PNWTIRC

Pacific Northwest Tree Improvement Research Cooperative

Annual Report 2011-2012

Oregon State University College of Forestry
Department of Forest Ecosystems and Society

Glenn Howe, Kori Ault, Scott Kolpak,
Lauren Magalska, Oguz Urhan

PNWTIRC

Photo courtesy of Noel Zia Lee

http://www.fsl.orst.edu/pnwtirc/

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Oregon State University College of Forestry
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2011-2012 Annual Report

Report editors
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Introduction

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Pacific Northwest Tree Improvement Research Cooperative

Annual Report 2011-2012

The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) was formed in 1983 to conduct research in support of operational tree improvement in the Pacific Northwest. Emphasis is on region-wide topics dealing with major coniferous species. Membership has included representatives from public agencies and private forestry companies in western Oregon, western Washington, and coastal British Columbia.

OUR MISSION IS TO:

- Create a knowledge base concerning genetic improvement and breeding of Pacific Northwest tree species.
- Develop reliable, simple, and cost-effective genetic improvement methods and apply these methods to solve tree-breeding problems.
- Promote effective collaboration and communication among public agencies and private industries engaged in tree improvement in the region.

All participants provide guidance and receive early access to research results. Regular and Associate members provide financial and in-kind support and are represented on the Policy/Technical Committee. This committee is responsible for making decisions on program strategy and support, identifying research problems, establishing priorities, and assisting in the planning, implementation and evaluation of studies. Because Contractual Participants provide less financial support, they have no voting rights on the Policy/Technical Committee. Liaison Members provide no financial support and have no voting rights. The PNWTIRC is housed in the Department of Forest Ecosystems and Society at Oregon State University.

PNWTIRC PARTICIPANTS

Regular Members

Bureau of Land Management

Cascade Timber Consulting

Green Diamond Resource Company

Hancock Timber Resource Group

Longview Timber Company

Olympic Resource Management

Oregon Department of Forestry

Oregon State University

Plum Creek Timber Company

Port Blakely Tree Farms

Rayonier

Roseburg Forest Products

Stimson Lumber Company

Washington State Department of Natural Resources

Associate Members

Starker Forests

Contractual Participants

Lone Rock Timber Company

Liaison Members

Inland Empire Tree Improvement Cooperative

Northwest Tree Improvement Cooperative

USDA Forest Service, Pacific Northwest Research Station

HIGHLIGHTS OF 2011-2012

- Lauren Magalska defended her thesis on September 16, 2011, which was entitled "Identifying site characteristics that explain variation in Douglas-fir site productivity and stem form." Additional progress on the site characterization project included completing the stem form genetic analyses and updating the site characterization dataset with newly available soils data from the Natural Resources Conservation Service (NRCS).

- We completed the acoustic velocity (wood stiffness) measurements at the remaining progeny test sites of Fir Grove (Douglas-fir) and Toledo (western hemlock). The relative merits of tools, measurement approaches, and sampling techniques were evaluated, and we compared stiffness between Douglas-fir and western hemlock.

- We completed the analysis of the flower, cone, and crown measurements at the Plum Creek Miniaturized Seed Orchard (MSO). These data have undergone statistical analysis, and an outline for the MSO publication has been completed. The MSO data from the Lebanon Forest Regeneration Center and Meridian Seed orchards have been acquired and summarized.

- The Phase II proposal for the Center for Advanced Forestry Systems (CAFS) was awarded. The CAFS webpage includes a description of research areas as well as highlights from current CAFS projects (http://cnr.ncsu.edu/fer/cafs/researchareas.html). There continues to be CAFS funding for the PNWTIRC project entitled "Early genetic selection for wood stiffness in Douglas-fir and western hemlock."

- We completed the construction of a Douglas-fir SNP genotyping array (Ilumina Infinium) as part of a joint project between the PNWTIRC and the USDA-funded Conifer Translational Genomics Network (CTGN). The resulting genotyping array ('SNP chip') can now be used to genotype 5,847 Douglas-fir SNPs. Furthermore, our SNP database may contain as many as ~200,000 true SNPs, and as many as ~69,000 SNPs that could be genotyped at ~20,000 gene loci using an Infinium genotyping array. Ultimately, these genomic resources will enhance Douglas-fir breeding and allow us to use genomic selection to enhance tree breeding.

MESSAGE FROM THE DIRECTOR

Over the next year, we'll complete two long-term projects. The Miniaturized Seed Orchard Study was begun more than a decade ago. The research proposal, entitled "Seed Orchard Research in Coastal Douglas-fir: Comparison of Macro, Micro, and Mini orchards," was written in 1999 by Tom Adams and Thimmappa Anekonda. The main goal of this research was to compare three miniaturized seed orchard (MSO) designs that differed in tree spacing and crown management. The bulk of the research was conducted at the Plum Creek seed orchard site. We thank Jim Smith for his expertise, hard work, and excellent record-keeping skills—all of which were critical to the success of this research. We also conducted satellite experiments at Roseburg Resource's Lebanon Forest Regeneration Center with the help of Mike Albrecht and Sara Lipow—and with the help of Jeff DeBell, integrated data from MSO and conventional orchards at the WaDNR Meridian Seed Orchard in Olympia, WA. This research would not have been possible without the substantial in-kind support available through the PNWTIRC. We also thank Marilyn Cherry who was involved early in the project, and other members of the MSO Advisory Committee, Margaret Banks, Randall Greggs, and Keith Jayawickrama. Finally, we thank Annie Simmonds, Kyle Pritchard, Elaine Blampied, Kori Ault, and Ron Rhatigan for helping us collect data in the field, and Shawn and Barbara Barnes for cone collection and seed processing. Scott Kolpak gave the final MSO presentation at the last annual meeting, and will submit the final publication summarizing all of this research during the next year.

The other project that will be completed this year is entitled "Early Genetic Selection for Wood Stiffness in Douglas-fir and Western Hemlock." This project was jointly funded by the PNWTIRC and Center of Advanced Forestry Systems (CAFS). The first phase of this project was conducted by Scott Kolpak, and the second phase was conducted by Oguz Urhan as part of his Master's thesis project. We thank Fred Pfund of Starker Forests, James Benson of Weyerhaeuser, Al Heimgartner and Jerry Anderson of Hancock, and Keith Jayawickrama of the Northwest Tree Improvement Cooperative for helping us gain access to the operational and genetic test plantations used in this study. We also thank Annie Simmonds, Lauren Magalska, Ron Rhatigan, Cameron Muir, Kyle Pritchard, and Sean Smith for help with the measurements. Oguz gave the final summary of this research at the last PNWTIRC annual meeting. During the next year, Oguz will complete his M.S. degree, and will work with Scott to submit a manuscript on both phases of the wood stiffness research.

So what now? Collaborative research between the PNWTIRC and the Conifer Translational Genomics Network laid the foundation for developing new genome-scale genetic markers that can be used to enhance Douglas-fir breeding. In particular, a breeding approach called 'genomic selection,' or 'whole-genome marker-assisted selection' could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. Genomic selection is a type of marker-assisted selection that uses tens of thousands of genetic markers to track alleles for most or all of the important genes in the genome. If very large numbers of markers are used, most or all genes will be linked to at least one marker, particularly in small populations. Genomic selection is now viable in forest trees because of new high-throughput technologies for genotyping single nucleotide polymorphisms (SNPs). SNPs are DNA sequence variations caused by single base pair changes at specific positions along a chromosome. Genomic selection has been widely adopted in livestock and crop breeding, and is beginning to play an important role in forest tree species. This will be an important new area of research for the PNWTIRC.

Glenn T. Howe, PNWTIRC Director

AGENDA – THURSDAY DECEMBER 6, 2012

– ANNUAL MEETING –
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE (PNWTIRC)

START TIME:	8:30 AM for coffee; 9:00 AM for presentations
LOCATION:	North Willamette Research and Extension Center, Aurora, OR
LUNCH:	Lunch provided

Time	Topic	Responsibility
8:30-9:00	Coffee	
9:00-9:10	Welcome and Introductions	Sara Lipow
9:10-9:20	Overview • *PNWTIRC accomplishments for 2011-12* • *PNWTIRC plans for 2012-13*	Glenn Howe
9:20-9:50	Miniaturized Seed Orchard Project	Scott Kolpak
9:50-10:10	Center for Advanced Forestry Systems • *Phase II project proposal* • *Genetic Markers for Western White Pine and Douglas-fir*	Glenn Howe
10:10-10:30	Break	
10:30-11:00	Center for Advanced Forestry Systems • *CIPS collaboration* • *Incorporating genetics into mechanistic growth models*	Doug Maguire Glenn Howe
11:00-11:30	Early genetic selection for wood stiffness in Douglas-fir and western hemlock	Oguz Urhan
11:30-12:00	Genetic and environmental control of Douglas-fir stem form	Lauren Magalska
12:00-1:00	Lunch	
1:00-1:50	Development and application of SNP markers in Douglas-fir • *Presentation* • *Discussion*	Glenn Howe
1:50-2:10	Budget and other business • *Budget presentation and vote* • *Elect new Policy/Technical Committee Chair*	Glenn Howe
2:10-2:30	Break	
2:30-2:55	Western Conifer Climate Change Consortium	Glenn Howe
2:55-3:00	Wrap-up and adjourn	Glenn Howe

PNWTIRC Annual Meeting 2012

Glenn Howe

Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University

PACIFIC NORTHWEST TREE IMPROVEMENT
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PNWTIRC personnel

2011-2012

- Director – ***Glenn Howe***
- Research Coordinator – ***Scott Kolpak***
- Program Manager – ***Liz Etherington, Kori Ault***
- Graduate students – ***Lauren Magalska, Oguz Urhan***
- Faculty Research Assistant – ***Lauren Magalska***
- Policy/Technical Committee Chair – ***Sara Lipow***

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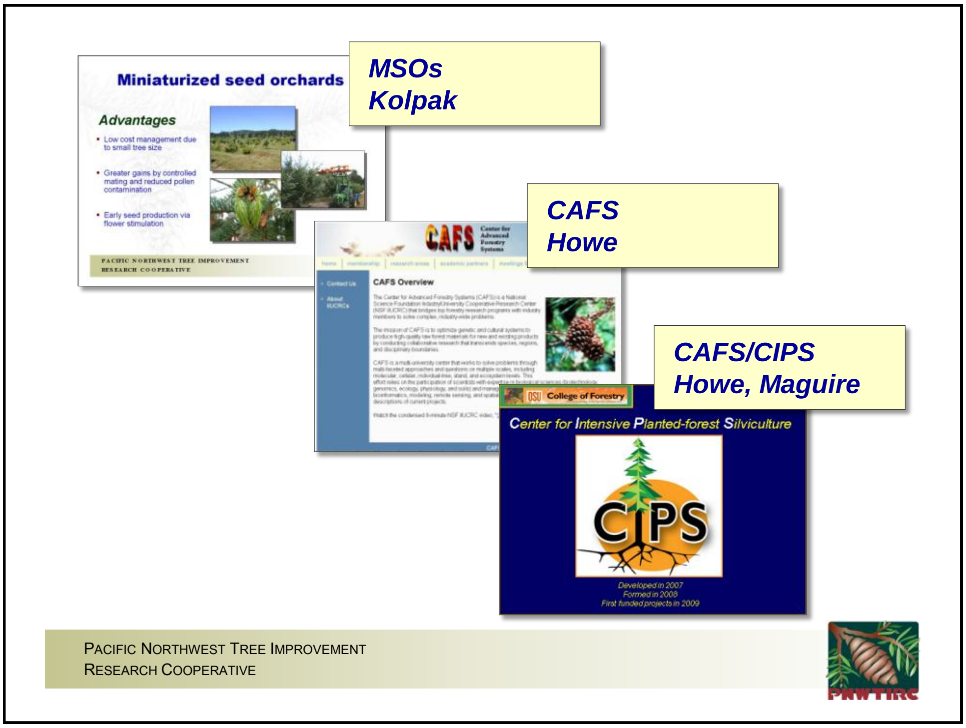
Miniaturized seed orchards
Advantages
Low cost management due to small tree size
Greater gains by controlled mating and reduced pollen contamination
Early seed production via flower stimulation
MSOs
Kolpak
CAFS
Howe
CAFS Overview
CAFS/CIPS
Howe, Maguire
College of Forestry
Center for Intensive Planted-forest Silviculture
CIPS
Developed in 2007
Formed in 2008
First funded projects in 2009
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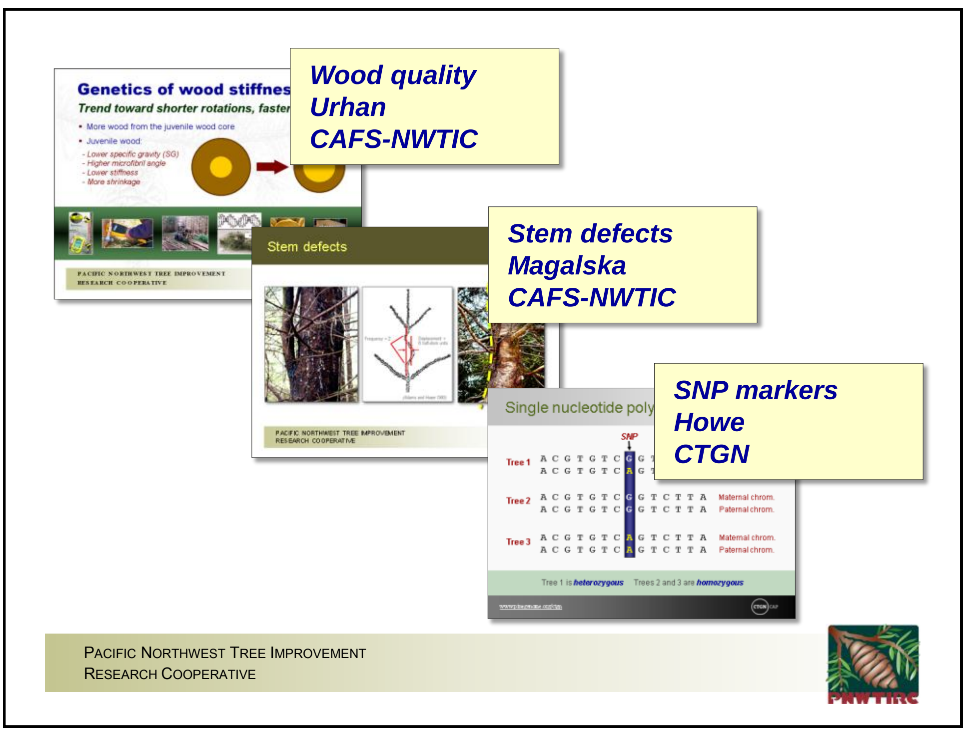
Genetics of wood stiffnes
Trend toward shorter rotations, faster
More wood from the juvenile wood core
Juvenile wood:
- Lower specific gravity (SG)
- Higher microfibril angle
- Lower stiffness
- More shrinkage
Wood quality
Urhan
CAFS-NWTIC
Stem defects
Stem defects
Magalska
CAFS-NWTIC
Single nucleotide poly
SNP
SNP markers
Howe
CTGN
Tree 1
Tree 2
Tree 3
Maternal chrom.
Paternal chrom.
Tree 1 is heterozygous
Trees 2 and 3 are homozygous
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Highlights of 2011-2012

Miniaturized seed orchards

- Completed flower, cone, and crown measurements at Plum Creek's MSO
- Completed the outline for the MSO publication
- Conducted statistical analysis of MSO data
- Acquired and summarized MSO data from the Lebanon FRC and Meridian seed orchards
- Scott will discuss

Plum Creek MSO

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CAFS Phase II was awarded

http://cnr.ncsu.edu/fer/cafs/researchareas.html

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OSU College of Forestry

Center for Intensive Planted-forest Silviculture

CIPS

Developed in 2007
Formed in 2008
First funded projects in 2009

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Highlights of 2011-2012

Wood quality research

- Continued CAFS funding for the proposal entitled "Early genetic selection for wood stiffness in Douglas-fir and western hemlock"
- Completed acoustic velocity (stiffness) measurements at the remaining test sites of Fir Grove (DF) and Toledo (WH)
- Analyzed the relative merits of tools and measurement approaches
- Compared estimated stiffness between Douglas-fir and western hemlock
- Evaluated sampling strategies
- Oguz Urhan will discuss

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Highlights of 2011-2012

Site characterization research – Stem defects

- Lauren defended her thesis on September 16, 2011: "Identifying site characteristics that explain variation in Douglas-fir site productivity and stem form."
- Completed the stem form genetic analyses and new site analyses
- Updated site characterization dataset with newly available soils data from the Natural Resources Conservation Service (NRCS)
- Lauren will present her latest results

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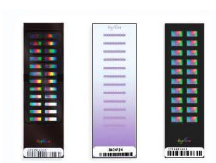
Douglas-fir SNP chip (Illumina Infinium)

Douglas-fir SNP chip is available

- 7256 SNPs can be assessed
- Many more potential SNPs are available



Numbers and percentages of putative Douglas-fir SNPs attempted and assayed with an Illumina Infinium SNP array (n = 260 trees).

SNPs attempted	8769
SNPs assayed by Illumina	8067
Percent of SNPs (assayed/attempted)	**92.0**
SNPs assayed by Illumina	8067
SNPs called (call frequency ≥ 0.85)	7256
Percent of SNPs (called/assayed)	**82.7**
SNPs called (call frequency ≥ 0.85)	7256
SNPs called that are polymorphic (MAF ≥ 0)	5847
Percent SNPs (called MAF > 0/called)	**80.6**
SNPs attempted	8769
SNPs called that are polymorphic (MAF ≥ 0)	5847
Percent SNPs (called MAF > 0/attempted)	**66.7**

PACIFIC NORTHWEST TREE IMPROVEMENT
RESEARCH COOPERATIVE

Highlights of 2011-2012

Publications by PNWTIRC personnel

- Howe, G.T., Yu, J., Knaus, B., Cronn, R., Kolpak, S., Dolan, P., Lorenz, W.W., and Dean, J.F.D. Submitted. A SNP resource for Douglas-fir: *De novo* transcriptome assembly and SNP detection and validation. Submitted to BMC Genomics.
- Lorenz, W. W., Ayyampalayam, S., Bordeaux, J.M., Howe, G.T., Jermstad, K.D., Neale, D.B., Rogers, D.L., and Dean, J.F.D. 2012. Conifer DBMagic: A database housing multiple de novo transcriptome assemblies for twelve diverse conifer species. Tree Genetics and Genomes 8:1477-1485.
- Magalska, L.E. 2011. Identifying site characteristics that explain variation in Douglas-fir site productivity and stem form. M.S. Thesis, Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR.

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Highlights of 2011-2012

Presentations by PNWTIRC personnel

- Colin, A.-L. Howe, G.T., St.Clair, J.B., and Maguire, D.A. 2012. Mechanistic growth models: Decomposing phenotypic models into their genetic and environmental components. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.
- Howe, G.T. 2012. Genomics, wood properties, and climate change: Technological advances and challenges for western tree improvement. Keynote address, Annual Meeting of the Inland Empire Tree Improvement Cooperative, February 29, 2012, Coeur d'Alene, ID.
- Howe, G.T. 2012. Plant conservation and climate change: Hitting a moving target. Invited talk and abstract In: Proceedings of the Second International Symposium on Biology of Rare and Endemic Plan Species, 23-27 April 2012 Fethiye, Turkey, p2.

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Highlights of 2011-2012

Presentations by PNWTIRC personnel

- Howe, G.T., Kolpak, S., Urhan. O., Cress, D., Jayawickrama, K., and Ye, T. 2012. Early genetic selection for wood stiffness in Douglas-fir and western hemlock. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.
- Magalska, L.E., Howe, G.T., Maguire, D.A. 2012. Site characteristics of Douglas-fir productivity and stem form. Poster presentation, Northwest Forest Soils Council Winter Meeting, 28 February 2012, Gifford Pinchot National Forest Headquarters, Vancouver, WA.
- Magalska, L.E., Howe, G.T., and Maguire, D.A. 2012. Site characteristics of Douglas-fir productivity and stem form. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.

