportunities for mitigation?*

2:10 - 3:25 **Contributed papers** (15 min presentations – including questions)
Art Fredeen *Net CO_2 fluxes from mountain pine beetle attacked pine forests in northern BC.*
Tony Trofymow *The effect of nitrogen fertilization on total ecosystem and component carbon fluxes in an age sequence of three coastal Douglas-fir stands in BC.*
Carolyn Smyth *Constraining the estimates of temperature sensitivity of soil heterotrophic respiration.*
Patrick von Aderkas *Experimental embryogenesis of plasticity in cold tolerance of interior spruce*
R. Soolanayakanahally *Phenological timing dictates carbon partitioning in poplar in different climates.*

3:25 - 3:45 Coffee

3:45 - 4:15 **Invited presentation:** Athena McKown - *The tank is half full: biofuel development gains from poplar tree physiology and genomics research*

4:15 - 5:30 Contributed Papers (15 min presentations – including questions)
Julie Nielsen *Streamside trees: responses of male and female cottonwoods to flooding.*
S. Keerthisinghe *MUSTACHES regulates bilateral symmetry generation in stomata.*
Chris Keeling *The Tria project: mountain pine beetle system genomics.*
Yuanyuan Liu *Regulation of secondary cell wall biosynthesis in Arabidopsis by a KNAT7 transcription factor complex.*
D. Yevtushenko *Genetic modification of the poplar defense pathway to enhance resistance against phytopathogens.*
6:00 Social gathering at University Club, Fireside Lounge, UVic campus.

ABSTRACTS ARE ARRANGED IN ORDER OF PRESENTATION – PAGES 2-14
POSTER ABSTRACTS IN ALPHABETICAL ORDER BY AUTHOR – PAGES 15-28
ESSENTIAL OILS WORKSHOP ABSTRACTS – PAGES 29-31

SYMPOSIUM SPEAKER:

A community genetics approach to ecosystem services, climate change, and restoration of forests

Thomas G. Whitham,

Department of Biological Sciences & Executive Director, Merriam-Powell
Center for Environmental Research, Northern Arizona University, Flagstaff, AZ

The community phenotypes and genetic structure of foundation species, often forest trees, are especially important to quantify as these species are by definition, “community and ecosystem drivers”. Using diverse examples from microbes to vertebrates, the community phenotypes of forest trees can be traced from the individuals possessing the trait, to the community, and to ecosystem processes such as leaf litter decomposition and N mineralization. Any agent of selection such as climate change that affects the distribution and genetic structure of foundation species is likely to have cascading impacts on the rest of the ecosystem. I develop specific examples that demonstrate the following points. 1). Climate change is an agent of selection on foundation species. 2). Changes in the genetic structure of foundation species alter community richness, abundance, composition, stability and biodiversity. 3). Changes in the genetic structure of foundation species affect diverse community members including mycorrhizal mutualists of plants, nurse plant associations, and arthropods occupying multiple trophic levels. 4). Changes in the genetic structure of foundation species alters the evolution of dependent community members. 5). Since many ecosystems are composed of multiple interacting foundation species, it is important to design experiments to study the combined effects of these interacting species, which could be very different from studies based on one species alone. Our findings and others argue that climate change has the potential to fundamentally change the evolutionary trajectories of whole communities and ecosystems. I propose an experimental approach involving provenance trials of interacting foundation species to provide the data to develop a management plan that would lessen the negative impacts of climate change and preserve the greatest biodiversity in the face of what could become a major extinction event.

Climatic response of pine and spruce populations evaluated in controlled climate chambers.

Pia Smets

Department of Forest Sciences, University of British Columbia

When replanting logged forest sites, appropriate seed source populations need to be chosen based on their adaptation to present climates as well as their capacity to respond well to future climates. Short term seedling experiments of several tree species in controlled climate chambers were used to derive population response curves to temperature at two levels of moisture. For lodgepole pine (*Pinus contorta*), response curves derived from comprehensive older field provenance trials and extrapolated to warmer conditions need to be confirmed. For interior spruce (*Picea glauca* x *P. engelmannii*), response curves derived from growth chamber trials are compared to those of field trials. Ongoing experiments with western larch (*Larix occidentalis*) and western redcedar (*Thuja plicata*) will provide baseline data on relative population performance in warmer climates in the absence of wide-ranging established field trials. According to climatic envelope models, the last two species have the potential to substantially increase their range with climate warming. As such, the predictions based on growth chamber performance may provide support for planting a larger variety of species, resulting in more resilient forests.

Assisted migration to address climate change: recommendations for aspen reforestation in western Canada

Laura Gray[1, 2], Tim Gylander[1, 2], Michael Mbogga[1, 2], Pei-Yu Chen[1, 2], and Andreas Hamann[1]

[1] Department of Renewable Resources, Faculty of Agricultural, Life, and Environmental Sciences, University of Alberta, Edmonton, AB, Canada,[2] These authors contributed equally to the study

Human-aided movement of species populations in large scale reforestation programs could be a potent and cost effective climate change adaptation strategy. Large-scale management interventions, however tend to entail the risks of unintended consequences, and we propose that three conditions should be met before implementing assisted migration in reforestation programs: (1) evidence of climate-related adaptation lag, (2) robust model projections of suitable habitat to target assisted migration efforts, and (3) observed biological impacts suggesting that the risk of inaction exceeds the risk associated with changing established management and conservation practices. In a case study of aspen *(Populus tremuloides)* we evaluate a reciprocal transplant experiment that reveals a significant adaptational lag of populations. Secondly, we report results from bioclimate envelope modeling that predicts suitable habitat for locally adapted genotypes under observed climate and predicted climate change. Lastly, we are able to detect remotely sensed declines in productivity in natural aspen populations. These results suggest that assisted migration could enhance forest productivity and health and recommendations can be made with reasonable confidence over a 10 to 20-year planning horizon, but model uncertainty doesn't allow for long-term planning, which means forest management will somewhat lag behind optimal prescriptions.

Implementing assisted migration of populations to mitigate the impacts of climate change: the assisted migration and adaptation trial (AMAT)

Greg O'Neill , Michael Carlson, Nicholas Ukrainetz, Vicky Berger and Alvin Yanchuk

Research Branch, BC Ministry of Forests and Range

Tree improvement in British Columbia has been underway for several decades, with many noteworthy successes with improvements for growth, stem quality and pest and disease resistance. However, with the expected changes in species' (and their populations) adaptive profiles under current climate change projections, climate based seed transfer (CBST) and 'facilitated migration' of populations is being gradually implemented. High quality traditional provenance and progeny testing remain the corner stone for seed transfer policy in both static and changing climates; however, the questions that climate change present to forest geneticists provide some new challenges which are being addressed with the establishment of a new genecology experiment called the Assisted Migration and Adaptation Trial (AMAT). First, better response functions for both wild and commercial 'synthetic' populations will be needed, which require a much broader range of climate sampling than has been conducted in the past. Second, the AMAT includes inter- and intra-specific variations, with large plots designed to better deal with competition over time and provide accurate growth and yield data – as such, it may be one of the first experiments that will allow geneticists to investigate some of the interesting questions in the developing area of community and ecosystem genetics, with changing climates.

An integrated genomic, proteomic and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce genotypes that are resistant or susceptible to white pine weevil

Dawn Hall[1], Jeanne Robert[1], Christopher I. Keeling[1], Sharon Jancsik[1], Dominik Domanski[2], Alfonso Lara Quesada[1], Britta Hamberger[1], Michael Kuzyk[2], Christoph Borchers[2] and Joerg Bohlmann[1]

[1]Michael Smith Labs, University of British Columbia; [2]Genome BC Proteomics Centre, University of Victoria

Conifer trees are constantly challenged by pathogens and herbivores and have evolved complex terpenoid chemical defense mechanisms to combat these threats. Hundreds of structurally diverse terpenoids are formed in one conifer tree, the biosynthesis of which is dependent on the tree's genotype and environment. Terpenoid diversity is related to the size of the terpene synthase (TPS) gene family, and the single- and multi-product profiles generated by these enzymes. The biosynthesis of the monoterpene (+)-3-carene in pest-resistant and susceptible genotypes of Sitka Spruce is controlled by a small subfamily of closely related (85-95% sequence identity) (+)-3-carene synthase (3CS) genes. Resistant trees produce a high level of (+)-3-carene whereas only trace amounts of this metabolite are detected in susceptible trees. Transcript profiling identifies one 3CS which is expressed in both tree genotypes, one 3CS which is expressed in resistant trees, and one 3CS which is expressed in susceptible trees. Selected reaction monitoring was used to quantify 3CS protein abundance in crude Sitka spruce extracts and confirms the differential expression of the 3CS proteins in these trees. A comprehensive kinetic analysis of the recombinant 3CS proteins suggests the basis of the unique (+)-3-carene profiles observed in resistant and susceptible Sitka spruce genotypes.