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Highlights of 2011-2012

Presentations by PNWTIRC personnel

- Rust, M.L. and Howe, G.T. 2012. Development of genetic markers for western white pine and Douglas-fir. Oral presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.
- Urhan, O.S. 2012. Genetic improvement of wood stiffness in young Douglas-fir and western hemlock. Oral presentation at the Forest Ecosystems and Society Graduate Student Symposium, 21 May 2012, Corvallis, OR.

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Collaborations and grants

- **CAFS** ***Center for Advanced Forestry Systems – Phase II.*** Howe, G.T., Maguire, D.A., and Strauss, S.H. National Science Foundation Industry/University Cooperative Research Center Program, 2012-2017, $300,000 (OSU).
- **USFS Rocky Mountain Research Station**. ***Developing a SNP panel for interior*** Douglas fir. Howe, G.T., and Cushman, S. USDA-Forest Service Joint Venture Agreement, $28,755 (2011-2013).

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Collaborations and grants

USDA AFRI. ***Western conifer forest systems: Strategies for climate change adaptation and mitigation.*** Howe, Glenn; Abatzoglou, John; Adams, Darius; Bentz, Barbara; Coleman, Mark; Crookston, Nick; Daley-Laurson, Steven; Ettl, Gregory; Fischer, Alexandra Paige; Gosz, James; Gray, Andy; Huang, Ching-Hsun; Johnson, James; Krankina, Olga; Lettenmaier, Dennis; Littell, Jeremy; Maguire, Doug; Mote, Philip; Oniel, Elaine; Robinson, Donald; Turner, Dave; Wang, Tongli; Waring, Richard. Submitted to the USDA National Institute of Food and Agriculture (NIFA) Program entitled Regional Approaches for Adaptation to and Mitigation of Climate Variability and Change in 2012 ($10M; declined).

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PNWTIRC plans for 2012-2013

- Oguz Urhan will defend his thesis and graduate with an M.S.
- Complete the wood stiffness study of young Douglas-fir and western hemlock and submit a manuscript for publication
- Complete the miniaturized seed orchard study and submit a manuscript for publication
- Complete the stem form analyses and submit a manuscript for publication
- Reanalyze the site characterization data using all available sites and new NRCS soils data
- Complete the analyses of mechanistic growth models with CIPS

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PNWTIRC plans for 2012-2013

- Begin the western white pine/Douglas-fir SNP marker study (CAFS)
- Form a subcommittee to guide new research on SNP marker-assisted selection, develop a research proposal, and begin implementing new SNP genotyping

PACIFIC NORTHWEST TREE IMPROVEMENT
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ABSTRACT: MINIATURIZED SEED ORCHARD STUDY

Scott Kolpak, Jim Smith, Sara Lipow, Mike Albrecht, Jeff DeBell, Glenn Howe

Plantations of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) are typically established using genetically improved trees derived from wind-pollinated seed orchards (Howe et al. 2006). Most of these orchards consist of large, widely spaced, grafted trees (clones) that are intensively managed for seed production. Miniaturized seed orchards (MSOs) have been proposed as desirable alternatives to conventional orchards in Douglas-fir and other conifers. In contrast to conventional orchards, MSOs are planted at much tighter spacings, and the trees are maintained at heights of only 2 to 4 m (Sweet 1995; Sweet and Krugman 1977).

The potential advantages of high-density MSOs include (1) greater per-hectare seed yields, (2) the ability to speed the production of seed crops, thereby increasing financial returns from tree breeding, (3) reduced land costs because of the greater planting density, (4) reduced costs of cone harvest and pest management because of the small sizes of the trees, and (5) increased genetic gains because of more effective pollination control (i.e., SMP, control mass pollination, or bloom delay) and reduced pollen contamination. However, the advantages of MSOs may be offset by the increased costs of crown management and flower stimulation. The goal of the Miniaturized Seed Orchard Study was to compare three alternative spacings and management regimes on a scale large enough to evaluate realistic management costs, seed yields, and seed quality.

We studied three miniaturized seed orchards of coastal Douglas-fir in Oregon and Washington. In Experiments 1 and 2, flowering and cone yields were greater using stem girdles plus stem injections of $GA_{4/7}$ compared to girdling alone, GA alone, root pruning, and girdling plus fertilization with $CaNO_3$. In Experiment 3, topping and pruning in the summer following flower stimulation minimized crown volume and maximized female and male flower densities. In Experiment 4, the widest spaced orchard (4x6 m) generally produced more flowers and cones per tree, and greater flower and cone densities compared to the higher density orchards (1x3 m and 2x4 m). Initially (i.e., in 2010), per-hectare cone yields were lowest in the 4x6 orchard, but were greatest in the 4x6 orchard by 2012. In general, per-hectare cone yields were similar or greater in the MSOs compared to nearby conventionally spaced orchards. Trees grafted using scions collected from juvenile trees (ages 7 to 8) generally had larger crowns, more female flowers and cones per tree, and greater female flower and cone densities compared to scions collected from middle-aged (ages 30 to 31) or mature (ages 56 to 101) trees. We found no evidence that clonal rows resulted in reduced seed quality relative to nearby conventional orchards.

Miniaturized Seed Orchard Study

Scott Kolpak[1], Jim Smith[2], Sara Lipow[3], Mike Albrecht[3], Jeff DeBell[4], Glenn Howe[1]

[1]Pacific Northwest Tree Improvement Research Cooperative
[2]Plum Creek Timber Company
[3]Roseburg Resources
[4]Washington State Department of Natural Resources

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MSO background

Miniaturized Seed Orchards (MSOs)

- MSOs (Sweet 1995) = orchards planted at close spacings and maintained at a height of 2–4 m

Benefits*

- Increased per hectare seed yields through higher stocking of orchards and crown management (e.g., fruit trees)
- Shift to earlier production of operational quantities of seed
- Increased genetic gains by facilitating controlled pollination (e.g., CMP, SMP) and by reducing pollen contamination via bloom delay
- Reduced costs of CMP, SMP, insect control, and cone harvest

Seed Orchard Research in Coastal Douglas-fir: Comparison of Macro, Micro, and Mini Orchards, July 1999, T.S. Anekonda and W.T. Adams.

MSO progress

Expected deliverables 2011 – 2012

- Seed orchard measurements
- MSO publication outline

Seed orchard accomplishments 2011 - 2012

Measurement or activity	Date	Status
Seed extraction results	December 2011	Completed
Flower stimulation production rates	March – May 2012	Completed
Flower counts	May 2012	Completed
Cone counts	August 2012	Completed
Crown volume – 2011 growth	Oct. – Nov. 2012	Completed
Crown volume – 2012 growth	Oct. – Nov. 2012	Completed
Flower & vegetative bud phenology		Cancelled
Crown pruning		Cancelled
Bloom delay		Cancelled

MSO progress

Expected deliverables 2011 – 2012

- Seed orchard measurements and activities – completed
- MSO publication outline – completed

  (1) Incorporated a decade of information from 3 MSOs
  - *WaDNR's Meridian Seed Orchard*
  - *Roseburg Resource's Lebanon Forest Regeneration Center (LFRC)*
  - *Plum Creek's Stewart Farm Orchard*

  (2) Seed orchard measurements at Plum Creek's Toledo MSO
  - *2010 - 2012*

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MSO publication outline

Incorporating a decade of information from three MSOs

Data source	Year	Location
Vaughn and PNWCTA flower stimulation trial	2001 – 02	Lebanon FRC
Meridian flower stimulation trial	2002 – 03	Meridian Seed Orchard
Vaughn pruning trial	2005 – 06	Lebanon FRC
Cost analysis of MSOs – Flower stim., cone collection, pruning, orchard establishment		Meridian & Stewart Farm orchards
Toledo MSO trial – 3 orchard spacings, 3 scion ages	2010 - 12	Stewart Farm Orchard

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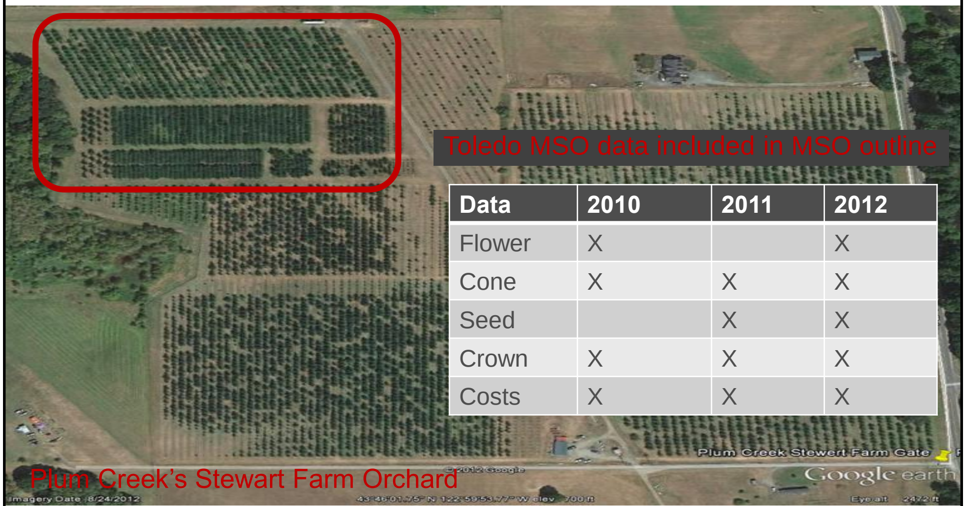
MSO publication outline

Plum Creek's Toledo MSO measurements



Data	2010	2011	2012
Flower	X		X
Cone	X	X	X
Seed		X	X
Crown	X	X	X
Costs	X	X	X

MSO materials, methods, and results

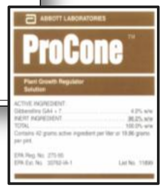
Data source	Year	Location
Vaughn and PNWCTA flower stimulation trial	2001 – 02	Lebanon FRC
Meridian flower stimulation trial	2002 – 03	Meridian Seed Orchard
Vaughn pruning trial	2005 – 06	Lebanon FRC
Cost analysis of MSOs – Flower stim., cone collection, pruning, orchard establishment		Meridian & Stewart Farm orchards
Toledo MSO trial – 3 orchard spacings, 3 scion ages	2010 - 12	Stewart Farm Orchard

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Flower and cone stimulation

- Vaughn and PNWCTA flower stimulation trial
  - *2001 – 2002*
  - *See Cherry et al. 2007*

> **Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*)[1]**
>
> Marilyn L. Cherry, Thimmappa S. Anekonda, Michael J. Albrecht, and Glenn T. Howe



- Meridian flower stimulation trial
  - *2002 – 2003*

Lebanon FRC flower stimulation trial

Materials and methods

- 2 orchards at 2.44 x 3.96 m spacing
  - *Vaughn*
  - *PNWCTA*
- 3 treatments + 1 control
- 36 trees / treatment / orchard
  - *9 clones x 4 ramets*

Treatment	Description
G	**Girdling** Stem girdles
GA	**4% $GA_{4/7}$** ProCone,1X rate (0.336 ul / mm^2)
G+GA	**Girdling + 4% $GA_{4/7}$** ProCone,1X rate (0.336 ul / mm^2)
Control	**Not stimulated**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Meridian flower stimulation trial

Miniaturized orchard

- 1.37 to 4.57 m (4.5 – 15 ft)
- Established (1987 to 1990)
- Pruned to 3 – 4 m (2000)
- 3 treatments / year
- 2 plots per treatment / year

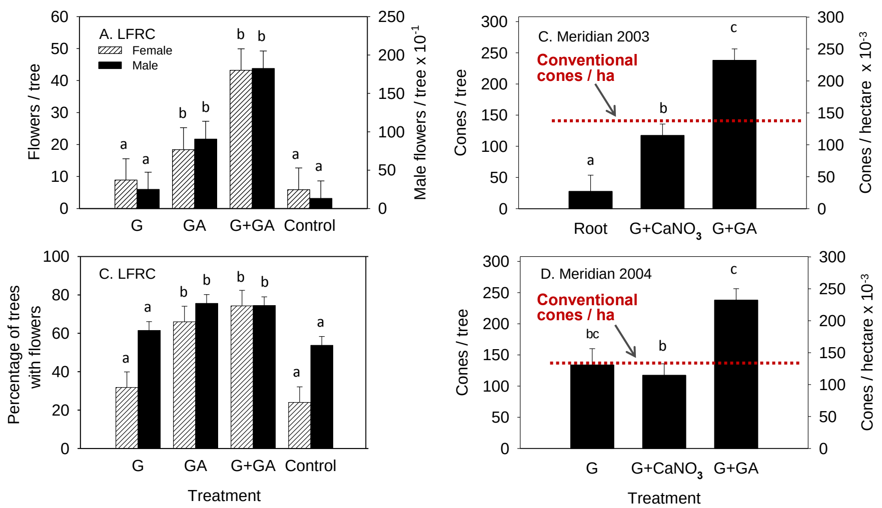
Conventional orchard

- Established - 1978 to 1988
- Rogued in 1998 (24 x 32 ft)
- Stimulated with G + $CaNO_3$

Treatment	2002	2003	Description
Root	X		**Root pruning** Parallel trenches, 1 meter from tree
G		X	**Girdling** Stem girdles
G+$CaNO_3$	X	X	**Girdling + N** 200 lbs. / acre N
G+GA	X	X	**Girdling + 4% $GA_{4/7}$** ProCone, 1 x rate (0.336 ul / mm²)

$GA_{4/7}$ + girdling enhances flower production

Flower production is less effective using $CaNO_3$ + girdling



MSO materials, methods, and results

Data source	Year	Location
Vaughn and PNWCTA flower stimulation trial	2001 – 02	Lebanon FRC
Meridian flower stimulation trial	2002 – 03	Meridian Seed Orchard
Vaughn pruning trial	2005 – 06	Lebanon FRC
Cost analysis of MSOs – Flower stim., cone collection, pruning, orchard establishment		Meridian & Stewart Farm orchards
Toledo MSO trial – 3 orchard spacings, 3 scion ages	2010 - 12	Stewart Farm Orchard

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Vaughn pruning trial

Materials and methods

- The study was done at the Vaughn MSO
  - *2005 – 2007*
- 6 treatments
  - *5 pruning treatments applied across a 2-year flower stimulation / cone production cycle*
  - *1 control (not pruned)*
- 126 trees per treatment
  - *18 clones x 7 ramets / clone*

Vaughn pruning treatments

Treatment		Description
1	**control**	*No pruning*
Treatments in the year of flower stimulation (Spring-Summer 2005, '07)		
2	**spr**	*Prune laterals and leaders before bud flush*
3	**spr/su**	*Prune laterals before bud flush; prune leaders in summer, after bud set*
4	**su**	*Prune laterals and leaders in summer, after bud set*
Treatments in the year of cone production (Summer-Fall 2006, '08)		
5	**su**	*Prune laterals and leaders in summer, after bud set*
6	**fall**	*Prune laterals and leaders in fall, after cone harvest*

Pacific Northwest Tree Improvement Research Cooperative

PNWTIRC

Vaughn pruning methods

Top pruning

- Main stem was cut to 2 m and terminal buds were removed from all but one branch located near the top of the pruned tree
  - *A small unpruned branch was chosen to form the new leader*

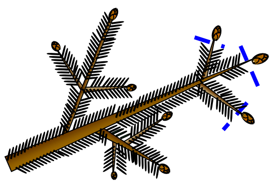
Large branches

- Main terminal buds and distal first-order branches were pruned from the branch

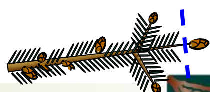
Smaller branches

- Main terminal buds were pruned from the branch but distal first-order branches were not removed

Large branch pruning



Small branch pruning



Pacific Northwest Tree Improvement Research Cooperative

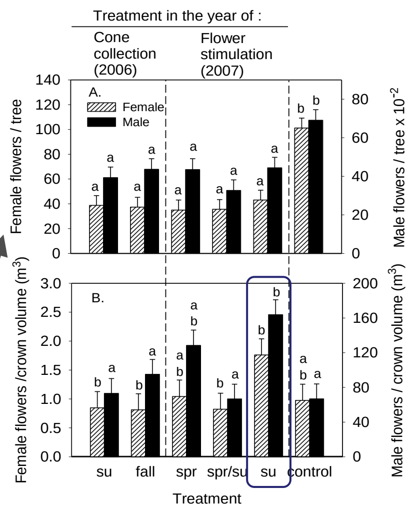
PNWTIRC

Flower efficiency was highest when trees were pruned after bud set in the year of stimulation

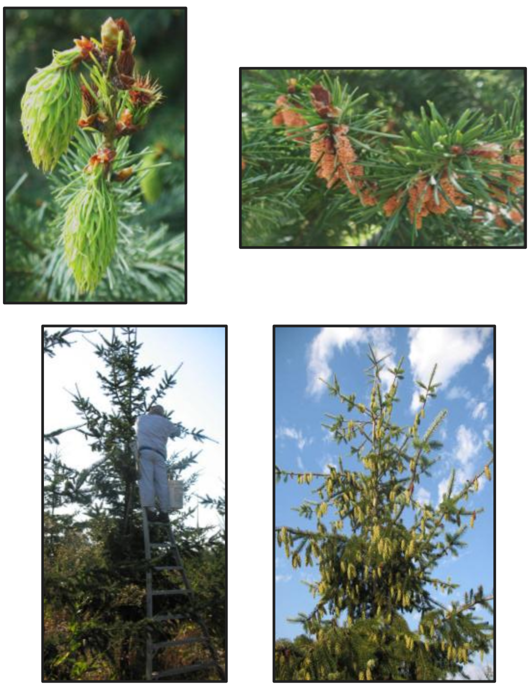
Flower production was similar among pruned treatments

Pruning and measuring activities

Date	Year of flower stimulation	Year of cone collection
March 2005	Treat. 2 pruned	
March / July 2005	Treat. 3 pruned	
July 2005	Treat. 4 pruned	
March 2006	Crown volume	
May 2006	Flower counts	
July 2006		Treat. 5 pruned
September 2006		Treat. 6 pruned
March 2007	Treat. 2 & 3 pruned	
September 2007	Treat. 4 pruned	
April 2008	Crown volume	
May 2008	Flower counts	



Flower and cone production at the Toledo MSO

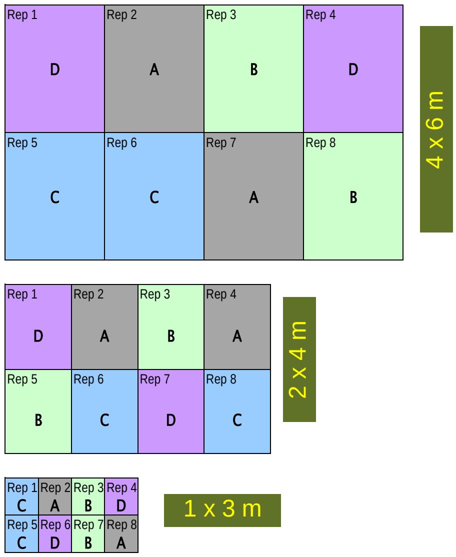


Plum Creek's Toledo MSO

Materials and methods

Spacing (m)	No. of trees	Stems / ha	Crown height (m)
4x6	640	416	NA
2x4	640	1,250	3
1x3	768	3,333	2

Clone category	~ Age of ortet at grafting	No. clones
Forward	10	12
Forward	33	4
Backward	60-103	8