MYB transcription factor in proanthocyanidin biosynthesis in poplar and apple

Andreas Gesell and C Peter Constabel

Centre for Forest Biology and Department of Biology, University of Victoria

Proanthocyanidins (PAs), also known as condensed tannins, commonly accumulate in leaves, roots and bark of woody plants such as poplar. PAs are a highly abundant natural product that can make up to 25% dry weight of plant tissues, and as such are an important carbon sink in trees. They are also found in fruit such as blueberry and apple, where they typically accumulate in the skin. Their abundance in many plants, together with their well known antioxidant and health-promoting activity in food plants, make studying PA synthesis and its regulation an important research target. We are studying MYB transcription factor that regulate PA biosynthesis, using promoter analysis and transcriptional activation by poplar or apple MYBs and other co-regulatory transcription factors. Based on expression analysis of poplar overexpressing PtMYB134 (a PA regulatory MYB factor), we have identified candidate WD40 and bHLH proteins predicted to cooperate with MYB regulators in activating a poplar anthocyanidin reductase (ANR) promoter. In addition, using in silico analysis of promoters of PA-specific and co-regulated genes, we hope to identify promoter regions that determine gene activation by specific MYB transcription factors. We are also exploring the possibility of using PtMYB134 to boost PA levels in heterologous systems such as apple and other functional foods. By comparing the activation abilities of MYB transcription factors from both poplar and apple transcription factor in trans-activation assays, we will test if heterologous systems are a practical way for the biotechnology industry to enhance PA synthesis in other crop plants.

Analysis of the Arabidopsis 4CL-like ACYL-CoA SYNTHETASE5 gene and co-expressed genes reveals an ancient biochemical pathway required for pollen development and sporopollenin biosynthesis.

SungSoo, Kim

University of British Columbia, Department of Botany

Formation of pollen and spore walls requires the deposition of sporopollenin, a poorly characterized mixed aliphatic/aromatic polymer with ester and ether linkages that contributes to the protective exine layer. We discovered that the Arabidopsis 4-coumarate:CoA ligase (4CL)-like enzyme Acyl-CoA synthetase5 (ACOS5) is required for pollen development. An acos5 mutant is sterile, devoid of visible pollen grains, and lacks sporopollenin. ACOS5 is transiently and exclusively expressed in tapetum cells and encodes an acyl-CoA synthetase with highest activity against medium chain hydroxy-fatty acids. In silico co-expression analyses identified Arabidopsis genes encoding potential enzymes that could work with ACOS5 to generate sporopollenin monomers. Previous studies and our analyses of co-expressed genes such as a dihydroflavonol-4-reductase-like1 (DRL1) and polyketide synthase (PKS) revealed that mutants in these co-expressed genes are also compromised in male fertility and sporopollenin deposition. Recent results suggest that co-expressed PKS enzymes use ACOS5-generated fatty acid starter molecules to produce polyketides that are reduced by DRL1 and incorporated into sporopollenin. Phylogenetic analyses showed that these genes are conserved in land plants including Physcomitrella. This work illuminates the outlines of an ancient but previously uncharacterized pathway involved in biosynthesis of sporopollenin monomer, one of the most robust cell wall matrices known in plants

A genomic resource for *Grosmannia clavigera* and insights into fungal tolerance towards tree defences

DiGuistini S.[1], Wang Y.[1], Alamouti SM.[1], Hesse U.[1], Hirst M.[2], Jones SJM.[2], Hamelin R.[3], Bohlmann J.[4], and Breuil C.[1]

[1] Dept. of Wood Science, University of British Columbia, Vancouver, BC, V6T 1Z4; [2] BC Cancer Agency Genome Sciences Centre, Vancouver, BC, [3] Natural Resources Canada, Ste-Foy, Quebec; [4] Michael Smith Laboratories, University of British Columbia, Vancouver, BC.

Grosmannia clavigera, is a lodgepole pine pathogenic fungus specifically associated with the Mountain Pine Beetle (MPB). The current MPB outbreak continues to kill lodgepole pine forests in British Columbia and Alberta, and is the largest in Canadian history. In British Columbia alone the beetle and its fungal associates have killed 620 million cubic meters of lodgepole pine forest. In our work to understand the role of *G. clavigera* in such outbreaks, we generated genomic resources including: a) the whole genome sequence, b) ESTs and c) RNA-seq data describing fungal gene expression when the fungus was exposed to tree phloem extract and terpenes. We annotated the protein coding genes on this fungal genome using these resources (e.g. ESTs and RNA-seq) and computational (gene prediction software) data. Finally, we are starting to clarify the relationships between lodgepole pine defences and this host-specific pathogen. We provide a preliminary evaluation of the *G. clavigera* protein coding gene families as well as the first insights into how this fungus tolerates host-specific defence metabolites including a comparison between the *G. clavigera* WT and an ABC transporter knockout. The ABC transporter was identified as the most strongly induced gene in the *G. clavigera* genome following terpene-treatment.

SYMPOSIUM SPEAKER:

Forests and carbon: positive feedback to climate change or opportunities for mitigation?

Werner A. Kurz,

Natural Resources Canada, Canadian Forest Service,
Victoria, BC, Canada

Forest ecosystem responses to global climate change will affect the net carbon (and non-CO_2 greenhouse gas) exchange between the atmosphere and the biosphere. Processes that increase net emissions, such as large-scale shifts in vegetation zones, increases in natural disturbances (fire, insects), higher decomposition rates, and melting of permafrost all provide “positive feedback” to climate change. In contrast, processes that increase sinks, such as higher growth rates and expansion of forest areas can provide “negative feedback”. Given the complexity of ecosystems, the diversity of ecological conditions, the long time scales, and the uncertainties in carbon cycle models, probabilistic model analyses are used to assess the range of possible forest responses to climate change. Results indicate that these responses can strongly affect the level of mitigation efforts required to reach atmospheric greenhouse gas stabilization targets. Climate mitigation objectives can be achieved through forest management and forest sector activities that maintain or increase carbon storage in forests, increase carbon storage in harvested wood products, use wood to substitute for more energy-intensive materials such as steel, concrete or plastics, and use bioenergy to substitute fossil fuels. Which mitigation strategies yield the greatest climate benefit is the subject of ongoing research.

Net CO_2 fluxes from mountain pine beetle attacked pine forests in northern BC.

Bowler, R., Fredeen, A.L., Brown, M., and Black, T.A.

Ecosystem Science and Management Program, Natural Resources and Environmental Studies Institute, University of Northern British Columbia

This presentation will provide an overview of the effects of mountain pine beetle (MPB) attack on the net CO_2 exchange (NCE) from MPB-attacked, pine-leading forests in northern BC. The overarching question that we address is how much of a sink or source these stands are in the first 3-5 years following attack. More specifically this presentation will address the importance of the residual live vegetation components (both tree and understory) on the net carbon balance. To assess the latter, we measured net photosynthesis in needles and leaves from residual vegetation at regular intervals in the field over the 2007, 2008 and 2009 growing seasons, scaled them up to the stand level spatially using biomass data for each vegetation layer, and temporally using empirically generated relationships between environmental variables and foliar net photosynthetic rates using continuous environmental data from the flux-towers. We compare and contrast the modeled net photosynthesis for the entire stand from this approach with the flux-tower, eddy-covariance estimates for the stand. We will conclude with our assessments of the importance of residual vegetation to the carbon dynamics and source to sink transitions in MPB affected stands in northern BC.

The effect of nitrogen fertilization on total ecosystem and component carbon fluxes in an age sequence of three coastal Douglas-fir stands in BC.

Trofymow, J.A.[1,3], Black, T.A.[2], Jassal, R.S.[2], Nesic, Z.[2], Roy, R.[3], and Spittlehouse, D.L.[4]

[1]Natural Resources, Canadian Forest Service, Victoria, B.C., V8Z 1M5; [2]Biometeorology and Soil Physics Group, University British Columbia, Vancouver, BC; [3]University of Victoria, Victoria, B.C.; [4]BC Ministry Forest and Range, Victoria, BC

Nitrogen fertilization, a common practice in various forest ages in coastal BC, has been suggested as an incremental management practice to increase C sequestration. On eastern Vancouver Island measurements of eddy covariance net ecosystem production (NEP), component C fluxes and stocks have been made since 2002 in three Douglas-fir dominated sites: regeneration planted 2000 (HDF00), juvenile planted 1988 (HDF88) and near-rotation planted 1949 (DF1949). In winter 2006-07 all three stands were urea fertilized, either helicopter broadcast 200 kg N/ha (DF49 and HDF88) or by hand at seedling dripline 40 kg N/ha (HDF00). Fertilization effects on NEP were determined from an empirical model fitted to multi-year pre-fertilization monthly fluxes to calculate unfertilized C fluxes in 2007-09 and compared to actual C fluxes. Component flux measurements were made in plots in fertilized and control unfertilized areas at each site. The largest net increase in NEP with fertilization was in DF49 (~180 g $C/m^2/yr$), then HDF88 (~160 g $C/m^2/yr$) and HDF00 (~50 g $C/m^2/yr$) and primarily due to increased GPP. In 2007, there was a slight increase in soil respiration and litter decay, and decreases in CH4 uptake. N2O loss was observed in 2007 but not in subsequent years. The fertilized vs control 3-yr above-ground growth increment increased in DF49 and HDF88 but decreased in HDF00.

Constraining the estimates of temperature sensitivity of soil heterotrophic respiration.

C. Smyth and W.A. Kurz

Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, **BC**

Plant detritus and soil organic matter are the largest carbon pools in terrestrial ecosystems. Annual natural fluxes of carbon in and out of terrestrial and ocean reservoirs are an order of magnitude larger than the perturbation from fossil fuels and land-use change (Schuur et al. 2008). Thus, understanding the dynamics of plant detritus and humified organic matter decomposition and their responses to global change is of critical importance for understanding the future greenhouse gas balance of terrestrial ecosystems. In this study, the temperature sensitivity of slowly decaying pools was estimated by comparing predictions of forest floor and mineral soil carbon stocks to measured stocks. Predictions were from the Carbon Budget Model of the Canadian Forest Sector (CBM-CFS3), an inventory-based model of forest carbon dynamics that is used to produce annual reports on greenhouse gas emissions and removals in Canada's managed forests. The measurements were from ~600 ground plots and included forest floor and mineral soil carbon stock estimates. Decay rates and temperature sensitivities were estimated by minimizing the residual error between predictions and measurements. Various combinations of parameters in the model can yield similar residual errors but result in different temperature sensitivities. National emissions from dead organic matter were estimated to year 2100 for various plausible temperature sensitivities with an A2 climate scenario.

Reference: Schuur, E. A. G., J. Bockheim, et al. (2008). "Vulnerability of Permafrost Carbon to Climate Change: Implications for the Global Carbon Cycle." BioScience **58**: 701-714.

Experimental embryogenesis of plasticity in cold tolerance of interior spruce

Marie Vance, Patrick von Aderkas, Lisheng Kong, Barbara Hawkins
Centre for Forest Biology, and Department of Biology, University of Victoria Victoria BC

Plasticity within key life history traits may allow spruce to persist in areas that are predicted to undergo significant changes in climate over the next century. In this study we exposed the somatic embryos of interior spruce (*Piceae glauca* x *P. engelmannii*) to non-freezing low temperatures during either early or late embryogenesis. Mature embryos were frozen at -6, -11, or 16 C and cold damage was assessed via the measurement of electrolyte leakage. Under the most extreme conditions (-16 C), pre-treatment with non-freezing low temperatures was found to increase cold tolerance by a significant amount. This effect was seen regardless of whether the treatment was applied during early or late embryogenesis. This suggests that cold tolerance acquisition may operate through mechanisms other than the developmentally associated mechanisms. It also confirmed spruce's broad phenotypic plasticity for cold tolerance acquisition. If plasticity allows for the maintenance of fitness under sub-optimal conditions, it may buffer against the predicted losses, giving these species time to adapt to the changing environment.

Phenological timing dictates carbon partitioning in poplar in different climates

Raju Y Soolanayakanahally [1, 2], Robert D Guy[1], Salim N Silim[2] and William R Schroeder[2]

[1]Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada,
[2]Agri-Environment Services Branch, Agriculture Agri-Food Canada, Indian Head, SK

The steady lengthening of the growing season observed worldwide results from higher temperatures in both spring and autumn periods. In nature the two main environmental cues that announce growth and dormancy in trees are temperature and day length (photoperiod). The role of temperature has been well documented. However, the role of photoperiod, a more reliable evolutionary cue with no variation over successive years, has been largely overlooked. Unlike bud flush, bud set is under tight photoperiodic control which thereby dictates height growth during active growing season. Here we report interactions between growing season length and photoperiodic response during growth phenophases (bud flush, leaf unfolding, bud set and green-cover period) in a range-wide collection of balsam poplar (*Populus balsamifera* L.) planted in two common gardens at similar latitude but with sharply contrasting climate. Our 3-year observations suggest that although balsam poplar possesses the plasticity to appropriately adjust phenology to warm springs by flushing earlier, it then also tends to reach photoperiodic competency earlier. This behavior results in an earlier bud set in an extended green cover period, thereby favoring carbon partitioning to roots over shoots. Enhanced partitioning to roots may contribute to increased belowground carbon storage and/or soil respiratory flux.

INVITED SPEAKER:

The tank is half full: biofuel development gains from poplar tree physiology and genomics research

Athena McKown

Department of Forest Sciences, Applied Genomics Innovation Program, University of British Columbia,

Both considerable public interest and green energy mandates in British Columbia are driving research in bio-based fuel alternatives to petroleum. An extensive project, spearheaded by UBC and UVIC, is focusing efforts on the possibility of obtaining lignocellulosic ethanol fuel from fast-growing, native poplar trees as a potential viable energy source in the Pacific northwest. The genomically sequenced cottonwood poplar (*Populus trichocarpa*) is a widespread tree and ranges from coastal Alaska to California, showing local adaptation in relation to growing season and climate. In order to apply native *P. trichocarpa* trees to biofuel research, current studies are investigating numerous avenues of basic tree research from adaptive physiology of *P. trichocarpa*, to structure and genetics of wood and lignin, to single nucleotide polymorphic differences in candidate genes, to continental population structure. A few thousand trees (representing replicate plantings of 499 collected genotypes of interest in 151 provenances) are being grown for studies in common gardens at UBC and Surrey. Trees have been monitored or sampled for numerous traits relating to seasonal phenological events, biomass and growth, nutrient analysis, wood, and preliminary candidate gene investigation. On-going results for this significant initiative are demonstrating that *P. trichocarpa* phenotypes and associated adaptative traits are highly linked to provenance origin.

Streamside trees: responses of male and female cottonwoods to flooding

Julie L. Nielsen[1], Stewart B. Rood[1]*, David W. Pearce[1], Matthew G. Letts[2], and Hester Jiskoot[2]

[1]Department of Biological Sciences, [2]Department of Geography, University of Lethbridge, Alberta, Canada, *Corresponding author

Cottonwoods, riparian poplar trees, are dioecious and prior studies have indicated that female poplars and willows are more abundant than males in low-elevation zones which are more frequently flooded. We investigated sex differentiation in response to flooding with clonal saplings of twelve male and nine female narrowleaf cottonwood (*Populus angustifolia*) genotypes grown in a greenhouse, along with three females of a native hybrid (*P. x jackii* = *P. deltoides x P. balsamifera*). Three constant water level treatments were provided and complete substrate inundation provided the flood treatment. In the non-flooded condition, the hybrids had 3.9-fold more dry weight than the narrowleaf cottonwoods ($P<0.01$) and in both cottonwood taxa, the flood treatment reduced stem heights and dry weights, root and leaf areas and weights, leaf chlorophyll, and stomatal conductance (all $P<0.01$). Inundation increased the foliar carbon-to-nitrogen ratio (+11%; $P<0.05$) but did not significantly alter leaf water potential (mean -1.5 MPa) or foliar $\delta^{13}C$, which was lower in *P. angustifolia* (-32.8‰) than *P. x jackii* (-31.5‰; $P<0.05$). The water level treatments influenced root distribution, and roots were sparse in the saturated substrate and abundant in the capillary fringe above. The male and female *P. angustifolia* genotypes performed similarly with the favourable water levels, but growth of the males tended to be more inhibited by flooding. The sapling dry weights of males were reduced by 56% versus a 44% reduction for females ($P=0.1$), and there were similarly lower reductions for leaf, stem and root dry weights in the females. These results demonstrate the inundation response of floodplain trees and indicate relative flood-tolerance as: *P. angustifolia* female > *P. angustifolia* male > *P. x jackii* female. This indicates that the narrowleaf cottonwood is highly flood-tolerant and that females are probably more flood-tolerant than males. We propose the concept of 'strategic positioning', whereby the seed-producing females could be better adapted to naturally flooded, low elevation streamside zones where seedling recruitment succeeds.

***MUSTACHES* regulates bilateral symmetry generation in stomata**

Sandra Keerthisinghe, J. Nadeau, J. Lucas, T. Nakagawa, and F. Sack

Departmentof Botany, University of British Columbia

Stomata are specialized epidermal structures, consisting of a pore surrounded by two bilaterally symmetrical guard cells (GCs), which regulate gas exchange across the plant epidermis. Stomata form through a dedicated cell lineage via one asymmetric and one symmetric division. The latter division, along with subsequent cell wall shaping, generates a mirror-like bilateral symmetry across the two GCs. Thus, *Arabidopsis* stomata provide an accessible system for analyzing the cell and molecular mechanisms responsible for the development of bilateral symmetry.

MUSTACHES (*MUS*) encodes a Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK). In *mus* mutants, the bilaterally symmetrical shape of normal GCs and the radial arrangement of the microtubule array are both disrupted. MUS appears to act early in stomatal morphogenesis, since it is expressed before symmetric division and the formation of radial microtubule arrays. In almost all cell types examined, MUS is expressed at the site of cytokinesis and new cell wall formation. However, in guard mother cells about to undergo a symmetric division, MUS is only expressed in the cell periphery. Thus, MUS signalling at the cell periphery appears to regulate the cell wall symmetry at the centre of the stomate, as well as the alignment and polarity of GC microtubule arrays.