Plum Creek's Toledo MSO

Flower stimulation

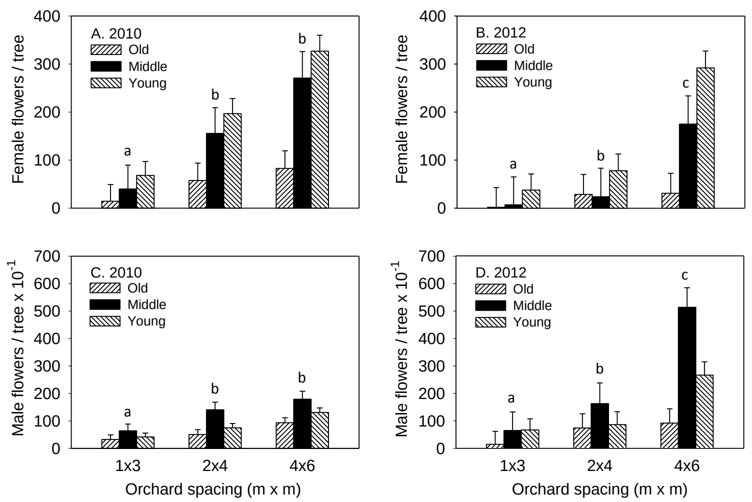
2010 and 2012

- 4x6 and 2x4 m = west replications stimulated
- 1x3 m = all replications stimulated

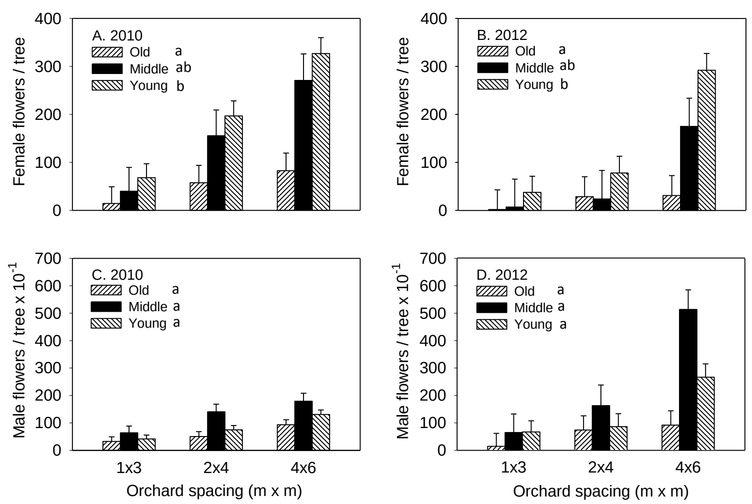
2011

- 4x6 and 2x4 m = east replications stimulated
- 1x3 m = not stimulated

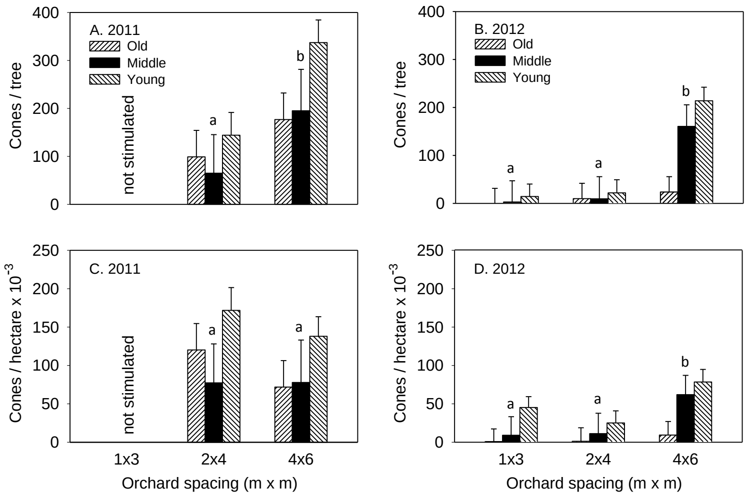
Wider spaced orchards produced more flowers per tree



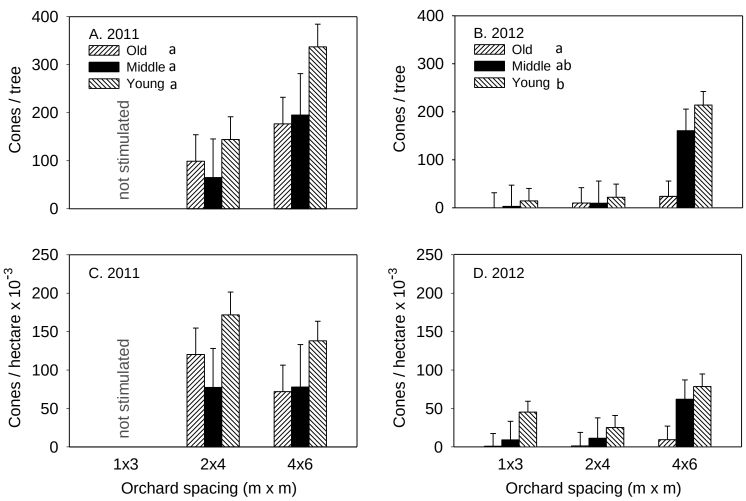
Young scions produced more female flowers per tree



The 4x6 m orchard produced the most cones



Youngest scions may produce more cones than the oldest scions



Crown volume measurements at the Toledo MSO

Is flower/cone production influenced by crown volume?

- Measured crown volume between 2010 and 2012
- Traits measured:
  - *Crown length*
  - *Crown shape*
  - *Maximum crown radius*

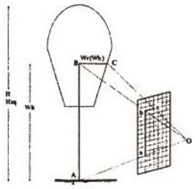
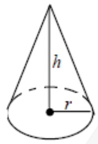


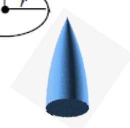
Fig. 1. Schematic presentation of the basic geometric relationships of the crown window device.

Old scion clone

Young scion clone

$$V = \frac{1}{3}\pi r^2 h$$

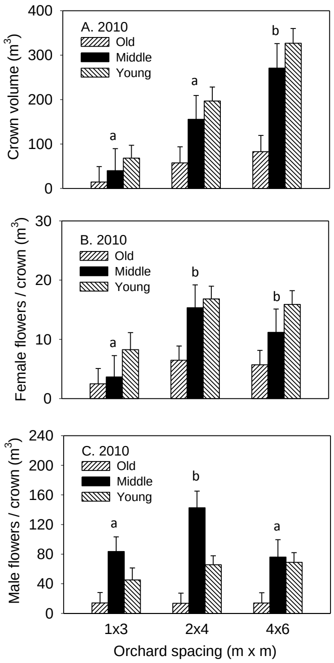




Vol = ½ π r²h

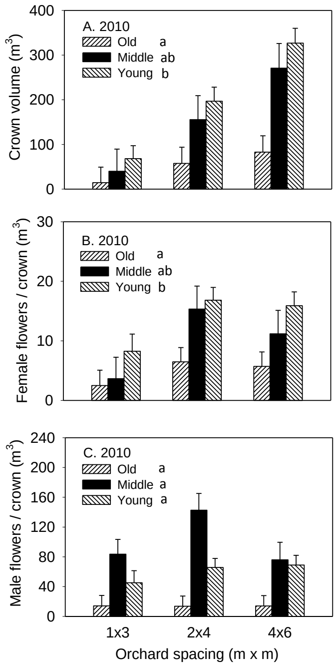
Spacing conclusions

- Flower efficiency (males and females) was higher in the wider spacings
- Crown volume was largest in the 4x6 m spacing



Scion age conclusions

- The youngest scions had higher female flower efficiency than the oldest scions
- The youngest scions were larger than the oldest scions



Conclusions

Flower stimulation

- $GA_{4/7}$ plus girdling enhances flower production in young orchards
  - *Caution: Cherry et al. 2007 found a 4–14 % increase in mortality and a reduction in relative growth rate, probably due to heavy cone production*
- Flower stimulation using girdling and $CaNO_3$ was less effective in the Meridian flower stimulation study
  - *$CaNO_3$ stimulation has been shown to be effective on larger trees*

Crown management

- Flower efficiency was highest when trees were pruned after bud set in the year of flower stimulation
- Timing of pruning did not influence short-term cone production in young trees

Conclusions

Orchard spacing

- Wider spaced orchards produce more flowers and cones per tree
- By 2012, the widest spaced orchard (4x6 m) produced the most cones/hectare
- Crown volume was largest in the 4x6 m orchard
- Flower efficiency (males and females) was greater at the wider spacings

Scion age

- The youngest scions may produce more flowers and cones per tree and have greater female flower efficiencies compared to the oldest scions
- The crowns of the youngest scions were larger than the crowns of the oldest scions

Implications

Role of MSOs in Douglas-fir tree improvement

- MSOs are promising for intensive control pollination programs that incorporate CMP or SMP to capture increased genetic gains
- By increasing trees/ha and stimulating young grafts with $GA_{4/7}$, orchard managers can reduce the time lag between orchard establishment and the production of commercial levels of seed
- MSOs require high intensity management and/or large capital investments to maintain the crowns for long-term cone production
  - *Labor needed for annual or bi-annual hand pruning*
  - *Mechanized pruning devices (e.g., sickle bar) require capital investments*

Implications

Role of MSOs in Douglas-fir tree improvement

- Seed orchard resources and the expected level of orchard management have declined since the inception of the MSO study
  - *CMP and SMP are used infrequently in the region*
  - *Seed orchard budgets are low and staffs are small*
  - *The benefits of MSOs are difficult to realize*
- The first operational harvest of WaDNR's MSOs (high-density orchards) occurred in 2012

Upcoming work

Future orchard activities

- Acquire operational seed extraction data (e.g., % filled, seeds/lb.)
- Acquire more seed orchard management costs (e.g., mechanized pruning rates at Meridian)

Expected deliverables 2012 – 2013

- Finish analyzing Toledo MSO data
  - *Recently acquired 2012 crown volume data and seed quality data*
- Incorporate a financial analysis of MSOs
  - *What is the cost of managing more trees per hectare?*
- Submit MSO manuscript – March 2013

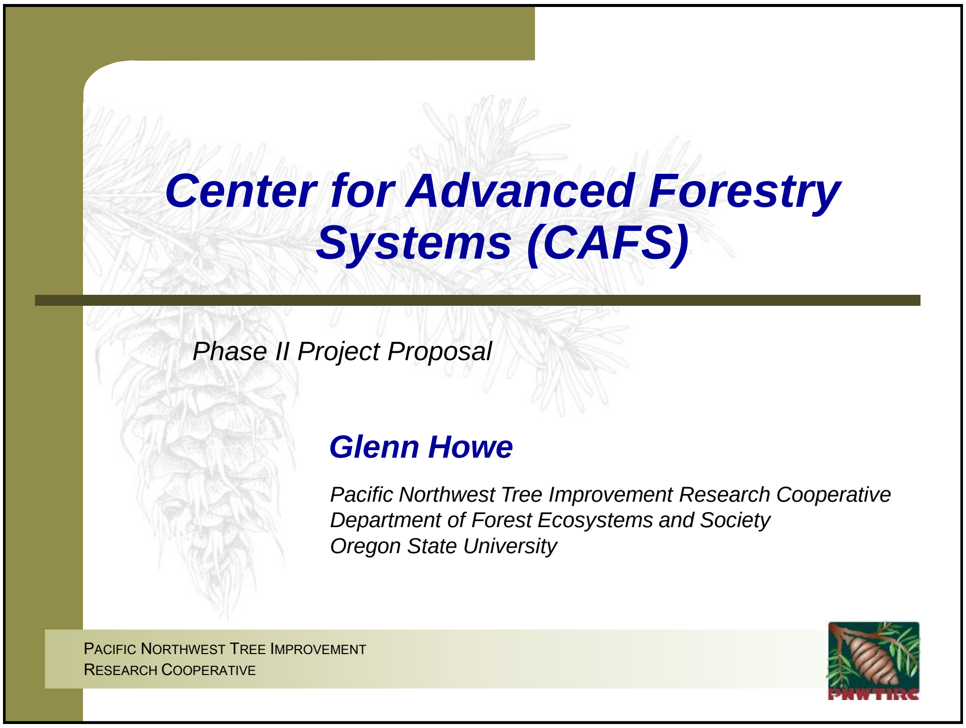
PACIFIC NORTHWEST TREE IMPROVEMENT
RESEARCH COOPERATIVE

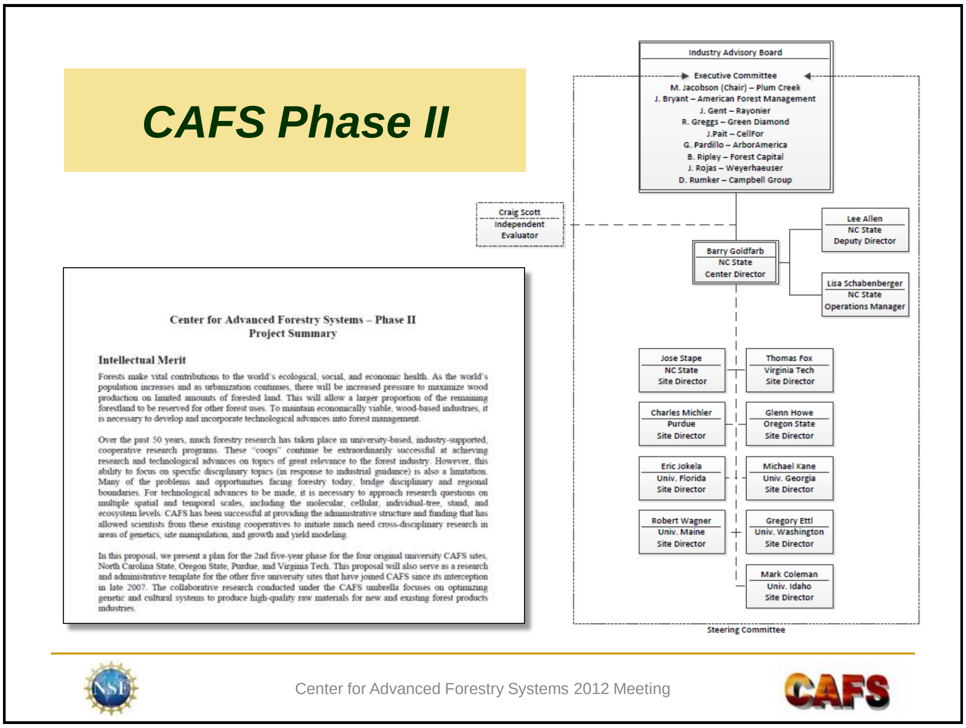
ABSTRACT: CENTER FOR ADVANCED FORESTRY SYSTEMS (CAFS) PHASE II PROJECT PROPOSAL*

Glenn Howe

Over the past 50 years, much forestry research has taken place in university-based, industry-supported, cooperative research programs. These "coops" continue be extraordinarily successful at achieving research and technological advances on topics of great relevance to the forest industry. However, this ability to focus on specific disciplinary topics is also a limitation. Many of the problems and opportunities facing forestry today bridge disciplinary and regional boundaries. Therefore, we must approach research questions on multiple spatial and temporal scales, including the molecular, cellular, individual-tree, stand, and ecosystem levels. CAFS has provided the administrative structure and funding that has allowed scientists from existing cooperatives to initiate cross-disciplinary research in the areas of genetics, site manipulation, and modeling. In this proposal, we describe the second five-year phase for the four original university CAFS sites, North Carolina State, Oregon State, Purdue, and Virginia Tech. This plan will also serve as a template for the other five universities that have joined CAFS since its inception in 2007. Research conducted under the CAFS umbrella focuses on optimizing genetic and cultural systems to produce high-quality raw materials for new and existing forest products industries.

This abstract contains excerpts from the project summary for the proposal entitled "Collaborative Research: Center for Advanced Forestry Systems—Phase II" by Barry Goldfarb, Howard Lee Allen, Harold E. Burkhart, Thomas R. Fox, Glenn T. Howe, Douglass Jacobs, Douglas A. Maguire, Charles H. Michler, Richard Meilan, Michael Saunders, Jose L. Stape, and Steven H. Strauss.


Center for Advanced Forestry Systems (CAFS)
Phase II Project Proposal
Glenn Howe
Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University
Pacific Northwest Tree Improvement Research Cooperative
PNWTIRC


CAFS Phase II
Center for Advanced Forestry Systems – Phase II
Project Summary
Intellectual Merit
Forests make vital contributions to the world's ecological, social, and economic health. As the world's population increases and as urbanization continues, there will be increased pressure to maximize wood production on limited amounts of forested land. This will allow a larger proportion of the remaining forestland to be reserved for other forest uses. To maintain economically viable, wood-based industries, it is necessary to develop and incorporate technological advances into forest management.
Over the past 50 years, much forestry research has taken place in university-based, industry-supported, cooperative research programs. These "coops" continue be extraordinarily successful at achieving research and technological advances on topics of great relevance to the forest industry. However, this ability to focus on specific disciplinary topics (in response to industrial guidance) is also a limitation. Many of the problems and opportunities facing forestry today, bridge disciplinary and regional boundaries. For technological advances to be made, it is necessary to approach research questions on multiple spatial and temporal scales, including the molecular, cellular, individual-tree, stand, and ecosystem levels. CAFS has been successful at providing the administrative structure and funding that has allowed scientists from these existing cooperatives to initiate much need cross-disciplinary research in areas of genetics, site manipulation, and growth and yield modeling.
In this proposal, we present a plan for the 2nd five-year phase for the four original university CAFS sites, North Carolina State, Oregon State, Purdue, and Virginia Tech. This proposal will also serve as a research and administrative template for the other five university sites that have joined CAFS since its interception in late 2007. The collaborative research conducted under the CAFS umbrella focuses on optimizing genetic and cultural systems to produce high-quality raw materials for new and existing forest products industries.
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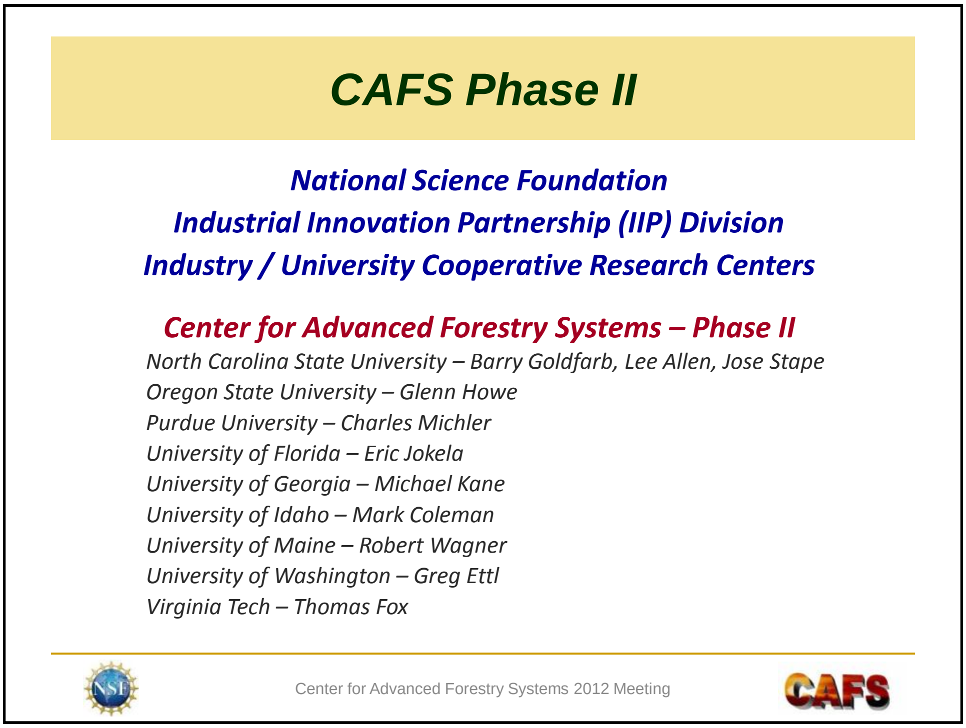
CAFS Phase II
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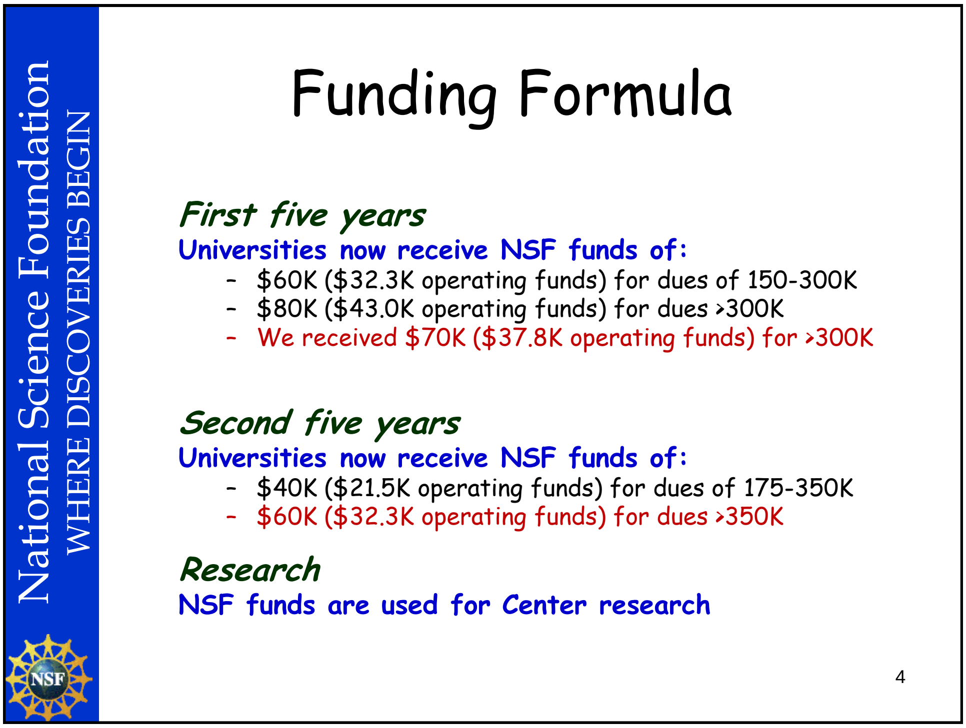
National Science Foundation
WHERE DISCOVERIES BEGIN
NSF
Funding Formula
First five years
Universities now receive NSF funds of:
- $60K ($32.3K operating funds) for dues of 150-300K
- $80K ($43.0K operating funds) for dues >300K
- We received $70K ($37.8K operating funds) for >300K
Second five years
Universities now receive NSF funds of:
- $40K ($21.5K operating funds) for dues of 175-350K
- $60K ($32.3K operating funds) for dues >350K
Research
NSF funds are used for Center research
4