The Tria project: mountain pine beetle system genomics

Christopher I. Keeling, Hannah Henderson, Maria Li, and Jörg Bohlmann
Michael Smith Laboratories, University of British Columbia

The mountain pine beetle (MPB) is having a devastating effect on the pines in Western North America, particularly in British Columbia. MPB is now entering into new habitats and onto new hosts on the Eastern side of the Rocky Mountains. To understand the interactions between the mountain pine beetle, its associated fungi, and the host pine trees, the Tria Project* seeks to develop genomic resources and generate new information for the MPB, MPB-associated fungi, and host pine trees. This information will be used to produce integrated genetic landscape maps of the MPB-fungal-tree complex and the combined genomic and genetic information will be incorporated into ecological risk models. Within the larger project, we specifically have developed expressed sequence tag (EST) and genome sequence resources for the beetle, the fungi, and the pines. In the beetle, we are studying the processes of olfaction and pheromone biosynthesis using functional genomics approaches.

The Tria Project Team
Project Leaders: Jörg Bohlmann[1] and Janice Cooke[3],
Co-Investigators: Brian Aukema[2,4], Colette Breuil[1], David Coltman[3], Barry Cooke[4], Nadir Erbilgin[3], Maya Evenden[3], Richard Hamelin[4], Robert Holt[5], Dezene Huber[2], Steven Jones[5], Christopher Keeling[1], Marco Marra[5], Brent Murray[2], and Felix Sperling[3]

[1]University of British Columbia, [2]University of Northern British Columbia, [3]University of Alberta, [4]Canadian Forest Service, Natural Resources Canada, & [5]Canada's Michael Smith Genome Sciences Centre

Regulation of secondary cell wall biosynthesis in Arabidopsis by a KNAT7 transcription factor complex

Yuanyuan Liu, Eryang Li, Shucai Wang, Jin-Gui Chen, and Carl Douglas

Department of Botany, University of British Columbia, Vancouver

The plant secondary cell wall is a composite network of complex polymers (cellulose, lignin, and hemicellulose) that provides protective and structural properties to the cell wall. The *Arabidopsis KNOX* gene *KNAT7* has been identified in transcriptional profiling and other experiments as a member of a transcriptional network regulating secondary wall formation during in xylem and fiber cell differentiation in *Arabidopsis* inflorescence stems. We have characterized the phenotypes of *knat7* mutants, which display an irregular xylem (*irx*) phenotype, as well as increased fiber wall thickness. KNAT7 interacts with members of the Ovate Family Protein (OFP) transcription co-regulators. We confirmed the KNAT7-OFP1 and KNAT7-OFP4 interactions by yeast two hybrid analyses and by biomolecular fluorescence complementation analyses *in planta*, and showed that the interaction enhances KNAT7 transcriptional repression activity *in planta*. Furthermore, an *ofp4* mutant exhibits similar phenotypes as *knat7*, and the pleiotropic effects of *OFP1* and *OFP4* overexpression depend upon *KNAT7* function. Co-expression and yeast two hybrid analyses suggest that BELL-LIKE HOMEODOMAIN (BLH) transcription factors could be part of a KNOX-BELL-OVATE transcription factor complex regulating aspects of secondary cell wall formation, together with KNAT7 and OFP1/4. We are further investigating the functional interactions of BLH partners with KNAT7 and OFP proteins through yeast two hybrid and *in planta* biomolecular fluorescence complementation analyses, and have identified a BLH partner that specifically interacts with KNAT7. To determine the function of this BLH protein, the phenotypic effects of *BLH* overexpression, *blh* loss of function, and *blh/knat7* double mutants on stem anatomy and cell wall biochemistry are being investigated.

Genetic modification of the poplar defense pathway to enhance resistance against phytopathogens

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A strategy that targets defense signal transduction pathways was used to enhance disease resistance in poplar. The nucleotide sequence encoding antimicrobial peptide MsrA2 (*N*-methionine-dermaseptin B1) was transcriptionally fused to the native poplar promoter, *win3.12T*, and introduced into commercial hybrid poplar *Populus nigra* L. x *P. maximowiczii* A. Henry via *Agrobacterium*-mediated transformation. This promoter contains several pathogen-responsive *cis*-acting elements, exhibits strong systemic activity in response to a variety of pathogens, and is thought to be a part of the poplar defense system. Stable transgene integration into plants regenerated on selective medium was confirmed by PCR and Southern analyses. Northern analysis showed accumulation of *MsrA2* transcripts in response to pathogen infection. Most importantly, the expression level of the MsrA2 peptide in transgenic plants, regulated by the *win3.12T* promoter, was sufficient to confer resistance against the poplar-specific pathogen *Septoria musiva*. In addition, a simple and efficient protocol for transformation, selection and regeneration of poplar was developed. It increased the number of transgenic plants up to 30-fold compared to previously reported systems for *Populus* transformation. The *win3.12T*-driven accumulation of MsrA2 peptide in transgenic poplars had no deleterious effect on plant growth and development. Expression of this peptide in poplar has the potential both for generating disease resistance and for molecular farming of antimicrobial therapeutics.

POSTERS

Cloning and functional characterization of a monoterpene transporter from lavender

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Lavenders are aromatic shrubs cultivated for their essential oils used in the manufacturing of cosmetic and personal care products. The essential oil is dominated by monoterpenes, which are synthesized in secretory cells and secreted into the storage cavity of glandular trichomes, or oil glands. Although numerous genes encoding monoterpene biosynthetic enzymes have been defined from various plants, the trafficking and secretion of these compounds is poorly understood. We hypothesize that monoterpene trafficking is facilitated by protein transporters, and intend to functionally characterize a putative ABC transporter that is strongly expressed in lavender glandular trichomes. This clone, which was isolated from a lavender flower cDNA library, contains all signature motifs present in known ABC transporters. Furthermore, the expression – at the level of transcription – of the gene strongly correlates that of known monoterpene synthases in lavender oil glands as depicted by qRT-PCR. We are currently in the process of recombinant expression, and functional characterization of this transporter in *Xenopus oocytes*.

Rhododendron leaf chlorophyll *a* fluorescence (Fv/Fm), dormancy of flower buds and vegetative buds, and greenhouse forcing - and a comparison with the chlorophyll Fv/Fm of senescing *Hydrangea* leaves.

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Chlorophyll *a* Fv/Fm of leaves of dormant *Rhododendron* plants was determined after plants were moved into a greenhouse following various times in the field. Chlorophyll fv/fm of *Hydrangea* leaves was also determined in the fall. A decrease in *Rhododendron* chlorophyll Fv/Fm could de detected, which coincided with a loss of dormancy in the field in the fall, and was indicated by a decreased flowering time in the greenhouse, and also by an increase in growth of vegetative shoots in the greenhouse. The decrease in *Rhododendron* Fv/Fm in the fall was not similar to that of senescing *Hydrangea* leaves in the fall, which was accompanied or preceded by a considerable drop in chlorophyll levels.

Trichoderma spp. as antagonists against _Phytophthora ramorum_, the Sudden Oak Death pathogen

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The pathogen *Phytophthora ramorum* causes a foliar blight in many host plant species. Isolates of *Trichoderma* spp. have shown effective biocontrol against other oomycete pathogens. We are investigating the potential of *Trichoderma* spp. to limit infection of plants by *P. ramorum*. Direct antagonism of *P. ramorum* was evaluated using a dual-culture assay in petri plates. Isolates of eight *Trichoderma* spp. were assayed. The highest rates of overgrowth were by *T. koningii, T. viride* and *T. virens* (7.8, 6.9, and 10.5mm/day), which also had the highest rates of lethal effect on *P. ramorum* (6.0, 5.4, and 10.5mm/day). A high rate of overgrowth did not always translate to a lethal effect on *P. ramorum.* These results suggest that certain species of *Trichoderma* have the ability to kill *P. ramorum* after it has established. Indirect effects of *Trichoderma* metabolites on *P. ramorum* are also being investigated in ongoing studies, using an *in vitro* assay of culture extracts. The results of these and other screening methods will be compared to see if they correlate and if they are predictive of leaf assays. Future work will investigate the possibility of combining promising *Trichoderma* isolates with other protective treatments in an integrated pest management approach.

Quantitative genetic variation and adaptive clines in Pacific dogwood (*Cornus nuttallii*): Evidence of weak local adaptation

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Local adaptation is widespread in temperate and boreal tree species. Adaptive clines in quantitative genetic traits along environmental clines have been demonstrated in many such species. Among-population differentiation in quantitative genetic traits (Q_{ST}) is also typically much higher than differentiation in neutral genetic markers (F_{ST}), suggesting that populations have undergone divergent selection. While local adaptation is well documented in widespread, wind-pollinated, commercially important conifers, this study is the first of its kind in Pacific dogwood (*Cornus nuttallii*), a relatively small, insect-pollinated hardwood that grows from California to southern British Columbia. A common garden consisting of individuals collected from range-wide provenances was used to look for patterns of local adaptation in several quantitative traits related to phenology and cold tolerance. In general, Q_{ST} values were only slightly higher than a previously estimated F_{ST} value. Adaptive clines were not found for all traits, and when found, were generally shallow. These results suggest that Pacific dogwood is only weakly locally adapted. Weak local adaptation may partially be the result of demonstrated low levels of genetic diversity in the species and hypothesized high levels of gene flow, and may put Pacific dogwood at greater risk of being outcompeted in the future by other species that will presumably be able to more successfully adapt to changing climates.

Target-specific PCR primers can detect and differentiate ophiostomatoid fungi from microbial communities associated with the mountain pine beetle *Dendroctonus ponderosae*

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The mountain pine beetle (MPB), *Dendroctonus ponderosae* is closely associated with a number of fungal species that include *ophiostomatoid* fungi that are difficult to differentiate morphologically. Of these, the most frequently isolated are the two pine-pathogens *Grosmannia clavigera* and *Leptographium longiclavatum*, the less pathogenic *Ophiostoma montium*, and an undescribed species in the genus *Ceratocystiopsis*. DNA sequencing can be used to identify species, but is expensive when many samples need to be tested. Because growing, isolating and extracting DNA from fungi vectored by MPB can be time and labour intensive, we designed three rDNA primer sets, Lepto, Omon, and CopMPB that specifically amplify short rDNA amplicons from the *Leptographium* clade, *O. montium*, and *Cop.* sp., respectively. We also designed two primer sets, Gclavi and Llongi, on a gene of unknown function that can differentiate *G. clavigera* and *L. longiclavatum*, two closely related species in the *Leptographium* clade. Primers were tested on 76 fungal isolates that included MPB associates. The primers rapidly and reliably identified their targets from DNA obtained from pure fungal cultures, pulverized beetles, beetle galleries, and from tree phloem inoculated with *G. clavigera*. These specific primers that detect fungi from DNA extracted from various substrates will facilitate large-scale work on the ecology of the MPB-fungal-lodgepole pine ecosystem, and applications for phytosanitary/quarantine sample screening.

Conifer ovular secretions: more than a pollination mechanism?

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Conifer ovules produce a liquid secretion as part of sexual reproduction. The secretion fills and protrudes from the micropyle in close coincidence with pollination. Termed ovular secretions, these exudates are widespread among modern conifers. To date they have been observed in all families of Coniferophyta except the Araucariaceae. Within the families that do contain drops, only the genera *Saxegothaea* (Podocarpaceae), *Abies* (Pinaceae), and some species of *Tsuga* (Pinaceae) do not produce secretions. Because gymnosperms lack the complex receptive tissues for pollen found in the angiosperms, ovular secretions are widely viewed as a mechanism to capture wind-borne pollen. Our lab has identified proteins in the ovular secretions of six conifer species in three families (Taxaceae, Pinaceae, Cupressaceae). These proteins represent a significant metabolic investment and suggest that ovular secretions function as more than a simple pollination mechanism. Putative functions of these proteins indicate the ovular secretion may play a role in protecting the ovule from microbial infection and in pollen tube growth and guidance. We are currently working towards confirming these functions.

Characterization of Western White Pine Candidate Genes for Resistance to Blister Rust Fungus *Cronartium ribicola*

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Western white pine (WWP, *Pinus monticola*) is severely damaged by white pine blister rust (WPBR) caused by the fungus *Cronartium ribicola*. Multiple phenotypic traits of resistance have been found in WWP populations, including major gene (*Cr2*) resistance. The purpose of this study is to identify essential WWP genes that contribute to resistance against the blister rust pathogen for more effective breeding programs. Firstly, as part of an effort to expand our understanding of the WWP transcriptome, a expressed sequence tag (EST) library was constructed from needles of a Cr2 family at early stages after *C. ribicola* infection. From 5,000 clones randomly sequenced, 3,034 unique ESTs were identified and annotated by similarity searches against DNA databases. Annotated transcripts were classified by the functional assignment of their Arabidopsis homologous genes. This functional categorization has revealed several putative signal transduction genes, transcription factor genes, and down-stream stress-responsive genes. Candidate genes for host resistance will be selected to examine their expression pattern and regulation during disease infection. These data will provide potential molecular markers for selection of WPBR resistance.

Functional Analysis of the Shikimate/Quinate Cycle

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The shikimate pathway connects primary metabolism with the synthesis of essential aromatic amino acids, i.e. phenylalanine, tyrosine and tryptophan. Both the end-products and intermediates are also precursors of a wide array of pivotal plant natural products including alkaloids and phenylpropanoids, which play important adaptive functions in plant development and defense. Branching out from the main trunk of the shikimate pathway, quinate is being formed in a single step reaction. It is thought to be a precursor of defense-related chemicals in plants, and may act as an intermediate in lignin biosynthesis. It is evident that quinate can be synthesized from both shikimate and dehydroquinate (precursor of shikimate), and enzymes involved in those two processes are quinate hydrolyase (QH) and quinate dehydrogenase (QD). However, genes encoding those two enzymes have not been identified to date. The reaction mechanisms of QD and DH resemble that of shikimate dehydrogenase (SDH) and dehydroquinate dehydrogenase (DHQD) respectively. DHQD/SDH is a bifuntional enzyme acting in the shikimate pathway in plants. The DHQD/SDH gene family comprises 5 members in poplar and we hypothesize that QD and QH are actually encoded by some of the five DHQD family members. The five DHQD/SDH genes have diverged into two phylogenetic groups, one of which does not include Arabidopsis homologues. Since quinate derivatives are not known to accumulate in Arabidopsis; it is plausible that the absent group of genes instead encode QD and QH. The main focus of our project is to characterize the DHQD/SDH gene family in poplar and to identify the genes involved in quinate biosynthesis employing a functional genomic approach that includes bioinformatics-based candidate gene identification and reverse biochemical characterization of the corresponding candidate genes.

ESTs of the beetle-vectored fungal tree pathogen *Grosmannia clavigera*

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Grosmannia clavigera (Gc), a beetle-vectored fungal pathogen of pines, causes wood discoloration and kills host trees by disrupting the flow of nutrients and water in phloem and sapwood. While our previous analysis revealed that Gc degrades antifungal pine terpenoids, no information is available on fungal genes involved in responding to phenolic plant defences. To obtain insight into how Gc overcomes tree defenses and grows on wood, we constructed seven cDNA libraries from eight strains grown under various culture conditions, and sequenced ~50,000 ESTs. Assembly resulted in 6,265 contigs and 2,459 singletons that mapped to 6,517 locations on the genome. We filtered the contigs to identify misassemblies caused by gene overlap. The 54 % of the unigenes that matched characterized proteins at the NCBI database indicate that expressed genes correspond to major metabolic pathways, cellular processes, and environmental and genetic information processing. Here, we describe the Gc unigene dataset, focusing on 54 P450s, genes expressed early in spore germination, and genes involved in responding to treatment with lodgepole pine phloem extract that contains phenolic compounds.

Characterization of dsRNA viruses in Canadian isolates of the Dutch Elm pathogen *Ophiostoma novo-ulmi*.

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Ophiostoma novo-ulmi, the casual agent of Dutch elm disease (DED), spreads throughout the Elm tree's vascular system eventually killing the tree. Two destructive pandemics of the disease have occurred in North America and Europe during the last century. It seems DED can't be stopped once it begins and there are currently no effective control methods available. In O. novo-ulmi double-stranded RNA (dsRNA) viruses have been associated with reduced virulence (hypovirulence), growth, and sporulation providing a possible route towards developing a biological control. Two isolates (O. novo-ulmi 93-1224 and O. novo-ulmi VA30) were examined by electron microscopy. Two different viruses were found in O. novo-ulmi 93-1224 isolate, while one virus was found in wild type isolate O. novo-ulmi VA30. All of these viruses are encapsulated, non-enveloped, long, rod-like with helical structure. The viruses were purified and separated by density gradient centrifugation. Mass spectroscopy was performed and partial sequences obtained from viral coat proteins; these were used for designing specific primers. We are currently in the process of identifying viral sequences. The goal of this research is to understand which virus causes hypovirulence of DED in Manitoba, and to make a phylogenetic comparison with other dsRNAs from other viral families. This information will eventually be used as a part of a biological control strategy.

Genetic and cell biological regulation of stomatal bilateral symmetry

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Arabidopsis stomata provide an accessible model system for studying how bilateral symmetry is established. Stomata, which are valves that regulate shoot gas exchange, consist of two 'kidney shaped' guard cells surrounding a pore. The bilateral symmetry of the stoma is essential for proper valve function. Stomata form through a dedicated cell lineage via one asymmetric and one symmetric division. The latter division along with cell wall shaping generates a mirror-like symmetry. To define the genetic and cell biological basis for this symmetry, mutations in various genes were identified that alter stomatal symmetry. Mutations in some genes cause stomatal cell walls and cytoskeletal arrays to be skewed, whereas those zin other genes induce centralized arrays. In addition, the effects of mutations on cytoskeletal dynamics will be discussed. Our data reinforce the view that the cytoskeleton is essential for establishing stomatal symmetry and highlight key organizational levels at which different "stomatal" genes act.