ABSTRACT: GENETIC MARKERS FOR WESTERN WHITE PINE AND DOUGLAS-FIR

Marc L. Rust, Anthony Davis, Glenn Howe

Members of the Center for Advanced Forestry Systems (CAFS) at the University of Idaho are engaged in a breeding program to develop blister rust resistant western white pine for operational reforestation and ecosystem restoration. This program currently uses a classical approach of selection, testing, and breeding to identify rust resistant genotypes for inclusion in seed orchards and breeding arboreta. While this approach is suitable, recent advances in genomics may provide important technologies that could be used alongside the classical approach to either improve the resistance levels in selected populations, or shorten the time for developing improved varieties. In Douglas-fir, Simple Sequence Repeat (SSR) markers are routinely used to identify mislabeled genotypes and measure pollen contamination. These markers work well, but still require a modest amount of hands-on work in the laboratory and during the interpretation of marker genotypes. Therefore, we propose to develop procedures that will allow tree breeders to transition from using SSRs to using SNPs for these and other analyses. This project will focus on developing procedures for using genetic markers called Single Nucleotide Polymorphisms (SNPs) to enhance existing operational tree improvement programs in western white pine and Douglas-fir. SNPs are DNA sequence variations caused by single base pair changes at specific positions along a chromosome. These markers can be used to (1) identify mislabeled genotypes in breeding programs and seed orchards, (2) measure and manage pollen contamination, (3) develop and evaluate advanced generation breeding materials using open-pollinated mating designs (i.e., without using controlled crosses), and if enough markers are available, (4) practice a marker-assisted breeding approach called genomic selection.

Center for Advanced Forestry Systems (CAFS)

Genetic Markers for Western White Pine and Douglas-fir

Marc L. Rust, Anthony Davis, Glenn Howe

Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Western white pine breeding program

- White pine blister rust was introduced into western North America in 1910 and reached Idaho in 1923
- Native stands were decimated by this exotic disease
- Efforts to eradicate *Ribes* spp., the alternate host, were much less successful in the mountainous west then in the east
- Phenotypically resistant western white pine were found in heavily infected stands as early as 1946

Rust on *Ribes*

Rust needle lesion

Rust canker

Phase I plus-tree

Center for Advanced Forestry Systems 2012 Meeting

Western white pine breeding program

- The USFS began a resistance breeding program in in 1950, under the supervision of Richard T. Bingham
- This program is a collaborative effort between the USFS and the Inland Empire Tree Improvement Cooperative (IETIC) at the University of Idaho
- The breeding program uses a classical approach of selection, testing, and controlled-cross breeding
- Good gains in resistance have been achieved

High rust family

Low rust family

Breeding

Seed orchard

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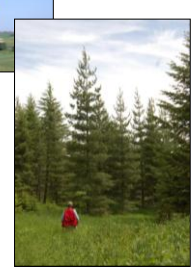
Western white pine breeding program

- Improved seed from first generation orchards is widely used for reforestation
- Seed from the IETIC's Richard T. Bingham seed orchard is estimated to be 65% resistant
- Performance of the Bingham seed source is variable, with some plantations performing better than expected and others worse
- The Bingham seed source has been planted extensively in the region for the last 30 years



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Douglas-fir

- Huge geographic and environmental range
  Two varieties, multiple species?
- 22 million hectares in the US/Canada
- Up to 120 m tall and 1,400 years old
- Planted in Europe, New Zealand, Chile
- 8 billion board feet of lumber in 2002
- Large breeding programs - more than:
  4 million progeny from
  34,000 parents on
  1,000 progeny test sites

Objectives

- Develop methods for the operational use of SNP genetic markers in western white pine and Douglas-fir
  - Identify mislabeled genotypes in seed or breeding orchards
  - Measure and manage pollen contamination in seed orchards
  - Identify good or poor performing parents from already established open-pollinated or bulk seed orchard plantations (esp. w. white pine)
  - Use open-pollinated mating designs for advanced generation breeding, thereby reducing or eliminating the need for controlled-cross breeding which is slow and expensive
  - Assess the potential for developing marker assisted breeding (e.g., genomic selection)

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What are genetic markers?

Any visible or measurable trait that can be used to track the inheritance of a particular gene or chromosomal location

- Markers must vary among individuals (i.e., must be 'polymorphic')
- Many kind of markers with different pros and cons
  - *Morphological*
  - *Monoterpenes*
  - *Allozymes*
  - *RFLP = Restriction fragment length polymorphism*
  - *RAPD = Randomly amplified polymorphic DNA*
  - *RAD = Restriction site associated DNA*
  - *SSR = Simple sequence repeat*
  - *SNP = Single nucleotide polymorphism*

www.pinegenome.org/ctgn

CTGN CAP

Trends in genetic markers

- Genomics research provides an almost unlimited supply of markers
- The costs of genotyping are declining rapidly
  - *New markers (SNPs)*
  - *New genotyping platforms*
- High-throughput genotyping can be outsourced
- Today's marker applications were only imagined a few years ago

Illumina GoldenGate platform

www.pinegenome.org/ctgn

CTGN CAP

Single nucleotide polymorphism (SNP)

Usually 2 alleles

Many many loci

SNP

Tree 1	A C G T G T C **G** G T C T T A	Maternal chrom.
	A C G T G T C **A** G T C T T A	Paternal chrom.
Tree 2	A C G T G T C **G** G T C T T A	Maternal chrom.
	A C G T G T C **G** G T C T T A	Paternal chrom.
Tree 3	A C G T G T C **A** G T C T T A	Maternal chrom.
	A C G T G T C **A** G T C T T A	Paternal chrom.

Tree 1 is ***heterozygous*** Trees 2 and 3 are ***homozygous***

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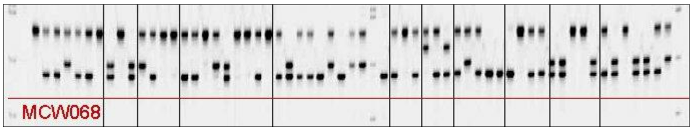
Markers in tree improvement

- Check the identity of genotypes (fingerprinting)
- Seed orchard management (parental analysis)
- Measure relatedness (pedigree reconstruction)
- Management of genetic diversity including inbreeding
- Marker assisted selection (MAS) and breeding (MAB)



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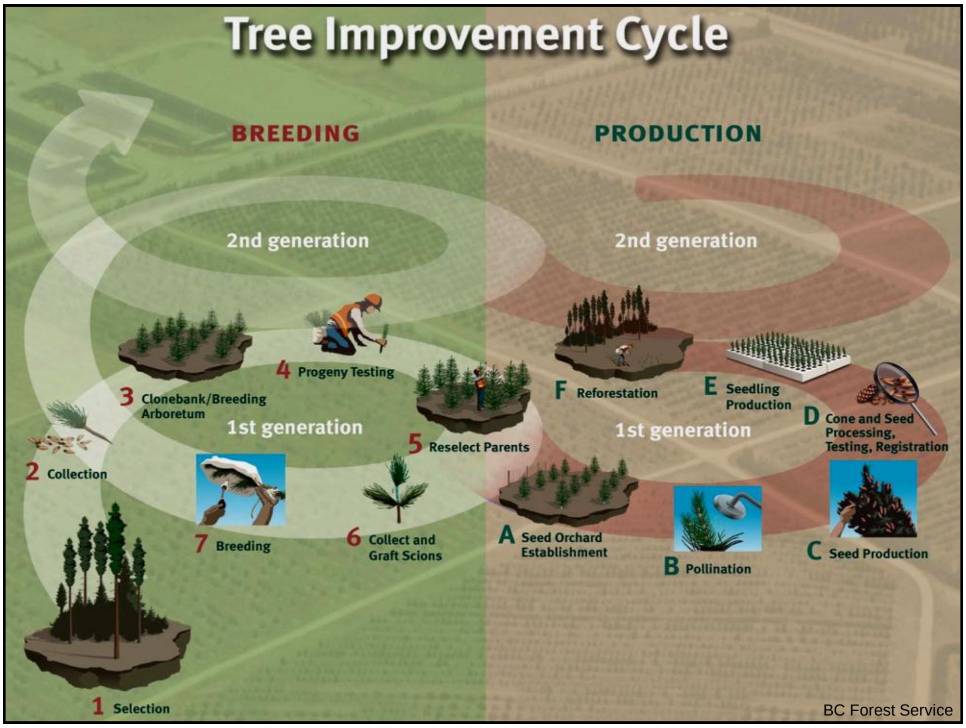
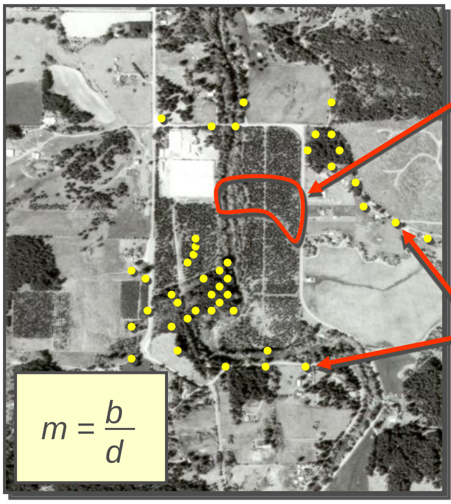


Seed orchard example



$$m = \frac{b}{d}$$

Trees genotyped

Test Block (all ramets)

60 trees from adjacent SO blocks (not shown)

44 trees from native stands

Slavov, G.T., Howe, G.T., Gyaourova, A.V., Birkes, D.S., and Adams, W.T. 2005. Estimating pollen flow using SSR markers and paternity exclusion: accounting for mistyping. Mol. Ecol. 14:3109-3121.

Breeding without crossing
Open-pollinated mating designs

‘Breeding without breeding’ (El-Kassaby et al 2007)

- Field test open-pollinated seed from seed orchard parents
- Use SNPs to fingerprint the top individuals in the best OP families to identify the fathers
- Saves time and the cost of full-sib crossing, and may increase gain
- Might be particularly valuable for identifying both parents of blister rust resistant trees in operational plantations?

**El-Kassaby,Y.A., M. Lstiburek, C. Liewlaksaneeyanawin, G.T. Slavov and G.T. Howe. 2007. Breeding without breeding: approach, example, and proof of concept. In: Proc. IUFRO, Low input breeding and genetic conservation of forest tree species. Antalya, Turkey. pp. 43-54.*

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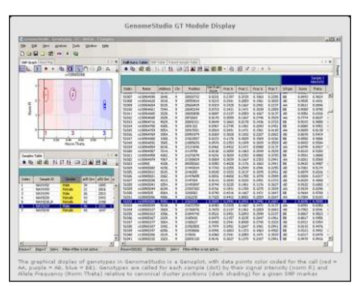
Status of white pine SNP markers

- 384 SNPs tested using the Illumina GoldenGate platform developed by P. Maloney, J. Wegrzyn, and D. Neale at UCD
- 164 SNPs passed quality thresholds based on genetic analyses using Illumina GenomeStudio software

GoldenGate
96-3,000 SNPs/run
96 samples/run



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Experimental plan

- Choose 40-80 SNPs to test using a low-cost genotyping platform
- For example, Sequenom is suitable for genotyping hundreds of SNPs in several thousand DNA samples
- SNPs will be chosen based minor allele frequency, call frequency, and probability of Hardy-Weinberg equilibrium

University of Washington genotyping costs

	Genotyping Cost for N Markers in Multiplex								
#Samples	N = 32	N = 33	N = 34	N = 35	N = 36	N = 37	N = 38	N = 39	N = 40
1-96	$675.84	$696.96	$718.08	$739.20	$760.32	$781.44	$802.56	$823.68	$844.80
97-192	$1290.24	$1330.56	$1370.88	$1411.20	$1451.52	$1491.84	$1532.16	$1572.48	$1612.80
193-288	$1843.20	$1900.80	$1958.40	$2016.00	$2073.60	$2131.20	$2188.80	$2246.40	$2304.00
289-384	$2334.72	$2407.68	$2480.64	$2553.60	$2626.56	$2699.52	$2772.48	$2845.44	$2918.40

Estimating pollen flow using SSR markers and paternity exclusion: accounting for mistyping

Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers

Gancho T. Slavov, Glenn T. Howe, and W. Thomas Adams

SEQUENOM

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CAFS

Experimental plan

- Plant materials
  - We will genotype at least 100 trees from each species
  - WWP and DF = seed orchard parents and progeny
  - WWP = disease resistant trees in plantations derived from SO parents
- Quantitative genetic analyses
  - We will estimate minor allele frequencies, probabilities of deviation from Hardy-Weinberg equilibrium, and detection probabilities
- Protocols for using SNPs in tree improvement programs
  - Protocols will be based on empirical results and simulations
  - Protocols = DNA fingerprinting, pollen contamination, mating systems
  - Evaluate open-pollinated breeding with parental analysis (breeding w/out crossing)

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CAFS

Project timeline

- Year 1
  - Recruit a graduate student at UI and write thesis proposal
  - Choose the most promising SNPs for western white pine and Douglas-fir
- Year 2
  - Collect samples from existing seed orchards and isolate DNA
  - Genotype SO trees using a cost-efficient platform such as Sequenom
- Year 3
  - Collect samples from plantations (WWP) and isolate DNA
  - Genotype trees using a cost-efficient platform such as Sequenom
  - Use empirical results and simulations to evaluate the potential for using open-pollinated breeding with parental analysis

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Expected deliverables – Year 1

- Graduate student thesis proposal
- Identification of the most promising 40-80 SNPs to test operationally in w. white pine and Douglas-fir
- Identification of the seed orchard parents, seed orchard progeny, and plantation trees to use in the study
- Confirmation of the genotyping platform and organization to use for genotyping
- Results from initial genotyping trials

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Budget

- A Master's level student will be recruited at the University of Idaho
- We request $50,000/year for 3 years with $40,000 being requested by UI and $10,000 being requested by OSU*
- IETIC and PNWTIRC and their members will provide access to seed orchards, breeding orchards, and plantations for DNA sampling

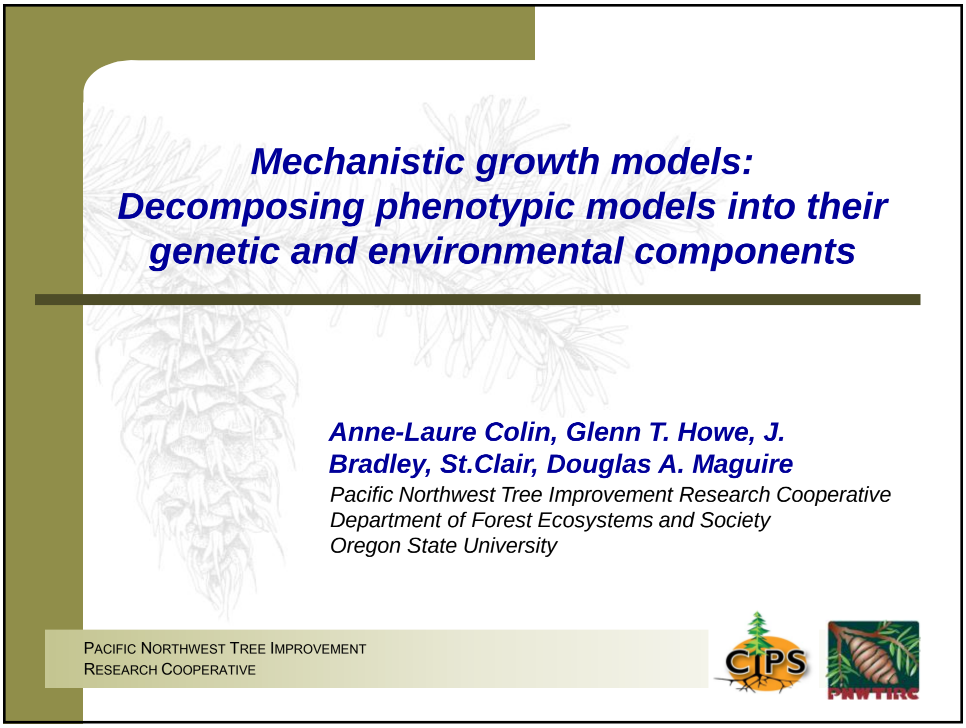
*In the written proposal, the UI request was $40,000 of total costs, but the OSU request was for direct costs only (~$5000). To be consistent with the UI request, the request for the OSU portion is now $10,000 (= total costs).

Center for Advanced Forestry Systems 2012 Meeting

ABSTRACT: MECHANISTIC GROWTH MODELS: DECOMPOSING PHENOTYPIC MODELS INTO THEIR GENETIC AND ENVIRONMENTAL COMPONENTS

Anne-Laure Colin, Glenn T. Howe, J. Bradley, St.Clair, Douglas A. Maguire

The long-term goal of this project is to develop mechanistic growth models that account for genetic and environmental effects. This integration is valuable for optimal deployment of genetically improved materials; making selection and breeding decisions; and altering the forest environment by modifying planting density, controlling vegetation, fertilizing, pruning, and thinning. We will accomplish this by (1) developing a conceptual model for tree growth that is based on available genetic data, (2) using Structural Equation Modeling (SEM) to understand the direct and indirect effects of these traits on tree growth, (3) developing a phenotypic growth model using the most important variables from SEM, (4) decomposing the phenotypic growth model into its genetic and environmental components using a selection index approach. This work is being accomplished in collaboration with the Center for Intensive Planted-forest Silviculture (CIPS).