Interim measures for the assisted range and population expansion of western larch for use as a climate change adaptation strategy in British Columbia: *bringing research into the realm of policy and practice*

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Climate change adaptation strategies such as climate-based tree species range and population expansion support British Columbia's Climate Action Secretariat that envisions that "British Columbia is prepared for and resilient to the impacts of climate change." This overarching vision includes the need to "make adaptation a part of the BC Government's business, ensuring that climate change impacts are considered in planning and decision-making across government" and "to assess risks and implement priority adaptation actions in key climate sensitive sectors." The BC Ministry of Forests and Range, Forest Stewardship Division has recently begun to re-focus its policy and practices through a climate change adaptation lens. The purpose of this initiative is to develop an interim measure for the assisted range and population expansion of western larch (Lw) in areas that are projected to be climatically suitable for the year 2030. The intended outcome of this initiative is to increase tree species and genetic diversity; maintain or enhance future timber supplies; and to reduce tree species vulnerability. In this case, the proposed interim measure aims to expand the tree species profile (i.e. Lw component) across British Columbia while managing for uncertainty given the current risk tolerance of decision makers in British Columbia.

vSEED – creating a virtual seed

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The ERA-NET project vSEED (www.vseed.org) aims at a dynamic mathematical description of seed dormancy, after-ripening and germination, using the related Brassicaceae species *Arabidopsis thaliana* and *Lepidium sativum* (garden cress) to develop a "virtual seed". The project integrates data from molecular biology, plant physiology, biomechanics and material sciences to identify key transcriptome networks, cell wall components and biophysical attributes of specific seed compartments that regulate testa and endosperm rupture and radicle emergence. Arabidopsis and cress both have a two-step germination, with testa rupture and endosperm rupture taking place sequentially. Only endosperm rupture is inhibited by abscisic acid (ABA). As cress seeds are about twenty times larger than Arabidopsis seeds, we concentrate our biomechanical analyses on cress. The micropylar endosperm of cress, which covers the radicle, weakens during germination, as could be shown by puncture force measurements. ABA inhibits this weakening. High magnification pictures of germinating cress and Arabidopsis seeds taken in an environmental scanning electron microscope help elucidate structural changes on the cellular and tissue level during testa- and endosperm rupture.

An investigation into the genetic transformation of western white pine for study of resistance against white pine blister rust.

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White pine blister rust (WPBR) caused by the rust fungus *Coronartium ribicola* J.C. Fischer is one of the most devastating disease of white pine (*Pinus monticola* Dougl.) trees of the Canadian forests. The results from our field screening program in British Columbia, Canada indicated partial resistance against *C. ribicola* with low incidence in some populations. Several candidate genes with expression rate regulated differentially between the resistant and susceptible families at the time of infection have been identified. RGA618 gene has been selected based on its homology to known R genes from other plants and its expression profiling. To examine the functionality of the gene in a closely related species, selected R candidate gene was introduced to SE cell cultures of Pinus strobus, originated from immature seed embryos. Transformation with the RGA gene was done through cocultivation of embryogenic tissue with Agrobacterium tumefaciens. From our first attempt we recovered 16 kanamycin resistant sublines. Of the first 16 sublines, we determined a considerable expression level of the candidate R gene in six sublines using absolute qPCR. Our preliminary results indicated a successful transformation and expression capability in the new host plant.

Characterization of resistance against white pine blister rust using in vitro techniques.

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Western white pine (*Pinus monticola* Dougl.) is one of the most significant conifers in western Canada for its economic, social and ecological value. White pine blister rust (WPBR) caused by the rust fungus *Coronartium ribicola* J.C. Fischer is one of the most devastating disease of western white pine trees. Different types of resistance with a very low frequency have been observed in a 4 yr field screen program. The resistant plants have been categorized in 4 major groups: difficult-to-infect (DI), bark reaction (BR), slow canker growth (SCG) and needle shed (NS). A disease assessment index (DAI), based on both *in vitro* and *in vivo* techniques, to evaluate specific reactions to the pathogen. The *in vitro* method provides a new approach to study inoculation in an axenic environment under controlled condition. The results from electron microscopic study of different tissues and organs from several samples of the DI resistant family collections indicated a significant difference ($p < 0.05$) in their needle structure with of those from control/ susceptible families. Basically, the statistical analysis of the amount of epicuticular wax indicated that there is a significant difference ($p < 0.05$) between DI resistant families more than the control families. Evaluation of epicuticular wax using chloroform method confirmed the electron microscopic results in that the majority of the stomata in DI resistant families were occluded.

Ecology, biology and control of some exotic-invasive weeds on federal lands in British Columbia, Canada.

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Scotch broom (*Cytisus scoparius*), Gorse (*Ulex europaeus*), Daphne (*Daphne laureola*), and English ivy (*Hedera helix*), are prominent, invasive plants posing a serious threat to Garry oak and associated ecosystems on federal lands in Victoria, British Columbia. These plants colonize disturbed areas quickly, form dense mono-specific stands, remain persistent, and defy easy eradication programs. They suppress and inhibit the growth of native plants and ultimately arrest forest succession. Several federal departments and Parks Canada have expressed great concerns regarding their rapid incursion, adverse impacts, and the resulting degradation of native habitats. We examined the population dynamics, phenology, and control methods of these invasive plants on federal lands near Victoria, B.C. Of the several methods of control tested, including manual cutting, application of a registered herbicide (triclopyr), a fungal bioherbicide (*Chondrostereum purpureum*), and a commercial plastic mulch, we found that some treatments (mulch and herbicide) provided 100% efficacy on control of sprouting of all four invasive species. While one bioherbicide (*Fusarium tumidum*) was very effective on Scotch broom in the greenhouse, it was not applied under field conditions. The other bioherbicide (*Chondrostereum purpureum*) produced a variable response when applied under the field conditions. Manual cutting was the least effective. Also a novel prospective bioagent (*Phomopsis sp*. denovo) was isolated from dying and dead samples of Daphne that holds a great promise for control of Daphne. A new technology using superheated water (Aquacide) to kill vegetative shoots of gorse did not offer long term control, nor was it found to be cost effective.

Characterization of ABCG26, an ATP-binding cassette transporter required for full male fertility and pollen exine formation in *Arabidopsis thaliana*

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The highly resistant biopolymer, sporopollenin, gives the outer wall (exine) of spores and pollen grains their unparalleled strength, shielding these structures from terrestrial stresses. Despite a limited understanding of sporopollenin, it appears that its synthesis in the tapetum requires transport of one or more sporopollenin constituents to the surface of developing microspores. We have identified a transport protein from the ATP-binding cassette (ABC) transporter superfamily, ABCG26, required for pollen exine formation in *Arabidopsis thaliana*. An *abcg26* mutant is severely reduced in fertility and mature anthers fail to release pollen. Electron microscopy revealed abnormalities in pollen wall formation first apparent in free uninucleate microspores (stage 8) as a lack of exine formation and sporopollenin deposition. Additionally, the highest levels of *ABCG26* mRNA were in the tapetum in stage 7 and 8, associated with early pollen wall formation, sporopollenin biosynthesis and sporopollenin deposition. Accumulations resembling the trilamellar lipidic coils in the *abcg11* and *abcg12* mutants defective in cuticular wax export were observed in *abcg26* anthers. Our results show that the transport protein ABCG26 plays a critical role in exine formation and pollen development, and support a model by which ABCG26 transports sporopollenin precursors from their site of synthesis in tapetal cells to the locule for polymerization on developing microspore walls.

Methane oxidation and methanotrophic bacteria in a New Zealand landfill soil

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Methane is the main greenhouse gas from agriculture in New Zealand, with dairy farming being the nation's primary agricultural industry. There is potential to use methanotrophic bacteria in biofilters to reduce methane emissions from dairy farms. However, the development of any biofiltration technology to mitigate methane emissions is fundamentally dependant on understanding the species composition of methanotrophic bacteria present in the filter media. Soil samples from an 8 year old landfill in New Zealand were studied for their ability to consume methane. The soil methanotrophic populations were characterized in 6 soil samples (Topsoil A, B, C and Subsoil A, B, C) by DNA extraction and PCR amplification. All samples indicated the presence of *pmoA* genes. *pmoA* libraries were constructed for all soils and the sequencing results indicate that Type II methanotrophs are dominant in them all. All sequenced clones from the Topsoil samples were most similar to uncultured methanotrophs related to *Methylocystis sp*. Although most of the Subsoil samples were also mostly similar to uncultured methanotrophs related to *Methylocystis sp*., some sequences were also related to other Type II methanotrophs such as *Methylosinus trichosporium* and *Methylocapsa acidiphila*. Only one *pmoA* clone from Subsoil A was related to a Type I methanotroph (*Methylococcus capsulatus*, 85%). Methanotrophic bacteria were successfully enriched from all soils using a nitrate mineral salt medium. *pmoA* clones from Subsoil B3 NMS enrichment were identified as an uncultured methanotroph most similar to another uncultured methanotrophic bacteria from a soil in China (95% similarity) but with only 83% similarity to the closest cultured *Methylococcus capsulatus*. These results were confirmed by electron microscopy which clearly showed the characteristic internal membranes typical of Type I methanotrophs.

Cloning and functional characterization of geraniol synthase from lavender

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We are developing *L. angustifolia* as a model system for investigating regulation of essential oil metabolism in higher plants. Here, we report the cloning and functional characterization of geraniol synthase (LaGERS) from a lavender flower cDNA library. The deduced amino acid sequence of LaGERS exhibits up to 71% identity to other terpene synthases. However, the encoded protein lacks an internal 73-amino acids fragment that includes the signature DDxxD cation binding motif. Expression of LaGERS in *E. coli* resulted in the production of soluble recombinant protein with the predicted molecular weight of 50 kDa. The recombinant enzyme produced geraniol using geranyl di-phosphate (GPP) as a substrate *in vitro*. Transcripts of *LaGERS* and linalool synthase were shown to be highly concentrated in lavender oil glands. Despite the strong and nearly identical transcriptional expression of both genes, *L. angustifolia* plants produce large quantities of linalool, but negligible amounts of geraniol. Given that both enzymes utilize the same substrate (GPP) our results suggest that LaGERS cannot compete with other functional terpene synthases *in vivo*.

In vitro* screening of several disinfectants to assess their efficacy in controlling mycelia growth and spore germination of *Phytophthora ramorum

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Phytophthora ramorum is the causal agent of sudden oak death, a very serious disease that causes die back and death of several important forest species in the USA, notably tanoak (*Lithocarpus densiflorus*). The pathogen has a wide host range including ornamentals such as *Rhododendron* and *Viburnum* that are of significant value and importance to the nursery industry. In addition to infected plant material, *P. ramorum* has also been recovered from nursery soil samples, so control of the pathogen in nurseries is an important component of any effective control program. Diligent use of effective disinfectants in nurseries can contribute to integrated management strategies for *P. ramorum*. In this study several disinfectants were evaluated *in vitro* for their efficacy in controlling the growth of *P. ramorum*. Hyperox® at a 1:128 dilution prevented growth of all *P. ramorum* isolates screened in this study. The isolates represented each of the three known clonal lineages, EU1, NA1, and NA2. Bleach (15%) and Chemprocide® (0.8 and 1.35%) were also effective but did not provide complete inhibition.

Flavonoid-specific poplar glycosyltransferases.

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Glycosylation involves the transfer of a sugar residue from a donor to an acceptor molecule and is often one of the last steps in the biosynthesis of plant phenolic compounds including anthocyanins and flavonoids. It is hypothesized that glycosylation of these compounds influences their stability, biological activity, and may be crucial for their targeting to compartments within a cell. Glycosylation is catalyzed by glycosyltransferases, and a specific subgroup, the uridine diphosphate glycosyltransferases (UGTs), transfer sugars from a UDP molecule to an appropriate acceptor molecule. Flavonoid-specific UGTs are characterized by the presence of a Plant Secondary Product Glycosyltransferase motif located at the C-terminal end of the protein. In microarray experiments done on poplar tissue, several flavonoid-specific UGT genes were highly upregulated in leaves after infection with Melampsora rust. The expression of these genes correlated with the upregulation of genes involved in the synthesis of proanthocyanidins suggesting a role for UGTs in proanthocyanidin synthesis. We have generated active recombinant proteins for two highly induced genes and found that both flavonols and anthocyanidins are substrates for these enzymes. We will continue to characterize the biochemical and physiological function of these UGTs. We also are using RNA interference to suppress the expression of poplar UGTs to investigate their roles in plant defense. Overall we hope to determine how UGTs are involved in plant phenolic metabolism and the defense response.

Do gymnosperm ovules divide defense between sporophyte and gametophyte?

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Insects that parasitize developing seed need to contend with a layered plant defense. Ovule organization plays a role, as the gametophyte is surrounded by the sporophyte. Physical defense barriers of the sporophyte include a waxy cuticle on the integument, and a sporopollenin-rich glycoprotein megaspore wall (part sporophyte/part gametophyte). Chemical defenses include resin, tannins and polyphenolics present in the integument, but not in the nucellus. Parasitic chalcids of conifers, such as *Megastigmus*, bypass these defenses ovipositing their eggs directly in the haploid eggs and surrounding megagametophyte tissue. The gametophyte offers no defense. The purpose of this presentation is to illustrate the differential distribution of defense mechanisms between sporophyte and gametophyte. This provides the basis for a new model for the evolution of seed habit and its defense systems.

Integrating environmental and genetic effects to predict responses of tree populations to climate

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Climate is a major environmental factor affecting the phenotype of trees and is also a critical agent of natural selection that has molded among-population genetic variation. Population response functions describe the environmental effect of planting site climates on the performance of a single population, whereas transfer functions describe among-population genetic variation molded by natural selection for climate. Although these approaches are widely used to predict the responses of trees to climate change, both have limitations. We present a novel approach that integrates both genetic and environmental effects into a single ‘‘universal response function’’ (URF) to better predict the influence of climate on phenotypes. Using a large lodgepole pine (Pinus contorta Dougl. ex Loud.) field transplant experiment composed of 140 populations planted on 62 sites to demonstrate the methodology, we show that the URF makes full use of data from provenance trials to: (1) improve predictions of climate change impacts on phenotypes; (2) reduce the size and cost of future provenance trials without compromising predictive power; (3) more fully exploit existing, less comprehensive provenance tests; (4) quantify and compare environmental and genetic effects of climate on population performance; and (5) predict the performance of any population growing in any climate. Finally, we discuss how the last attribute allows the URF to be used as a mechanistic model to predict population and species ranges for the future and to guide assisted migration of seed for reforestation, restoration, or afforestation and genetic conservation in a changing climate.

***Agrobacterium*-meditated gene disruption using split-marker in *Grosmannia clavigera,* a mountain pine beetle associated pathogen**

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Grosmannia clavigera is a fungal pathogen associated with the mountain pine beetle (*Dendroctonus ponderosae*) which is devastating large areas of western Canada's conifer forests. This fungus also produces a dark melanin pigment that discolors pine sapwood. In *G. clavigera,* melanin is produced through dihydroxynaphthalene (DHN) pathway. We have generated the genome sequence of *G. clavigera*; however, functional characterization of genes identified in the genome requires an efficient gene disruption method. Here, we report a gene replacement method for *G. clavigera* using *Agrobacterium*-mediated transformation in conjunction with linear or split-marker deletion cassettes. In addition, we used long flanking regions up to 3 kb from both sides of the targeted genes in our deletion cassettes. We assessed this gene disruption method with two genes from the melanin DHN biosynthesis pathway that produce easily detectable white and red/brown mutant phenotypes: polyketide synthase (PKS) and scytalone dehydratase (SD). The approach yielded *G. clavigera* gene replacements with homologous recombination higher than 50%. This method can now be applied to efficiently identify genes involved in *G. clavigera* fungal pathogenicity and will facilitate understanding how the fungus overcomes the host defense system.

Long-term effects of harvesting, thinning and fertilization on soil methane oxidation and diversity of methane-oxidizing bacteria in a Coastal Douglas-fir forest.

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Forest soil has an important role in the methane cycle, since it has been reported to be a sink for atmospheric methane (CH_4). Although tree harvesting has showed a decrease on soil CH_4 oxidation by bacteria, little is known on the long-term effects of different forest management practices. The goal of this research is to ascertain whether or not long-term harvesting, heavy thinning and fertilization affect the capacity of forest soil to consume CH_4 and if these practices have an impact on the diversity of methane-oxidizing bacteria. To address this, we used static gas chambers to measure CH_4 fluxes, and sequencing of methane monooxygenase genes (*pmoA*) to characterize the methanotrophs composition in a Douglas-fir forest soil on southern Vancouver Island. Methane oxidation rates were measured under a fertilized, a thinned and a control environment after 37 years of forest management, and in three other aged stands (70,100 and 250-year old). Average rates ($\mu gCH_4 consumed.m^{-2}.h^{-1}$) were similar between fertilized (103.7±35.5), thinned (110.4±43.5) and control (100.1±31.9) plots. However, CH_4 fluxes were significantly different between age stands, mainly during the summer, where the 100-year old forest (154.5±42) showed higher rates than the 70-year (107±31) and 250-year (85.2±48). Analysis of the diversity of *pmoA* clones showed that the majority of the CH_4 oxidizers in the soil (91%) belong to the uncultivated 'upland soil cluster alpha', which is distinctly related to *Methanocapsa acidiphila*. Methanotrophs composition was slightly different after 37 years of fertilization and thinning, but the long-term effect of these practices did not affect atmospheric methane oxidation in the soil. Differences in methane oxidation and methanotrophs composition were stronger between age stands, which might indicate an impact on microbial composition, particularly in the methanotrophic guild.

Insect repellent properties of unique lavender essential oils produced in British Columbia, Canada.