Mechanistic growth models:
Decomposing phenotypic models into their genetic and environmental components
Anne-Laure Colin, Glenn T. Howe, J. Bradley, St.Clair, Douglas A. Maguire
Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University
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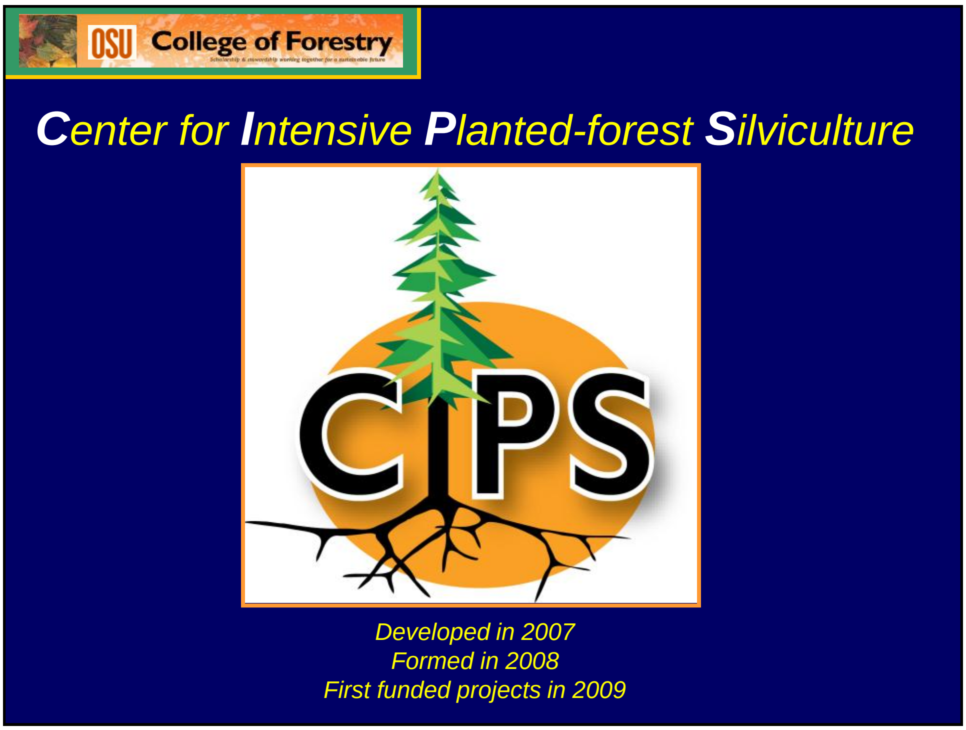
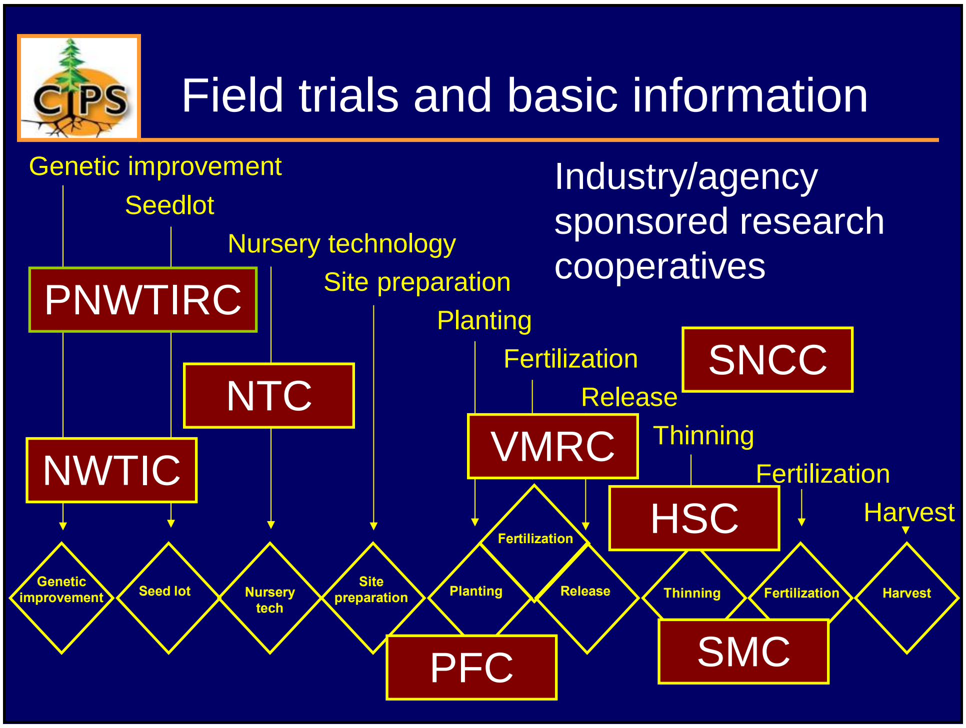
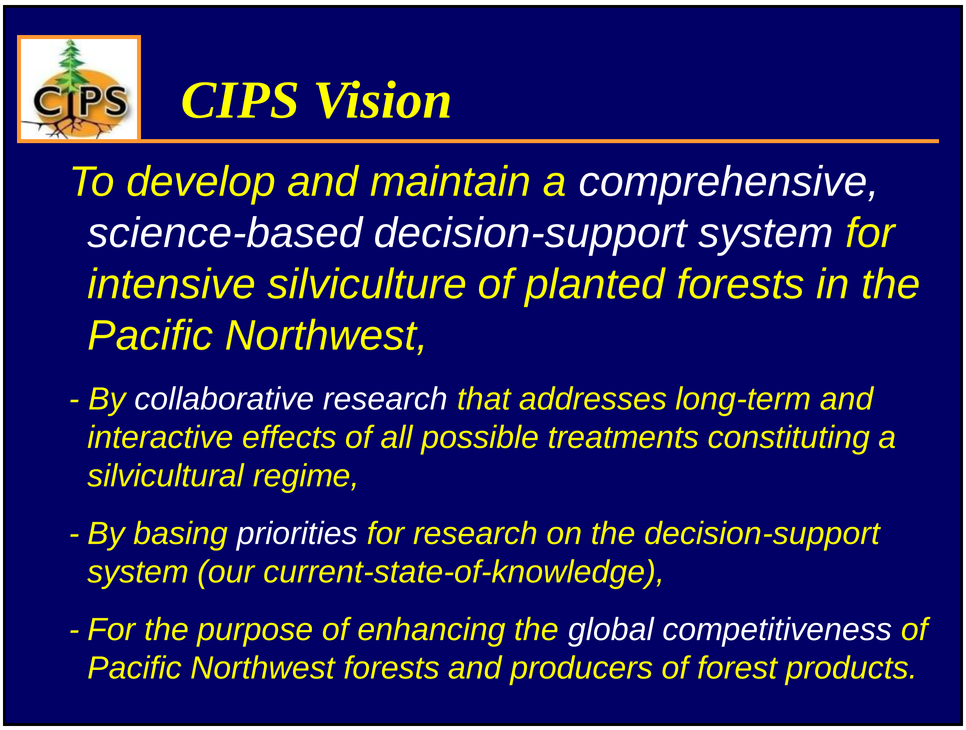
PNWTIRC


OSU College of Forestry
Center for Intensive Planted-forest Silviculture
CIPS
Developed in 2007
Formed in 2008
First funded projects in 2009


CIPS
Field trials and basic information
Genetic improvement
Seedlot
Nursery technology
Site preparation
Planting
Fertilization
Release
Thinning
Fertilization
Harvest
Industry/agency sponsored research cooperatives
PNWTIRC
NTC
NWTIC
SNCC
VMRC
HSC
PFC
SMC
Genetic improvement
Seed lot
Nursery tech
Site preparation
Planting
Fertilization
Release
Thinning
Fertilization
Harvest


CIPS
CIPS Vision
To develop and maintain a comprehensive, science-based decision-support system for intensive silviculture of planted forests in the Pacific Northwest,
- By collaborative research that addresses long-term and interactive effects of all possible treatments constituting a silvicultural regime,
- By basing priorities for research on the decision-support system (our current-state-of-knowledge),
- For the purpose of enhancing the global competitiveness of Pacific Northwest forests and producers of forest products.

CIPS Strategic Framework

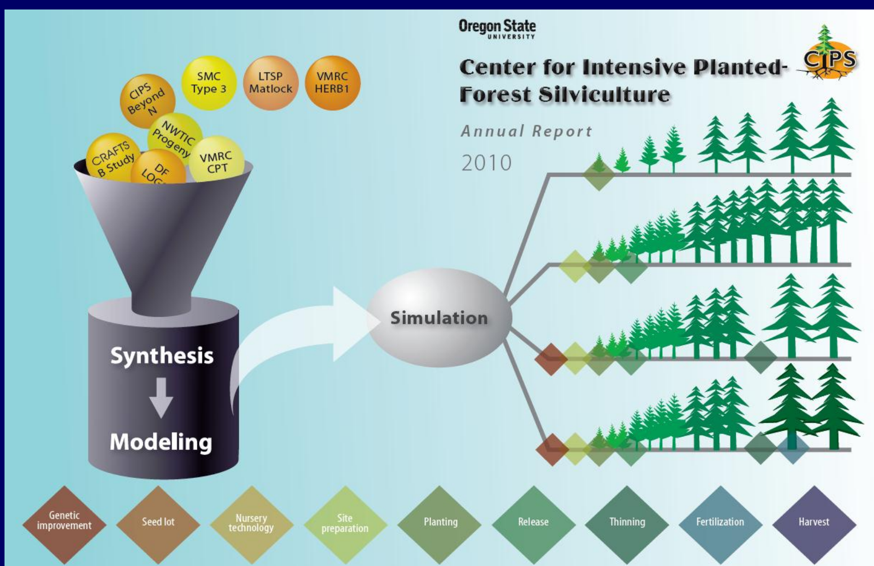
NEEDS:

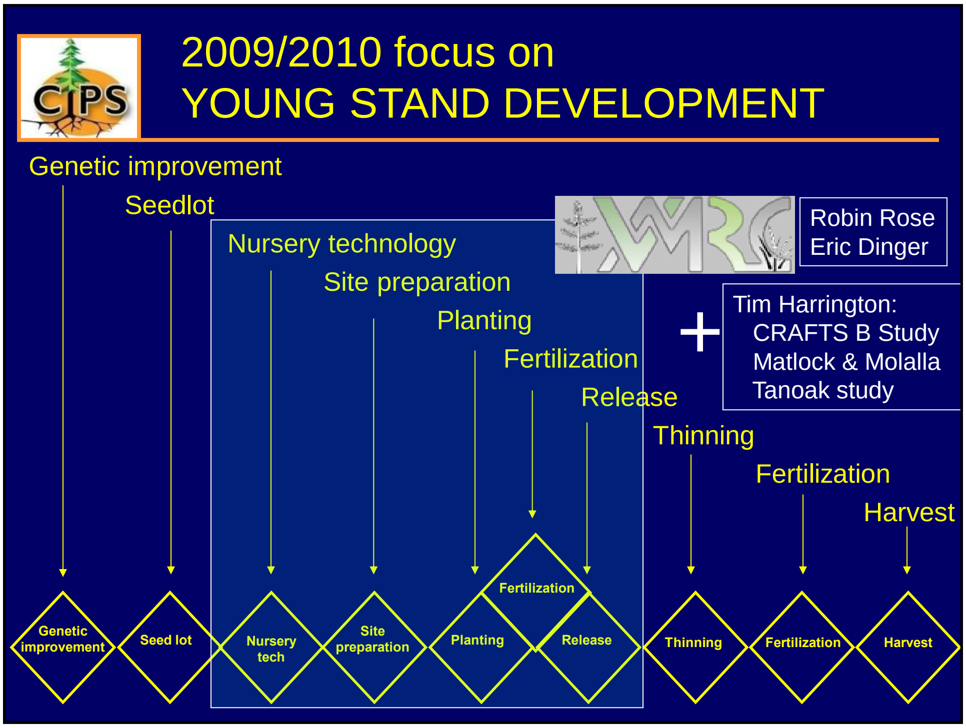
- Develop a growth model with mechanisms that are just adequate to integrate effects of environmental conditions, stand and tree structure, and silvicultural practices on growth and final yield by tree/log size class

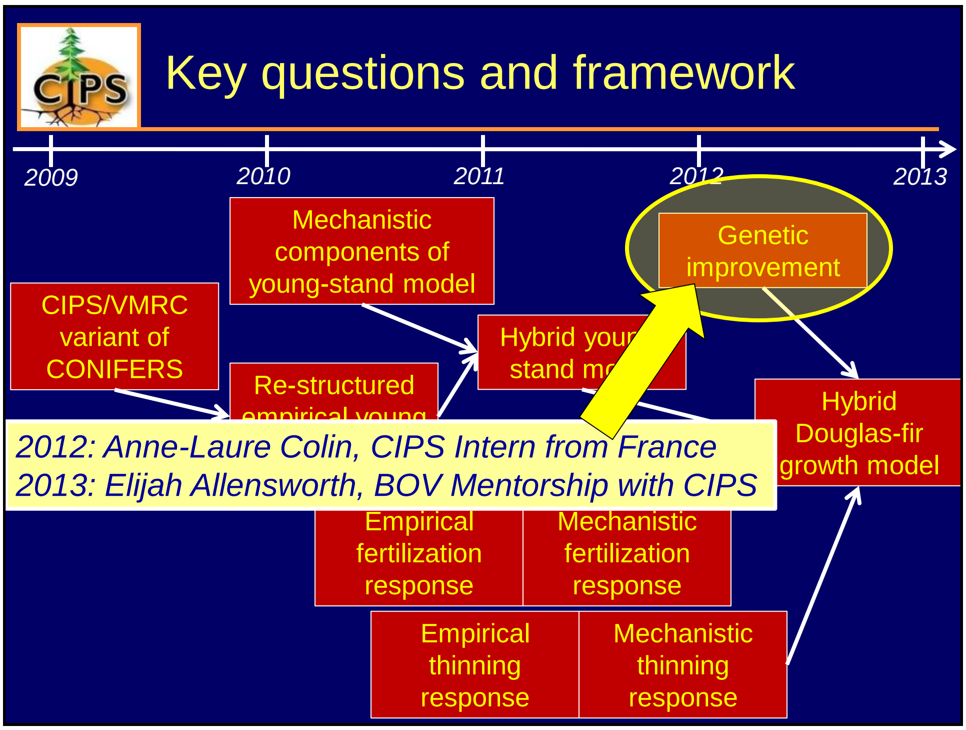
- Improve our ability to characterize the productive potential of a site

- Represent genetic tree improvement by direct links to some structural attribute or physiological process or parameter that influences growth model

2010 CIPS Annual Report




CIPS
2009/2010 focus on
YOUNG STAND DEVELOPMENT
Genetic improvement
Seedlot
Nursery technology
Site preparation
Planting
Fertilization
Release
Thinning
Fertilization
Harvest
VMRC
Robin Rose
Eric Dinger
+
Tim Harrington:
CRAFTS B Study
Matlock & Molalla
Tanoak study
Genetic improvement
Seed lot
Nursery tech
Site preparation
Planting
Fertilization
Release
Thinning
Fertilization
Harvest


CIPS
Key questions and framework
2009
2010
2011
2012
2013
Mechanistic components of young-stand model
Genetic improvement
CIPS/VMRC variant of CONIFERS
Hybrid young stand model
Re-structured empirical young
Hybrid Douglas-fir growth model
2012: Anne-Laure Colin, CIPS Intern from France
2013: Elijah Allensworth, BOV Mentorship with CIPS
Empirical fertilization response
Mechanistic fertilization response
Empirical thinning response
Mechanistic thinning response

Outline

- Introduction
- Goal
- Materials and methods
- Preliminary results
- Conclusions

Anne-Laure Colin (M.S.)
Engineering School of Agronomy and Food Sciences, Nancy, France

Pacific Northwest Tree Improvement Research Cooperative

Introduction

- Douglas-fir growth models
- Tree-improvement programs produce genetically superior reforestation stock
- How to incorporate the effects of genetic gain into growth models?

Goal

Our ***long-term goal*** is to develop mechanistic growth models that account for genetic and environmental effects

We will develop and test a ***new approach*** for partitioning the genetic and environmental components of mechanistic growth models

Introduction

Growth models use phenotypic coefficients to predict the phenotypic value of a target trait (e.g., volume growth)

- Target trait = α*trait1 + β*trait2 + δ*trait3 + …
- trait1, trait2, and trait3 are predictor traits
- α, β, δ describe the relationships between predictor traits and the phenotypic value of the target trait
- α, β, δ can be decomposed into their genetic and environmental components
- Target trait = $(\alpha_g + \alpha_e)$*trait1 + $(\beta_g + \beta_e)$*trait2 + $(\delta_g + \delta_e)$*trait3 + …

Pacific Northwest Tree Improvement Research Cooperative

Introduction

Genetic selection index

- Target trait (G) = α_g*trait1 + β_g*trait2 + δ_g*trait3 + …

Environmental index

- Target trait (E) = α_e*trait1 + β_e*trait2 + δ_e*trait3 + …

Combine

- Target trait (P) = (α_g + α_e)*trait1 + (β_g + β_e)*trait2 + (δ_g + δ_e)*trait3 + …

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Why?

Target trait (P) = (α_g + α_e)*trait1 + (β_g + β_e)*trait2 + (δ_g + δ_e)*trait3 + …

- How will growth be affected if I change the genetics of one or more predictor traits, but leave the environment unaffected?
  - *What is the impact of tree breeding?*
  - *Which traits are the most important to target in breeding programs?*
- How will growth be affected if I change the environment, but the leave the genetics unchanged?
  - *What is the impact of silvicultural treatments?*
  - *Which silvicultural treatments are best to implement?*
- Can we use mechanistic models to extrapolate to new conditions?

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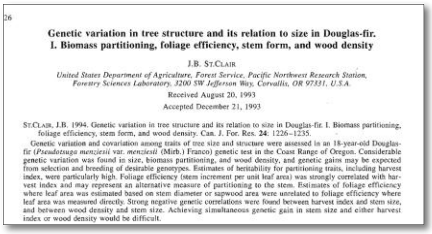
Materials

Data from a progeny test measured by Brad St.Clair

- 18-year-old Douglas-fir test near Newport, OR


26

Genetic variation in tree structure and its relation to size in Douglas-fir. I. Biomass partitioning, foliage efficiency, stem form, and wood density

J.B. St.Clair

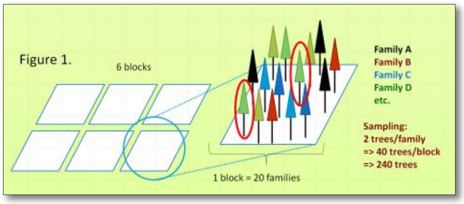
United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, U.S.A.

Received August 20, 1993

Accepted December 21, 1993

St.Clair, J.B. 1994. Genetic variation in tree structure and its relation to size in Douglas-fir. I. Biomass partitioning, foliage efficiency, stem form, and wood density. Can. J. For. Res. **24**: 1226–1235.

Genetic variation and covariation among traits of tree size and structure were assessed in an 18-year-old Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) genetic test in the Coast Range of Oregon. Considerable genetic variation was found in size, biomass partitioning, and wood density, and genetic gains may be expected from selection and breeding of desirable genotypes. Estimates of heritability for partitioning traits, including harvest index, were particularly high. Foliage efficiency (stem increment per unit leaf area) was strongly correlated with harvest index and may represent an alternative measure of partitioning to the stem. Estimates of foliage efficiency where leaf area was estimated based on stem diameter or sapwood area were unrelated to foliage efficiency where leaf area was measured directly. Strong negative genetic correlations were found between harvest index and stem size, and between wood density and stem size. Achieving simultaneous genetic gain in stem size and either harvest index or wood density would be difficult.



What to measure?

Example target traits

- Height growth
- Diameter growth
- Volume growth

What to measure?

Examples of predictor traits

- Crown length
- Crown width
- Dry weight of leaves
- Specific leaf area (needle leaf area / needle dry weight)
- Average branch angle
- Branch number
- Branch dry weight

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Steps

To decompose mechanistic phenotypic growth models into their genetic and environmental components we will…

- Develop a conceptual model for tree growth based on available genetic data
- Use ***Structural Equation Modeling*** (SEM) to understand the direct and indirect effects of these traits on tree growth
- Develop a phenotypic growth model using the most important variables from SEM
- Decompose the phenotypic growth model into its genetic and environmental components using a selection index approach

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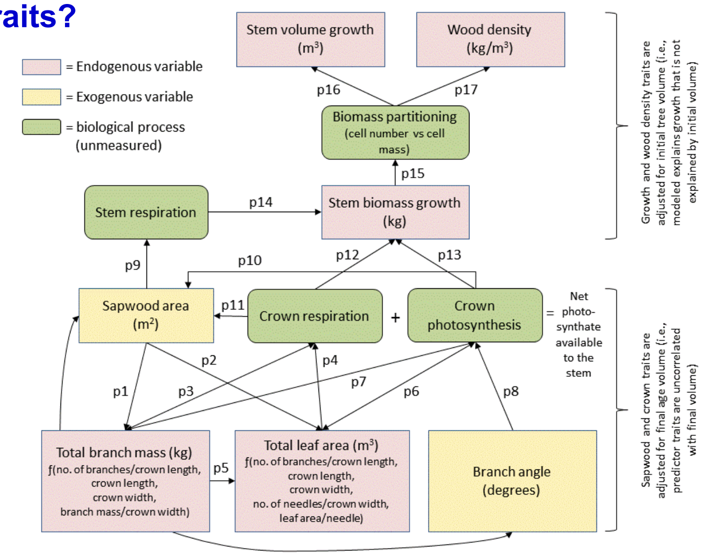
Conceptual model and structural equation modeling

Which traits?



Conceptual model and structural equation modeling

Path analysis or other methods of ***structural equation modeling*** (SEM) can be used to help choose model variables that best represent ***mechanistic (i.e., causal)*** relationships between the measured variables and tree growth

To explore mechanistic relationships, we will use predictor traits that are not directly associated with tree size *per se*

Methods

Phenotypic growth models

- The second step is to develop a phenotypic growth model that predicts volume growth (ΔV_p) from the most important tree morphological traits
- This step is used to refine choice of traits and variable transformations
- The b_{pi} are the phenotypic coefficients for each predictor trait (P_i), and the P_i are the measured phenotypic traits expressed as deviations from the population mean

$$[1] \qquad \Delta V_p = b_{p1} P_1 + b_{p2} P_2 + \ldots + b_{pn} P_n$$

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Methods

Genetic growth model = selection index

- A corresponding genetic model for the target trait can be estimated as a linear function of phenotypic observations using a selection index approach (Falconer 1989)

$$[2] \qquad \Delta V_g = b_{g1} P_1 + b_{g2} P_2 + \ldots + b_{gn} P_n$$

- Matrix form: G = P * b

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Methods

Genetic selection index

G = P * b

$$\begin{bmatrix} \sigma_1 \\ Cov_G(1,2) \\ \vdots \\ Cov_G(1,n) \end{bmatrix} = \begin{bmatrix} \sigma_1 & Cov_P(1,2) & \dots & Cov_P(1,n) \\ Cov_P(1,2) & \sigma_2 & & Cov_P(2,n) \\ \vdots & & \ddots & \vdots \\ Cov_P(1,n) & \cdots & \cdots & \sigma_n \end{bmatrix} * \begin{bmatrix} b_1 \\ b_2 \\ \vdots \\ b_n \end{bmatrix}$$

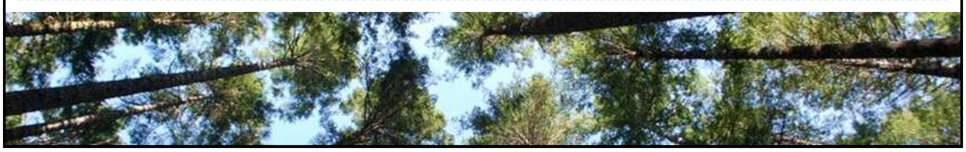
- Index: G = P * b
- Solution: $b = P^{-1} * G$

Methods

Genetic selection index

- Genetic and phenotypic covariances estimated using MIXED procedure in SAS

```
proc mixed data=A
       covtest asycov noitprint noclprint noinfo method = reml;
       class trait fam rep tree;
              model y = trait trait*rep;
              random trait/type=UN subject=fam g gcorr;
              repeated/type=UN subject=tree(rep fam) r rcorr;
       ods output covparms=_varcomp;
run;
```



Methods

Environmental growth models

- A corresponding environmental model for the target trait can be estimated using an analogous approach to the selection index
- ΔV_e is the environmental value of the target trait expressed as a deviation from the population mean
- b_{ei} are the coefficients associated with each predictor trait (P_i)

$$[3] \qquad \Delta V_e = b_{e1} P_1 + b_{e2} P_2 + \ldots + b_{en} P_n$$

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Preliminary results

	CV	TCRDW	LFDEN	GE	CWL	BRDEN	SLAZ	LACPA	LACSA
HT18 = Genetic selection index	0.29	-0.35	-0.20	0.02	-0.06	-0.01	-0.02	0.31	0.28
HT18 = Environmental index	0.52	-0.34	-0.22	0.44	-0.27	0.18	-0.09	0.07	0.47
HT18 = Phenotypic index	0.82	-0.69	-0.42	0.46	-0.33	0.17	-0.11	0.38	0.76

- *CV = crown volume*
- *TCRDW = crown dry weight*
- *LFDEN = leaf density (leaf dry weight per unit crown volume)*
- *GE = foliage growth efficiency*
- *CWL = slenderness ratio (crown width per crown length)*
- *BRDEN = branch bulk density = branch dry weight per unit crown volume*
- *SLAZ = specific leaf area*
- *LACPA = leaf area per crown projection area*
- *LACSA = leaf area per crown surface area*

Next steps

Refine trait selection using structural equation modeling

Transform traits as needed prior to analysis (e.g., logarithm)

Re-estimate genetic, environmental, and phenotypic models

ABSTRACT: EARLY GENETIC SELECTION FOR WOOD STIFFNESS IN JUVENILE DOUGLAS-FIR AND WESTERN HEMLOCK*

Oguz Urhan, Scott Kolpak, Glenn Howe

Wood modulus of elasticity (MOE), also known as wood stiffness, is one of the most important wood properties. Wood stiffness is a measure of the resistance to deflection, and is important because some products such as laminated veneer lumber, plywood, and dimension lumber require stiff and strong wood. Incorporating wood stiffness into breeding programs could help maintain acceptable wood quality and increase economic profits for wood producers. There is limited information on the genetics of wood stiffness in young Douglas-fir plantations, and the genetics of wood stiffness has not been studied in western hemlock. Therefore, my objectives were to use young (8- to 12-year-old) genetic test plantations of Douglas-fir and western hemlock to (1) determine the best approach for measuring acoustic velocity, and then use the best approach to (2) estimate additive and non-additive genetic variation, heritabilities, and potential genetic gains, (3) estimate genetic and phenotypic correlations between acoustic velocity and growth traits, and (4) discuss implications of these results for operational tree improvement. I studied acoustic velocity at two genetic test plantations of Douglas-fir (Fir Grove and Roaring River) and one test plantation of western hemlock (Toledo) using the TreeSonic and Microsecond Timer standing-tree tools, and two vertical placements of the sensors. These tools can be used to measure acoustic velocity in standing-trees, an indirect measure of wood stiffness. My results show that (1) the effects of standing-tree tool, vertical placement, and DBH-adjustment methods were non-significant, (2) acoustic velocity had significant genetic variation in Douglas-fir and western hemlock, (3) heritability of acoustic velocity was higher than the heritabilities of growth and form traits, and (4) substantial genetic gains in acoustic velocity are possible. My results also indicate that the mean acoustic velocity and modulus of elasticity were higher in Douglas-fir than in western hemlock. Although mean stiffness was higher for Douglas-fir, the distributions of acoustic velocity and modulus of elasticity overlapped between the species. These results indicate that comparable genetic gains are possible using both the TreeSonic and Microsecond Timer tools. Because of practical considerations, and higher measurement rates, I recommend that breeders use the TreeSonic and the same-face approach. I found positive genetic correlations between growth and acoustic velocity in western hemlock. This provides an opportunity to focus on improving wood stiffness in western hemlock so that it can better compete with Douglas-fir for products in which stiffness is important. Near optimal genetic gains are possible using 10 trees per family for wood stiffness. Because dominance variation was non-significant for Douglas-fir and western hemlock, near optimal gains in wood stiffness and growth traits can be obtained by collecting open pollinated seed from orchards (i.e., without control crossing) as long as pollen contamination is not a problem.

*This is the abstract from Oguz Urhan's M.S. thesis entitled "Early Genetic Selection for Wood Stiffness in Juvenile Douglas-fir and Western Hemlock."

Early Genetic Selection for Wood Stiffness In Juvenile Douglas-fir and Western Hemlock

Oguz Urhan, Scott Kolpak, Glenn Howe

Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University

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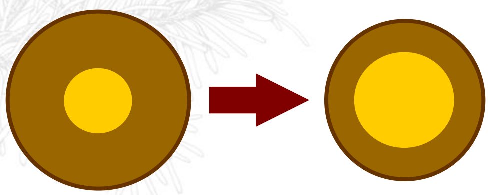
Genetics of wood stiffness

Trend toward shorter rotations, faster growth

- More wood from the juvenile corewood
- Juvenile wood:
  - *Lower wood density*
  - *Higher microfibril angle*
  - *Lower stiffness*
  - *More shrinkage*



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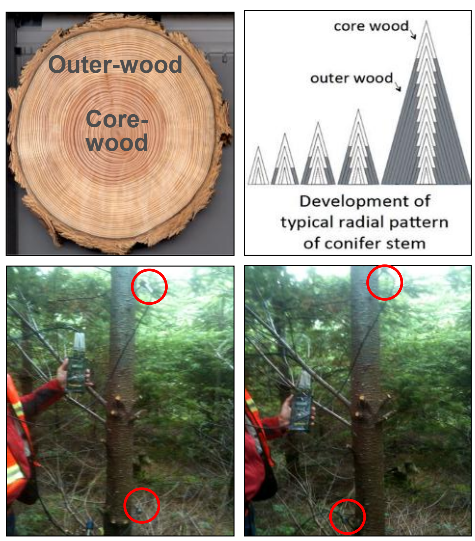
Background

Past research on wood quality traits of mature Douglas-fir (Cherry et al 2008, Jayawickrama et al 2011, Vikram et al 2011)

- Stiffness is heritable and substantial gains are possible
- HM200 modulus of elasticity (MOE) has a high genetic correlation (rg=0.92) with bending MOE
- ST300 MOE has a moderate genetic correlation (rg=0.57) with bending MOE
- Acoustic velocity can be used instead of dynamic MOE to evaluate wood stiffness (no strong need to measure greenwood density)
- Selection for bending stiffness or acoustic velocity has no large adverse effects on growth traits

Background

- Genetic variation and heritabilities are higher for corewood in *Pinus radiata* (Dungey et al 2006)
- Acoustic velocity for standing trees can be measured using same-face (SF) or opposite-face (OF) approaches (Mahon et al 2009, Wagner et al 2003)
- Mahon et al and Wagner et al both suggested OF is better



Rationale

- Strong interest in improving wood stiffness in young trees (e.g., ages 6-12)
- Genetic improvement of wood stiffness in western hemlock has not been studied
- Challenges
  - *Wood anatomy is different in young trees = mostly corewood and sapwood*
  - *Branches on small trees may adversely affect measurements*
  - *We don't know which measurement approaches or acoustic tools are best*

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Work phases

This project has two phases

- Phase 1: Phenotype
  - *Evaluate acoustic tools and different approaches in operational plantations*
- Phase 2: Genetics
  - *Develop measurement protocols and selection scenarios for improving juvenile wood stiffness*
  - *Estimate genetic parameters and genetic gains using the best tool and measurement approach in genetic tests*

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Phase 1: Objectives

- Compare acoustic tools with a log based tool (HM200)
  - *TreeSonic with standard sensors*
  - *TreeSonic with SD-O2 sensors*
  - *Microsecond Timer with SD-O2 sensors*
  - *Ultrasonic Timer with SD-O2 sensors*
- Evaluate horizontal sensor placement
  - *Same-face*
  - *Opposite-face*
- Evaluate vertical sensor placement
  - *Interwhorl = shorter flight-paths (< 1 meter), more clear wood*
  - *Across-whorl = longer flight-paths (~ 1 meter), acoustic waves travel across branch whorls*

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Phase 1: Acoustic tools

Tools

Sensors

Standing-tree tools, Fakopp Enterprises, Hungary

Log-based tool Fibre-gen

Phase 1: Plant materials

Table 1: Operational plantations

Plantations (Starker Forests')	**Years planted (tree age)**	**DBH (cm)**	**No. of Douglas-fir**	**No. of Western hemlock**
Peeler Greene	2004-05**(7)**	4.5	12	13
Rhubarb 2	2002-03**(9)**	6.9	13	12
Ellmaker Parkview	2001-02**(10)**	6.2	12	13
Edward Spring	1998-99**(13)**	13.4	12	9
Elephant Foot	1996-1997**(15)**	14.4	13	14

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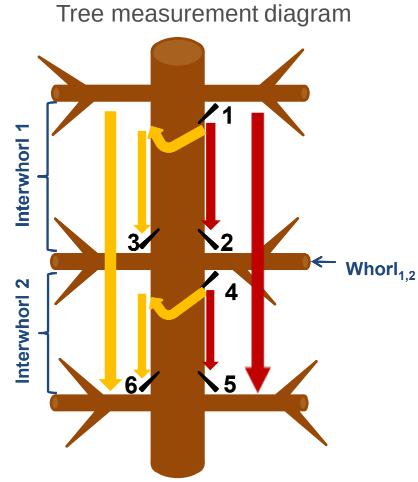
Phase 1: Methods

- Time-of-flight (TOF) measurements taken using 4 standing-tree tools
  - *4 tools x 3 locations x 2 sensor placements x 3 TOFs = 72 TOFs / tree*
- Resonance acoustic velocities taken using HM200
  - *Minimum log length 2 meters*
- We took wood disks at breast height
  - *Wrapped in plastic*
  - *Kept frozen until green wood density (DEN) measurements were taken*

Tree measurement diagram



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Phase 1: Methods

- Calculate six potential path lengths and AVs as described by Mahon et al (2009)
- Calculate dynamic MOE for each tree as
  - *$DEN*AV^2 = MOE$*
- Calculate correlations between standing-tree AV and log MOE

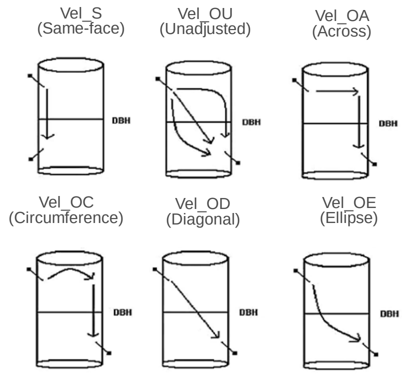

Figure 1. Hypothesized stress wave flight pats for the same-face and distance adjusted values (Mohan et al 2009)

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Phase 1: Results

Table 2. Correlations among acoustic tools and sensor placements for Douglas-fir and western hemlock pooled across five sites. Douglas-fir correlations are below the diagonal and western hemlock are above the diagonal.

Sensor Placement		Acoustic tool	Opposite face sensors: Micro Timer	Tree-Sonic 1	Tree-Sonic 2	Ultra Timer	Same face sensors: Micro Timer	Tree-Sonic 1	Tree-Sonic 2	Ultra Timer
	Opposite	Micro Timer	–	0.76	0.44	0.36	0.45	0.32	0.27	0.34
		TreeSonic 1	0.34	–	0.52	0.25	0.35	0.44	0.27	0.32
		TreeSonic 2	0.42	0.39	–	0.10	0.24	0.25	0.77	0.25
		Ultra Timer	0.04	-0.07	-0.04	–	0.20	0.10	0.02	0.40
	Same	Micro Timer	0.20	0.16	0.34	0.10	–	0.65	0.46	0.53
		TreeSonic 1	0.26	0.32	0.26	0.05	0.45	–	0.48	0.50
		TreeSonic 2	0.27	0.27	0.50	-0.04	0.42	0.60	–	0.36
		Ultra Timer	0.22	0.15	0.23	0.05	0.46	0.51	0.39	–

Correlation coeficients (r) are among averages for interwhorls 1 and 2.

Phase 1: Results

Table 3-a. Repeatability of tools and methods between interworls and whorls. Correlations between acoustic velocities measured on (1) two successive interwhorls and (2) interwhorls versus intervening whorl in Douglas-fir and western hemlock.

Sensor Placement		Acoustic tool	Douglas-fir: Interwhorl 1 versus interwhorl 2	Douglas-fir: Whorl versus interwhorls	Western hemlock: Interwhorl 1 versus interwhorl 2	Western hemlock: Whorl versus interwhorls
	Opposite	Micro Timer	0.26	0.44	0.66	0.72
		TreeSonic 1	0.14	0.43	0.53	0.60
		TreeSonic 2	0.17	0.48	0.30	0.45
		Ultra Timer	-0.08	0.11	0.64	0.58
	Same	Micro Timer	0.44	0.53	0.68	0.67
		TreeSonic 1	0.59	0.70	0.60	0.46
		TreeSonic 2	0.44	0.71	0.29	0.58
		Ultra Timer	0.42	0.44	0.43	0.37

Phase 1: Results

Table 3-b. Comparison of standing-tree tools and measurements within interwhorls versus across whorls for Douglas-fir and western hemlock. Values are pooled within-plantation correlation coefficient (r_w) and overall across-plantation correlation coefficients (r) between acoustic velocity (AV) measured with four standing-tree tools and acoustic MOE estimated using HM200. Opposite-face correlation are averages of the four diameter adjustment methods described in Mahon et al 2009.