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Several lavender (*Lavandula*) species are grown for the production of essential oils (EO) used in cosmetics and alternative medicines. The EOs of a few lavender species and other plants are also used for deterring insects and other household pests. The specific application of lavender EO depends on the presence of certain chemical constituents, e.g. linalool, linalool acetate, and camphor, and the production of EO constituents in plants may be modulated through chemical mutagenesis and biotechnology (1). In an attempt to improve EO yield and composition, we have regenerated over 600 mutagen-treated lavender plants through tissue culture (1, 2). Many of these plants produce EOs of unique composition, including a few lavandin (*L. x intermedia*) lines that accumulate very high amounts of linalool, but trace quantities of linalool acetate. The EO of these mutants was shown to repel herbivorous *Drosophila melanogaster*, and may have applications as pest repellent in organic farming. In addition, these plants provide novel scientific tools for studying the regulation of essential oil production in higher plants. Future research will focus on characterizing key genes that control the biosynthesis of EO constituents in plants.

1. Desautels A, Biswas K, Lane A, Boeckelmann A, Mahmoud SS. (2009) Suppression of linalool acetate production in *Lavnadula intermedia*. *Natural Product Communications* 4: 1533-1536.
2. Mahmoud SS, Williams S, and Croteau R. (2004) Cosuppression of limonene-3-hydroxylase in peppermint promotes accumulation of limonene in the essential oil. *Phytochemistry* 65, 5: 547-554.

Do tannins act as anti-herbivore compounds?

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Condensed tannins (CTs, syn. proanthocyanidins) are polymeric flavonoids found in many tree and other plant species. They have the ability to precipitate proteins, and are often discussed as being defensive phytochemicals with detrimental effects on both mammalian and insect herbivores. However, there are many conflicting reports as to their actual impact on defense. Poplars and aspen (*Populus tremuloides*) can contain significant levels of CTs in leaves and other vegetative organs, which often vary depending on genotype and environmental conditions. In aspen, their synthesis can be induced by herbivore damage or wounding of leaves. We have identified a gene in poplar that regulates this induced response, and overexpression of this gene in transgenic poplar plants leads to dramatically increased levels of CTs in leaves. These plants are an excellent opportunity to test the ecological effects of tannins. Surprisingly, choice tests with forest tent caterpillars showed that high tannin plants were preferred. This suggests that i) the larvae use CTs as a feeding cue, or ii) that other shifts in the phytochemical profile are more significant in determining larval food choice. We are currently investigating these possibilities using purified phytochemicals, as well as considering additional potential roles of CTs in poplar.

Antifungal activity of a *Pinus monticola* – antimicrobial peptide 1 (Pm-AMP1) and its accumulation in *Cronartium ribicola*-infected western white pine

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A 79 amino acid long, basic protein termed *Pinus monticola*-antimicrobial peptide 1 (Pm-AMP1) induced in cankered bark of western white pine (WWP) infected with the blister rust fungus *Cronartium ribicola* was found to show homology with other antimicrobial agents. Application of recombinant Pm-AMP1 on the growing hyphal margin of *C. ribicola*, *Phellinus sulphurescens,* and *Ophiostoma montium* resulted in visible growth inhibition 3 to 12 days post-treatment. Spore germination of various fungi was also inhibited 44%-97% five days post-treatment. Seven WWP families showing quantitative, bark reaction resistance were assessed for associations between Pm-AMP1 and presence of stem symptoms. Overall there was a significant difference ($p<0.001$) in mean Pm-AMP1 between families. The three full-sib bark reaction families (#1, #2, #5) showed higher average Pm-AMP1 levels than the half-sib families (#6, #7) and the susceptible family (#10) showed the lowest levels ($p<0.05$). Family #1 showed a significant association between Pm-AMP1 and overall seedling health ($P=0.014$), with higher levels observed in healthy seedlings as opposed to severely infected ones. Our results suggest that Pm-AMP1 is involved in the WWP defense response as observed by its inhibitory activity and through its association with different indicators of disease resistance.

ESSENTIAL OIL WORKSHOP PRESENTATIONS:

Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products

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In spite of intensive research on plant natural products and insect-plant chemical interactions over the past three decades, only two new types of botanical insecticides have been commercialized with any success in the past 15 years – those based on neem seed extracts (azadirachtin) and those based on various plant essential oils. Certain plant essential oils, normally obtained through steam distillation and rich in mono- and sesquiterpenes and related phenols, are widely used in the flavouring and fragrance industries and in aromatherapy. Some aromatic plants have traditionally been used for stored product protection, but the potential for development of pesticides from plant essential oils for use in a wide range of pest management applications has only recently been realized. One US company, EcoSMART Technologies, had led the commercial development of pesticide products based on common plant essential oils, in part because several of the oils are exempt from registration by the EPA. Many plant essential oils and their major terpenoid constituents are neurotoxic to insects and mites and behaviourally active at sublethal concentrations. For consumer and industrial use, they are toxic to important pests including cockroaches, termites, ants and houseflies, but they may also be useful as flushing agents, perimeter barriers and as fumigants. They are toxic and repellent to mosquitoes including vectors of West Nile Virus. Animal health is another area where essential oil pesticides can be valuable. Certain products of this type are effective on companion animals against fleas and ticks, for fly control in dairy barns, and possibly for fly and beetle control in poultry production. In all of these applications, there is a premium on human and animal safety that takes priority over absolute efficacy. In agriculture, the main market niche for essential oil-based pesticides is in organic food production, at least in developed countries, where there are fewer competing pest management products.

Current research on essential oil crops: focusing on lavender

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Essential oils (EO) are predominantly comprised of two classes of low molecular weight secondary metabolites known as the monoterpenes and the sesquiterpenes. In plants that accumulate large quantities of EO, the biosynthesis and storage of EO constituents is restricted to specialized structures known as glandular trichomes or oil glands. The mono- and sesquiterpenes are very important to plants, for example as pollinator attractants and pest deterrents. They are also economically very significant and find numerous applications in cosmetics, personal hygiene products, industrial and household cleaners, and medicines. As a result, a great deal of research has focused on improving the yield and composition of the essential oil in agronomically important plants including several species of lavender. Current research can be categorized in the three general areas of plant breeding, mutagen treatment, and molecular biology. This presentation will summarize recent developments in these areas, with emphasis on modern genomics-based research that aims to elucidate the molecular basis of EO formation, secretion and storage in lavenders.

Lavender farming in British Columbia

Andrea McFadden

Okanagan Lavender Herb Farm

Outline

1. Introduction
2. Know your climate, soils and growing conditions
3. Do your research
4. Know your market – how & where will you sell what you produce?
5. Know the regulations of the ALR, your health authority and city
6. Find your niche
7. Cross marketing with other like minded businesses.

Okanagan Lavender Herb Farm was first conceived in 1994. The first four years were spent researching and locating lavender varieties, planting test blocks and developing a line of finished products. Next, the farm was introduced to the central Okanagan through the local farmer's market and the opening of an on-site location in 1999. The farm has grown through many evolutions, adding more plantings and products as the market demanded. It is currently in the midst of a large expansion which will see another 2 ½ acres planted in the gardens and a custom built retail/production facility.

Systematic work on the genus *Lavandula* - recent advances and future prospects

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Recent research into the systematics of *Lavandula* has produced a recent monograph (Upson & Andrews 2004) providing a modern and comprehensive overview of the genus, recognising 39 species and a total of over 80 taxa and an accounts of nearly 400 cultivars. Phylogenetic research has established relationships within the genus with a new subgeneric classification of 3 subgenera and 8 sections and the family Lamiaceae, in subfamily Nepetoideae and related to the tribe Ocimeae (the Basils). This work has also established key economic species to be exceptionally variable in their morphology which has traditionally been exploited by selecting superior clones. These advances provide an important base from which a deeper understanding of the genus can now be developed and future breeding guided. A key future step is to establish a species level phylogeny to provide further systematic insights and to allow an understanding of biochemical pathways that have given rise to the great diversity of essential oils both between species and within populations. *Lavandula* continues to offer opportunities to develop improved cultivars, to find and utilise its essential oils in both traditional and novel ways.

Upson, T.M. & Andrews, S. (2004). The Genus *Lavandula*. A Botanical Magazine Monograph. The Royal Botanic Gardens, Kew.

What is the future of essential oil distillation in the unique and far-flung region of Canada's northwest coast?

Lana Wilhelm

NTFP Working Group, Coastal First Nations

The remote central and north coastal areas of BC hold enormous challenges and opportunities in the distillation of essential oils. Coastal First Nations are in the process of building a "green" economy on the coast, and currently at the forefront of a sustainable effort to capture essential oils from the needles of salvaged conifer trees to craft a respectful product from the waste of resource industries. The presentation will discuss the objectives of this work, challenges encountered to date and some promising findings.

PRESENTERS AND ATTENDEES

Name

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Bohlmann, Jorg
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Breuil, Colette

Carneiro, Joyce Silva
Carruthers, Christopher
Charleson, Lee
Chedgy, Russell
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Curtis-McLane, Sierra

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