	Douglas-fir				Western hemlock			
	Interwhorl		Whorl		Interwhorl		Whorl	
Acoustic tool	r_w	r	r_w	r	r_w	r	r_w	r
Same-face								
Ultrasonic Timer (UT)	0.51	0.70	**0.44**	**0.70**	0.20	0.35	**0.46**	**0.54**
Microsecond Timer (MT)	0.45	0.70	**0.66**	**0.83**	0.14	0.37	**0.22**	**0.47**
TreeSonic SD02 (TS-SD02)	0.43	0.69	**0.65**	**0.84**	0.39	0.54	**0.39**	**0.44**
TreeSonic STD (TS-STD)	0.55	0.78	**0.77**	**0.89**	0.08	0.33	**0.40**	**0.53**
Opposite-face								
Ultrasonic Timer (UT)	-0.01	0.42	-0.08	0.23	-0.03	0.13	-0.07	0.14
Microsecond Timer (MT)	0.18	0.41	0.66	0.84	0.01	0.29	0.02	0.41
TreeSonic SD02 (TS-SD02)	0.12	0.37	0.63	0.84	0.01	0.30	0.00	0.43
TreeSonic STD (TS-STD)	0.35	0.54	0.74	0.90	-0.17	0.09	0.26	0.48

Phase 1: Results from Table 2 and Table 3a-b

- Correlations among the tools are generally higher using the same face method
- Correlations among interwhorls are about the same (WH) or consistently higher (DF) using the same-face method compared to the opposite-face method
- Measuring across a whorl of branches seems to have little adverse effect in these young trees
- Across-whorl measurements usually have higher correlations, presumably because of longer flight paths
- Correlations between standing tree acoustic tools and the log-based acoustic tool are modest to high (0.44 to 0.89)

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Phase 1: Conclusions

- Dropped Ultrasonic - Ultrasonic correlations are weak and the measurement distance between the sensors is short
- Dropped TreeSonic with ‘standard’ sliding hammers
- TreeSonic or Microsecond timer with SD02 sensors have good correlations and are easier to use on small trees
- Whorls - measuring acoustic velocity across whorls is not a problem
- Horizontal sensor placement - we do not have adequate information to judge effectiveness so this was repeated in the genetic study

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Phase 1: Remaining questions

- Tools
  - *Microsecond Timer?*
  - *TreeSonic (with SD-O2 sensors)?*
- Horizontal placement
  - *Same-face?*
  - *Opposite-face?*
- Adjustment methods
  - *Same-face (SF) adjusted method?*
  - *Opposite-face unadjusted (OU) method?*
  - *Opposite-face across adjusted (OA) method?*
  - *Opposite-face circumference adjusted (OC) method?*
  - *Opposite-face diagonal adjusted (OD) method?*
  - *Opposite-face ellipse adjusted (OE) method?*
- Genetic parameters
  - *Heritabilities?*
  - *Potential genetic gains?*

Phase 2: Objectives

- Develop measurement protocols and selection scenarios for improving juvenile wood stiffness
- Estimate genetic parameters and genetic gains using the best tool and horizontal sensor placement in genetic tests

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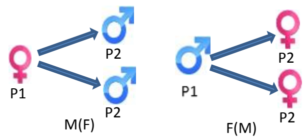
Phase 2: Plant materials

Table 4. Genetic test plantations

Genetic test	Species	Age	DBH (cm)	# of families	Mating design
Fir Grove	Douglas-fir	8	5.7	54	nested
Roaring River	Douglas-fir	12	11.3	78	nested
Toledo	Western hemlock	12	10.4	80	nested

- From the available trees, we constructed two nested mating designs (cross-types) to choose families
  - *Males nested in female M(F)*
  - *Females nested in male F(M)*
  - *Allows estimation of non-additive genetic variances*



- Each parent used in only one cross

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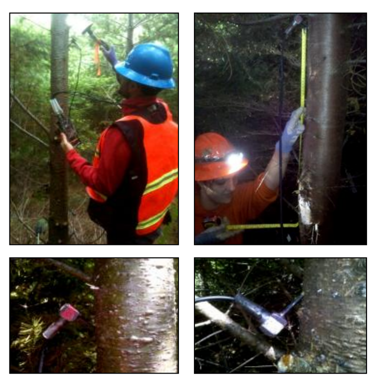
Phase 2: Methods

Application of sensors and measurements

- Two consecutive TOF for each tool and horizontal sensor placement
- Placement of sensors and penetration depth
  - *Always on the same aspect*
  - *45 +/- degree angle*
  - *20-25 mm penetration depth*
- Sensor distance
  - *No fixed distance (75 to 125 cm)*
- Diameter
  - *We measured DBH*



Phase 2: Methods

- First, we calculated six flight path lengths (P) as described Mahon et al 2009 (P_{OU}, P_{OA}, P_{OD}, P_{OC}, P_{OE} and P_{SF})
- Second, squared acoustic velocity (AV^2) was calculated for each potential flight path ($AV_{OU}^2, AV_{OA}^2, AV_{OD}^2, AV_{OC}^2, AV_{OE}^2, and\ AV_{SF}^2$)
  - $AV^2 = P/TOF$
- Third, we calculated individual-tree narrow sense heritability (h_i^2) for each AV^2 and acoustic tools
  - *Additive genetic variance (σ_A^2) was estimated as 2*σ_f^2. The estimate of σ_A^2 includes two times the maternal or paternal effects and one-half of the dominance variance ($\sigma_f^2 = \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2 \ldots$)(Becker 1984).*
- Finally, we chose the best tool and approach based on heritabilities

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Same-face is better than opposite-face approach

Table 5. Estimates of narrow sense heritabilities (h_i^2) at Toledo, Roaring River and Fir-Grove plantation using two standing tree acoustic tools (TreeSonic and Microsecond Timer) for 6 acoustic velocity variables ($AV_{OU}^2, AV_{OA}^2, AV_{OD}^2, AV_{OC}^2, AV_{OE}^2, and\ AV_{SF}^2$; $km^2 \cdot s^{-2}$).

	Heritabilities					
	Opposite-face [a]					Same-face [a]
	OU	OA	OD	OC	OE	SF
TreeSonic						
Toledo (WH)	0.491	0.564	0.503	0.551	0.533	0.511
Roaring-River (DF)	0.526	0.528	0.530	0.501	0.538	0.560
Fir-Grove (DF)	0.906	0.852	0.903	0.807	0.899	0.899
Microsecond Timer						
Toledo (WH)	0.492	0.554	0.504	0.534	0.531	0.493
Roaring-River (DF)	0.533	0.536	0.536	0.513	0.543	0.587

[a] We used five opposite-face adjustment methods (OU = unadjusted, OA = across adjusted, OD = diagonally adjusted, OC = circumference adjusted, and OE = ellipse adjusted) and one same-face adjustment method (SF = same-face) (Mahon et al 2009).

- Based on heritabilities
  - *Tools are not significantly different*
  - *Same-face approach is slightly better overall (SF=0.658 vs. OF=0.642)*
  - *If someone wants to use opposite-face approach, OA and OE may be slightly better than other opposite-face adjustments*

TreeSonic is better than Microsecond Timer

- Based on the advantages of the TreeSonic (Table 6) and production rates (Table 7), we recommend the TreeSonic acoustic tool
- For remaining analyses we used TreeSonic and same-face approach

Table 6. Advantages and disadvantages of tools

	Advantage	Disadvantage
Microsecond timer	▪ Smaller and more practical	▪ On/off switch and reset button ▪ Reset last reading each time ▪ SD-02 probe bending
TreeSonic	▪ Faster ▪ Automatically switch on/off ▪ No button on the unit ▪ SD-02 sensor more practical than original TreeSonic	▪ Bigger and heavier ▪ SD-02 probe bending

Table 7. Production of measurements

	Same-face and Opposite-face		Same-face (Estimated)	
	Pruned site	Not pruned site	Pruned site	Not pruned site
Microsecond Timer	27 tree/h	15 tree/h	41 tree/h	23 tree/h
TreeSonic	32 tree/h	19 tree/h	49 tree/h	29 tree/h

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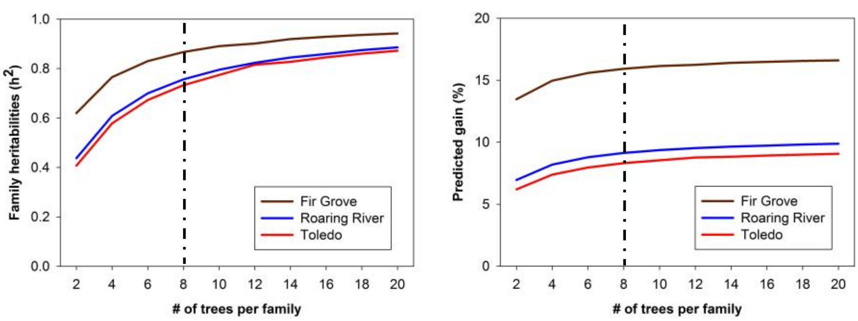
Use variance components to determine optimal sample size



- Using more than 8 trees per family does not substantially improve family heritabilities or genetic gains

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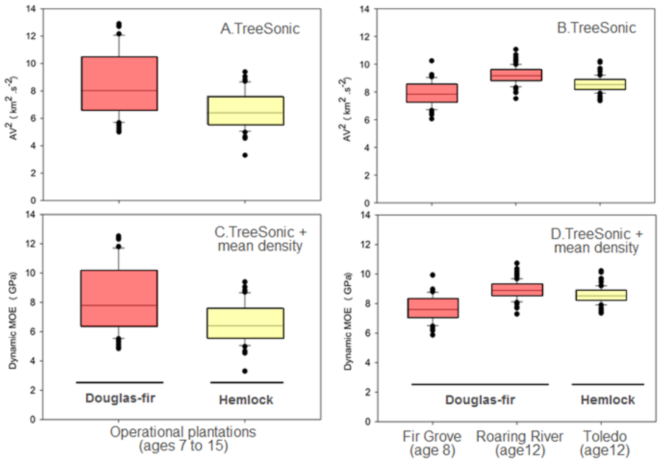
Douglas-fir and western hemlock overlapped



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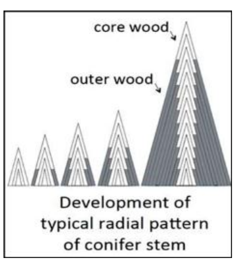
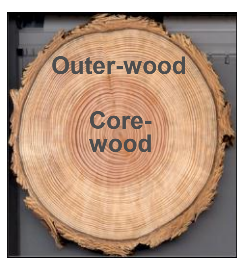
Phase 2: Heritabilities were higher on younger trees

- Fir Grove (0.89) vs. Roaring River (0.56) and Toledo (0.51)
  - *More corewood proportion*
  - *More variation and higher heritabilities in wood stiffness in corewood (Dungey et al. 2006)*





Phase 2: Our results suggested that same-face approach is better

- Mahon et al recommend that OF was better
  - *No heritability calculation*
  - *Hit to hit variation was less using OF*
  - *Velocities did not vary with DBH with OC adjustment method*
  - *100 Loblolly pine trees*
  - *DBH (16.8 to 35.8)*
- Wagner et al (2003) suggested that opposite face is better
  - *Phenotypic correlation*
  - *DBH ranged from 30.2 to 37.3 cm*
  - *Different technique (only accounted for DBH)*
  - *24 Douglas-fir trees*
- Our study
  - *Younger trees and smaller DBH (5.7, 10.4, and 11.3 cm)*
  - *Robust analysis*
  - *Based on heritabilities*

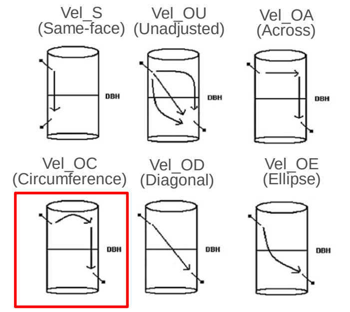
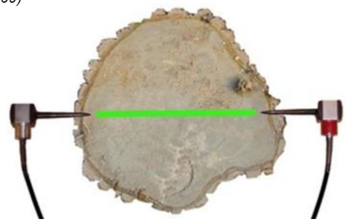


Figure 1. Hypothesized stress wave flight pats for the same-face and distance adjusted values (Mohan et al 2009)



Conclusions

- Wood stiffness is under high genetic control in both species
- Measuring across a whorl of branches has little adverse effect on the AV measurements, and should be used if one can use a much longer flight path
- The opposite-face approaches did not improve our wood stiffness measurements or potential genetic gain
- If someone wants to use the opposite-face approach OA and OE may be slightly better than other opposite-face adjustments
- Overall, we recommend:
  - *Same-face method (for efficiency)*
  - *TreeSonic-STD, TreeSonic-SD02, or Microsecond Timer*
  - *The SD-02 sensor seems to be more practical for the smallest trees*

Biological conclusions

- Interpretation of variance components
  - *Assuming higher-order interactions are zero…*
  - $\sigma^2_{P1} = \frac{1}{4}\sigma^2_A$
  - $\sigma^2_{P2(P1)} = \frac{1}{4}\sigma^2_A + \frac{1}{4}\sigma^2_D$
- No maternal effects
  - σ^2_{P1} *in M(F)* ≈ σ^2_{P1} *in F(M)*
  - $\sigma^2_{P2(P1)}$ *in M(F)* ≈ $\sigma^2_{P2(P1)}$ *in F(M)*
- No dominance variance
  - $\sigma^2_{P1} \approx \sigma^2_{P2(P1)}$
  - $\frac{1}{4}\sigma^2_A \approx \frac{1}{4}\sigma^2_A + \frac{1}{4}\sigma^2_D$
  - $\sigma^2_D \approx 0$

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Future work

Calculate genetic correlations between

- AV^2 vs. growth traits
- AV^2 vs. taper

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ABSTRACT: GENETIC AND ENVIRONMENTAL CONTROL OF DOUGLAS-FIR STEM FORM*

Lauren Magalska

The value of wood products is determined by tree volume and stem quality. Stem form defects, such as forks and ramicorn branches, reduce stem quality and, therefore, tree value. Foresters in the Pacific Northwest have observed that the frequency of stem form defects seems to be associated with rapid growth and proximity to the coast. In addition, past research studies have indicated a positive genetic correlation between growth and stem defects. Nonetheless, the relative roles of genotype and environment on the frequency of stem form defects are still unclear. The objectives of this study were to i) identify environmental characteristics that explain variation in the frequency of forks and ramicorn branches; ii) examine whether rapid plantation growth is associated with an increase in stem defects; iii) determine how much variation in stem defects can be explained by differences in growth; iv) examine whether there is a relationship between stem form and proximity to the coast; v) estimate genetic and environmental correlations between stem defects and growth traits; and vi) estimate heritabilities and genetic gains for stem defects and growth traits.

To achieve these objectives, data from 22 first generation operational breeding programs within the Northwest Tree Improvement Cooperative (NWTIC) were analyzed. We examined 40 environmental characteristics (climate, soils, and topography), and did not find any evidence that they explain variation in stem form defects. We found that the frequency of stem form defects increased with increased growth and increased proximity to the coast. We also found that forks and ramicorn branches were heritable and were generally positively genetically correlated with growth. However, genetic correlations were variable among programs. Direct backward selection on stem form traits could result in a decrease in defect frequency between 3 and 28%. Selection solely on growth traits (i.e., indirect backward selection) had a small potential to increase the frequency of stem form defects (1-4%). Because of the variability in genetic correlations between growth traits and stem form defects, it is also possible to select simultaneously for growth and fewer defects.

We now have a better understanding of the genetic and environmental control of Douglas-fir stem form. There is ample evidence to suggest that stem form should be included as a selection criterion in operational breeding programs, particularly in breeding programs that plan to deploy material close the coast or on "high" sites. The genetic correlations between stem form and growth traits should be examined in later generations, as the potential to increase the frequency of stem form defects may change, particularly in programs that had large positive genetic correlations between growth and the frequency of stem defects.

*This is the abstract from the presentation Lauren gave at Forest Genetics 2013: Magalska, L., Howe, G.T., and Maguire, D.A. 2013. Genetic and environmental control of Douglas-fir stem form. Abstract In Proceedings of Forest Genetics 2013, Joint Meeting of the Canadian Forest Genetics Association, Western Forest Genetics Association, IUFRO Population, Ecological and Conservation Genetics (Working Group 2.04.01), IUFRO Breeding and Genetic Resources of Pacific Northwest Conifers (Working Group 2.02.05), July 22-25, 2013, Whistler, B.C.

Genetic and Environmental Control of Douglas-fir Stem Form

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Genetic and environmental control of Douglas-fir stem form

- Introduction
- Site analyses
- Genetic analyses
- Conclusions and recommendations

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Introduction

- Why is the relationship between stem form and growth important?
- Previous work
- Objectives of this study
- Traits

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Why?

- Stem quality is second only to volume when determining tree value
- The relative roles of genotype and environment are still unclear
- Positive correlations between growth and frequency of stem forks and ramicorns

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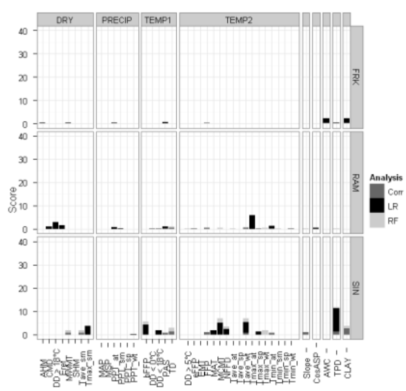
Previous work

- Site characteristics study
- Nehalem study
- Other studies

Site characteristics study

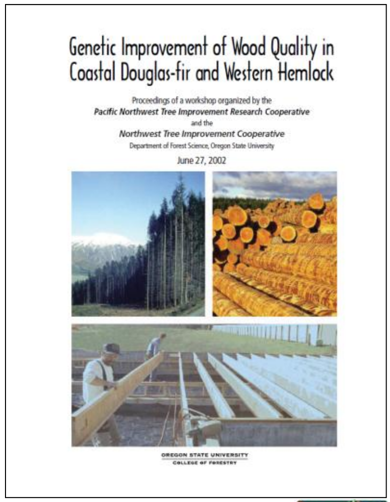
- Sought to identify site characteristics that explain variation in stem forks, ramicorns and sinuosity
- 40 site characteristics were considered
- Three different parameter importance measures were calculated and summarized
- None of the site characteristics consistently explained the variation in stem defects



Nehalem study

Previous study that examined the genetics of stem quality in coastal Douglas-fir

- Heritabilities
- Genetic correlations
- Environmental correlations
- Effects of selecting solely for increased growth



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Stem defects are heritable

Heritabilities in the Nehalem program

Heritabilities of growth and stem form traits in a NWTIC first-generation progeny test in the Nehalem breeding zone.

Trait	Age	Individual h^2	Family h^2
Growth traits			
Height	5	0.25	0.86
Height	11	0.27	0.87
Height growth	5 -11	0.23	0.84
Diameter	11	0.23	0.84
Volume	11	0.25	0.84
Stem form traits			
Ramicorns	11	0.20	0.81
Crookedness	11	0.16	0.78

Stem defects are associated with increased growth

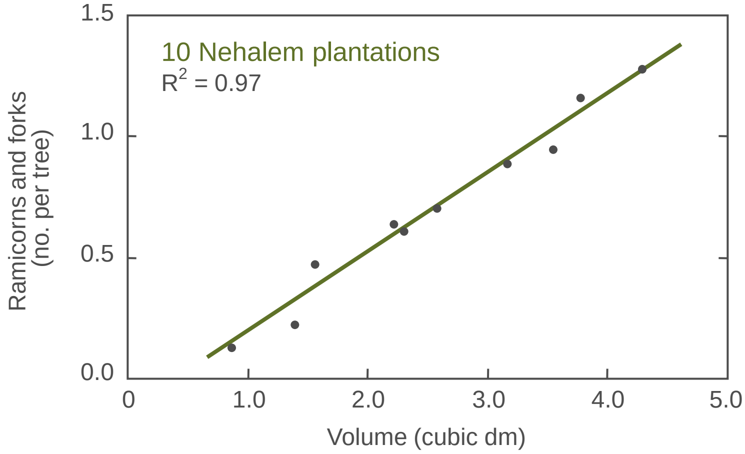
Genetic correlations in the Nehalem program

Correlations between growth and stem form traits in a NWTIC first-generation progeny test in the Nehalem breeding zone.

		Genetic correlation		Site correlation	
Growth trait	Age	Ramicorns	Crookedness	Ramicorns	Crookedness
Height	5	0.36	0.44	0.98	0.77
Height	11	0.36	0.45	0.95	0.86
Height growth	5 -11	0.33	0.42	0.88	0.87
Diameter	11	0.43	0.37	0.97	0.84
Volume	11	0.45	0.41	0.99	0.79

Ramicorns and stem forks are correlated with plantation growth

Nehalem study



Selection for increased growth will increase ramicorns and stem forks

Nehalem study

Potential for reducing ramicorns and forks is large		
Direct selection to reduce ramicorns and forks		
	Absolute change when:	
Response (%)	Mean 0.13	Mean 1.3
–	–	–
−47	−0.06	−0.61
−19	−0.02	−0.24
−84	−0.11	−1.08

Selecting only for increased growth will increase ramicorns and forks			
Correlated increase in ramicorns and forks by selecting only for increased growth			
		Absolute change when:	
Trait selected	Response (%)	Mean 0.13	Mean 1.3
Height (age 13)	+12	+0.02	+0.16
DBH (age 9)	+28	+0.04	+0.36
DBH (age 12)	+9	+0.01	+0.12
Volume (age 11)	+38	+0.05	+0.49

(Adams & Bastien 1994; Schermann et al 1997; Temel & Adams 2000; Nehalem data)

Other studies

- Temel & Adams (2000)
- Vargas-Hernandez et al (2003)
- Xiong et al (2010)

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Other studies

Temel & Adams (2000)

- Age 12 and 24
- Individual tree and family heritabilities for whorls with steep angle branches (WSAB) at age 12 were 0.08 and 0.33, respectively.
- Individual tree and family heritabilities for WSAB at age 24 were 0.05 and 0.23, respectively
- Mean genetic correlation between WSAB and diameter growth was $r_A = 0.46$
- Selection on diameter alone at age 12 would cause an increase in WSAB at age 24 by 9.3%

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Other studies

Vargas-Hernandez et al (2003)

- Family heritabilities for WSAB at ages 12 and 24 were 0.42 and 0.5,1 respectively
- Genetic correlations between steep WSAB and diameter (0.93), height (0.50), bole volume (0.88) at age 12
- Selection on age 12 height alone would cause an increase in WSAB by 10%

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Other studies

Xiong et al (2010)

- Studied loblolly pine populations in 7 different regions
- Individual tree and family heritabilities averaged 0.05 and 0.76, respectively, across all regions
- Genetic correlations between forking and height in loblolly pine ranged from $r_g = 0.12$ to 0.29
- Selection on height alone would result in an unfavorable increase in forking
- Selection against forking resulted in expected reductions between 12% and 23%

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Other studies

Conclusions

- Stem defects are heritable
  - *Individual tree heritabilities between 0.05 and 0.20*
  - *Family heritabilities between 0.23 and 0.81*
- The genetic correlation between stem defects and growth is consistently positive (0.12 to 0.93)
- Stem defects are expected to increase if selection is based solely on growth
- Stem defects can be reduced through direct selection

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Objectives of this study

Extend analyses to many more programs and sites
Understand general relationships

- Understand the environmental relationships between stem defects and growth traits
  - *Is rapid growth associated with stem defects?*
  - *How much variation in stem defects can be explained by differences in growth?*
- Examine whether there is a relationships between stem defects and distance to coast

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Objectives of this study

Extend analyses to many more programs and sites
Understand general relationships

- Estimate genetic correlations between stem defects and growth traits
- Estimate heritabilities of stem defects and growth traits
- Estimate genetic gain in stem defects
  - *Direct selection to reduce stem defects*
  - *Potential adverse effects of selecting solely for increased growth*

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Traits

- Stem defects
  - *Average number of stem forks and ramicorns per tree per year*
- Growth
  - *Average annual growth for DBH, HT, and VOL*

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Genetic and environmental control of Douglas-fir stem form

- Introduction
- Site analyses
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Site analyses

- Do fast growing plantations have more stem defects?
- Are stem defects more frequent near the coast?

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Do fast growing plantations have more stem defects?

- Examined 6 relationships (2 stem defects * 3 growth traits)
- Relationships examined across 22 programs
- Program treated as a random effect
- Short answer = yes

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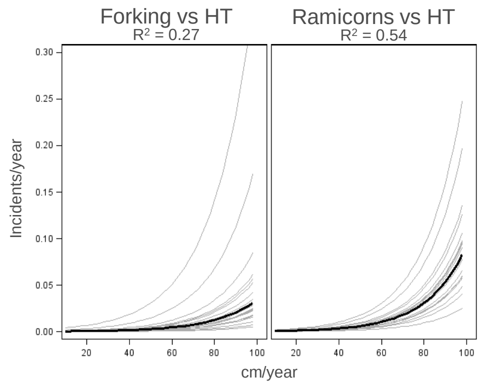
Both stem forks and ramicorns increase with increasing height growth

- R^2 is greater for HT than for DBH and VOL, which is consistent with biology
- For every additional cm/year
  - *Stem forks/year increase 5%*
  - *Ramicorns/year increase 6%*



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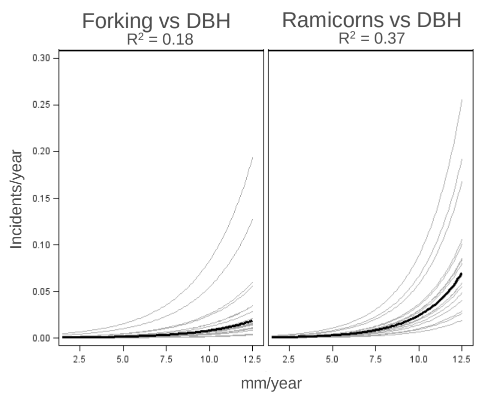
Both stem forks and ramicorns increase with increasing diameter growth

- R^2 is less than R^2 for HT
- For every additional mm/year
  - *Stem forks/year increase 40%*
  - *Ramicorns/year increase 50%*



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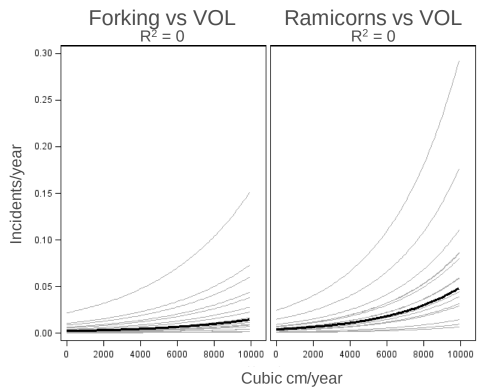
Both stem forks and ramicorns increase with increasing volume growth

- No variation in stem defects explained by volume growth
- Inconsistent with original Nehalem analysis (R^2 = 0.97)
  - *Ramicorns and stem forks were combined in Nehalem study*
  - *Relationship across 22 programs*



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Are stem defects more frequent near the coast?

- Calculated the distance to coast for 125 sites in 22 programs
- Regressed the incidents of stem defects per tree per year on the distance

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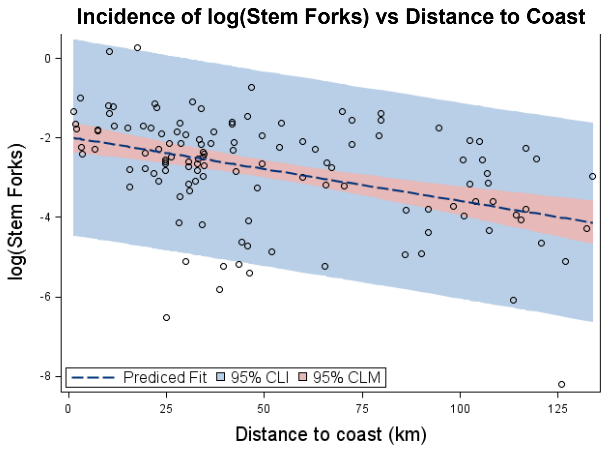
Stem forks are more frequent near the coast

- $R^2 = 0.18$
- For every 10km further inland, the number of stem forks decreases by 15%



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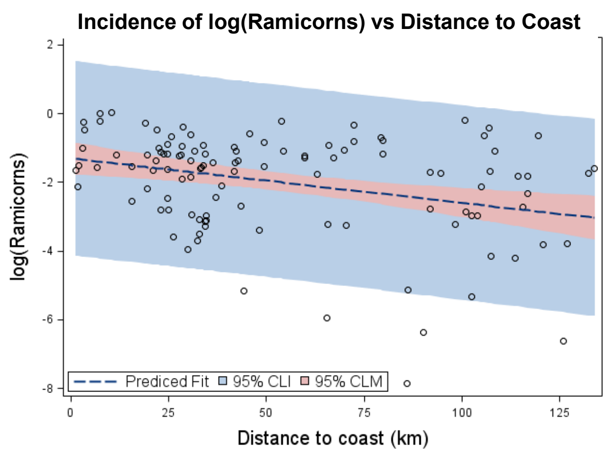
Ramicorns are more frequent near the coast

- $R^2 = 0.10$
- For every 10km further inland, the number of ramicorns decreases by 12%



Pacific Northwest Tree Improvement Research Cooperative

Genetic and environmental control of Douglas-fir stem form

- Introduction
- Site analyses
- Genetic analyses
- Conclusions and recommendations

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Genetic analyses

- Heritabilities
- Genetic correlations
- Genetic gain
  - *Reduction of stem defects through direct selection*
  - *Adverse effects of selecting solely on growth*

PACIFIC NORTHWEST TREE IMPROVEMENT
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Stem defect heritabilities are low but variable

Table 1 Individual tree heritabilities for growth and stem form traits in 9- to 18-year old coastal Douglas-fir, where n=the number of programs or the number of sets for which the trait was measured. Families within a program were grouped into sets.

			Individual tree h^2			Family h^2		
Trait	**Type [a]**	**n**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**
Growth								
DBH	P	22	0.058	0.180	0.394	0.476	0.733	0.880
DBH	S	181	0.000	0.188	0.778	0.000	0.707	0.937
HT	P	21	0.095	0.228	0.460	0.628	0.794	0.899
HT	S	159	0.031	0.238	0.735	0.303	0.771	0.942
VOL	P	19	0.046	0.183	0.368	0.443	0.713	0.862
VOL	S	151	0.000	0.196	0.749	0.000	0.698	0.938
Form								
SQFRK	P	22	0.000	0.023	0.084	0.001	0.275	0.624
SQFRK	S	182	0.000	0.021	0.166	0.000	0.228	0.775
SQRAM	P	18	0.007	0.046	0.170	0.140	0.397	0.776
SQRAM	S	146	0.000	0.045	0.280	0.000	0.343	0.865

[a] Statistics were calculated at both the program (p) and set (s) levels.

PACIFIC NORTHWEST TREE IMPROVEMENT
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[a] Statistics were calculated at both the program (p) and set (s) levels.

Nehalem

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Stem defects and growth are genetically correlated

Table 2 The genetic and environmental/error correlations between growth and stem form traits in 9- to 18-year old coastal Douglas-fir.

		SQFRK			SQRAM		
	Type [a]	Min	Mean	Max	Min	Mean	Max
DBH	G	-0.477	0.242	1.000	-1.000	0.165	0.617
DBH	E	-0.076	0.012	0.091	-0.042	0.036	0.142
HT	G	-0.733	0.277	1.000	-0.033	0.219	0.488
HT	E	-0.130	-0.038	0.083	-0.073	0.003	0.099
VOL	G	-0.643	0.324	1.000	-0.685	0.190	0.518
VOL	E	-0.081	-0.001	0.092	-0.044	0.022	0.137
SQFRK	G				-0.136	0.627	1.000
SQFRK	E				-0.199	0.015	0.474

[a] G = genetic correlations; E = environmental correlations

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Little adverse effect of selection on growth

Table 3 Genetic gains and correlated responses to selection for backward selection of parents based on progeny performance in n distinct progeny tests.

Parental selection intensity (%)	Indirect growth trait	SQFRK n	Min	Mean	Max	SQRAM n	Min	Mean	Max
Response to direct backward selection (ΔG, %)									
12.5		22	0.0	-2.9	-13.6	18	-0.5	-6.4	-19.7
2.5		22	0.0	-4.2	-19.4	18	-0.7	-9.1	-28.1
Correlated response in form trait from indirect backward selection on growth trait (Δ CR, %)									
12.5	DBH	22	-1.8	0.8	3.4	18	-2.7	2.2	8.5
2.5	DBH	22	-2.5	1.2	4.9	18	-3.8	3.1	12.2
12.5	HT	21	-1.9	0.9	3.4	17	-0.3	2.1	7.8
2.5	HT	21	-2.7	1.3	4.9	17	-0.4	3.0	11.2
12.5	VOL	19	-1.9	1.0	3.4	15	-1.7	2.7	9.2
2.5	VOL	19	-2.7	1.4	4.8	15	-2.5	3.8	13.2

PACIFIC NORTHWEST TREE IMPROVEMENT
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Genetic and environmental control of Douglas-fir stem form

- Introduction
- Site analyses
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PACIFIC NORTHWEST TREE IMPROVEMENT
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Conclusions and recommendations

- Stem forks and ramicorns increase with increasing growth
- Stem forks and ramicorns increase near the coast
- Stem defects are heritable
- On average stem defects are positively genetically correlated with growth (which is bad), but genetic correlations are variable among programs and sets
- Selecting solely for growth has a small potential to increase stem defects
- It is possible to select for growth and fewer defects

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References

Temel, F., and Adams, W.T. 2000. Persistence and age-age genetic correlations of stem defects in coastal Douglas-fir (Pseudotsuga menziesii var. menziesii (Mirb.) Franco). Forest Genetics **7**(2): 145-153.

Vargas-Hernandez, J.J., Adams, W.T., and Joyce, D.G. 2003. Quantitative genetic structure of stem form and branching traits in Douglas-fir seedlings and implications for early selection. Silvae Genetica **52**(1): 36-44.

Xiong, J.S., Isik, F., McKeand, S.E., and Whetten, R.W. 2010. Genetic Variation of Stem Forking in Loblolly Pine. Forest Science **56**(5): 429.

Pacific Northwest Tree Improvement
Research Cooperative

ABSTRACT: DEVELOPMENT AND APPLICATION OF SNP MARKERS IN DOUGLAS FIR*

Glenn Howe

Genomics research and new technologies allow tree breeders to use an almost unlimited supply of genetic markers to enhance tree breeding programs. In particular, genetic markers called Single Nucleotide Polymorphisms (SNPs) are being widely used in breeding programs of livestock, agricultural crops, and forest trees. The main goal of this research was to develop a SNP resource large enough to facilitate genomic selection in Douglas-fir breeding programs. To accomplish this, we developed a 454-based reference transcriptome for coastal Douglas-fir, annotated and evaluated the quality of the reference, identified putative SNPs, and then validated a sample of those SNPs using the Illumina Infinium genotyping platform. We assembled a reference transcriptome consisting of 25,002 isogroups (unique gene models) and 102,623 singletons from 2.76 million 454 and Sanger cDNA sequences from coastal Douglas-fir. We identified 278,979 unique SNPs by mapping the 454 and Sanger sequences to the reference, and by mapping four datasets of Illumina cDNA sequences from multiple seed sources, genotypes, and tissues. The Illumina datasets represented coastal Douglas-fir (64.00 and 13.41 million reads), interior Douglas-fir (80.45 million reads), and a Yakima population similar to interior Douglas-fir (8.99 million reads). We assayed 8067 SNPs on 260 trees using an Illumina Infinium SNP genotyping array. Of these SNPs, 5847 (72.5%) were called successfully and were polymorphic. Based on our validation efficiency, our SNP database may contain as many as ~200,000 true SNPs, and as many as ~69,000 SNPs that could be genotyped at ~20,000 gene loci using an Infinium II array—more SNPs than are needed to use genomic selection in tree breeding programs. Ultimately, these genomic resources will enhance Douglas-fir breeding and allow us to better understand landscape-scale patterns of genetic variation and potential responses to climate change.

*This abstract contains text from the manuscript entitled "A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation" by Howe, G.T., Yu, J., Knaus, B., Cronn, R., Kolpak, S., Dolan, P., Lorenz. W.W., Dean, J.F.D. BMC Genomics 14:137 (2013).

Development and Application of SNP Markers in Douglas-fir

Glenn Howe

Pacific Northwest Tree Improvement Research Cooperative
Department of Forest Ecosystems and Society
Oregon State University

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PNWTIRC

Genetic Markers in Tree Breeding

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Single nucleotide polymorphism (SNP)

Usually 2 alleles

Many many loci

SNP

Tree 1	A C G T G T C **G** G T C T T A	Maternal chrom.
	A C G T G T C **A** G T C T T A	Paternal chrom.
Tree 2	A C G T G T C **G** G T C T T A	Maternal chrom.
	A C G T G T C **G** G T C T T A	Paternal chrom.
Tree 3	A C G T G T C **A** G T C T T A	Maternal chrom.
	A C G T G T C **A** G T C T T A	Paternal chrom.

Tree 1 is ***heterozygous*** Trees 2 and 3 are ***homozygous***

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Trends in genetic markers

- Genomics research provides an almost unlimited supply of markers
- The costs of genotyping are declining rapidly
  - *New markers (SNPs)*
  - *New genotyping platforms*
- High-throughput genotyping can be outsourced
- Today's marker applications were only imagined a few years ago

Illumina GoldenGate platform →

We are using Illumina Infinium (more capacity)

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Acknowledgements

Submitted manuscript

Douglas-fir SNPs; Howe et al. 1

A SNP resource for Douglas-fir: *de novo* transcriptome assembly and SNP detection and validation

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Transcriptome sequencing

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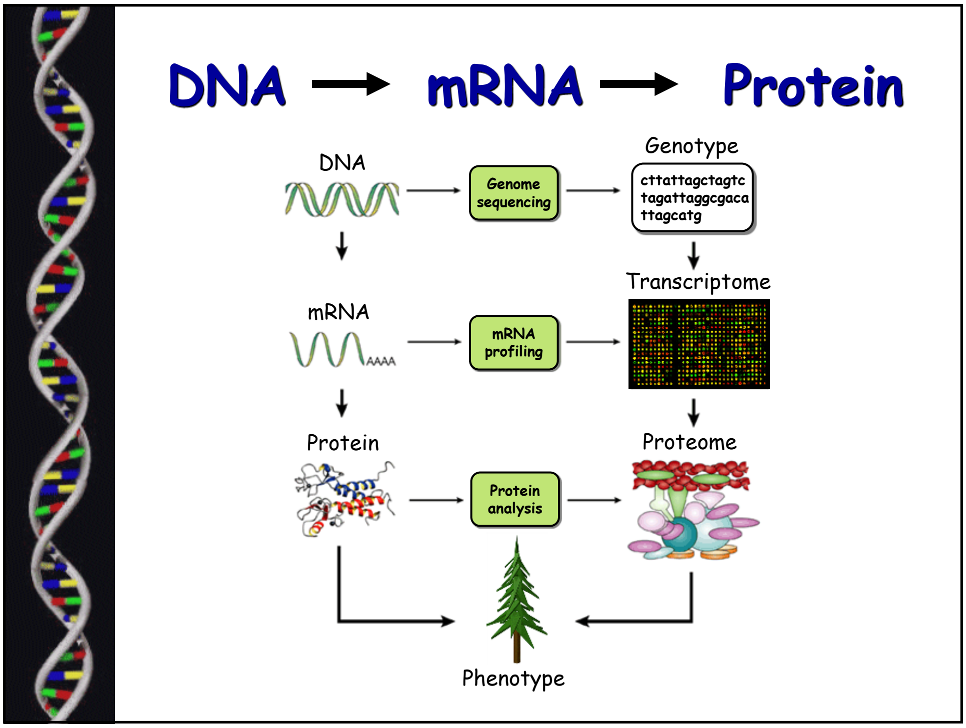

Transcriptome sequencing
Coastal Douglas-fir

Transcriptome reference from the 454 sequences

SNP discovery from the 454 and Illumina sequences

- Seedlings acclimating to cold
  - *Sanger sequences from microarray project*
  - *Some samples used for Illumina sequencing*
- Single mature genotype, multiple tissues
  - *Roche 454 sequences from Joint Genome Institute*
- Seedlings from 79 seed sources harvested throughout the year
  - *Roche 454 sequences for the transcriptome reference*
  - *Illumina sequences for SNP discovery*

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Transcriptome sequencing
Interior Douglas-fir

Develop SNP markers for interior Douglas-fir (var. glauca)

- Collaboration with Sam Cushman, USFS Rocky Mountain Station
- Include ‘interior’ SNPs on the SNP chip
- Multi-genotype sample used for Illumina sequencing
  - *Trees in BCMoF provenance test (Barry Jaquish, BCMoF)*
  - *Cherrylane SO in N. Idaho (Marc Rust, IETIC)*
  - *Needles, stems, and buds*

IETIC
The Inland Empire Tree Improvement Cooperative

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Douglas-fir reference transcriptome

Table 1 Sequence datasets used to construct the Douglas-fir reference transcriptome. For each dataset, the numbers of reads filtered using the SnoWhite pipeline (Figure 3, Step 3) are shown by sequence type.

Plant materials (dataset ID) *Collection information*	Method[*] *cDNA library*	Total reads in dataset (%)	Number of reads filtered from the input dataset (% of library total)					
			Short or low-quality	Adapter or vector	Chloro-plast	Mitochon-drial	rRNA	Retro-transposon
Multi-genotype #1 ($MG1_{SANG}$) *Cold season* *Greenhouse*	Sanger *Normalized* *Non-normalized*	12,157 (100)	57 (0.47)	0 (0.00)	2 (0.02)	2 (0.02)	0 (0.00)	1 (0.01)
Multi-genotype #2 ($MG2_{454}$) *Cold and warm seasons*	GS-FLX Titanium *Normalized*	1,709,211 (100)	6649 (0.39)	1893 (0.11)	8570 (0.50)	5519 (0.32)	7264 (0.42)	11,114 (0.65)
Single-genotype (SG_{454}) *July 8, 2008*	GS-FLX Titanium *Non-normalized*	1,241,260 (100)	6582 (0.53)	1826 (0.15)	11,070 (0.89)	10,463 (0.84)	86,828 (7.00)	21,849 (1.76)
All datasets		2,962,628 (100)	13,288 (0.45)	3719 (0.13)	19,642 (0.66)	15,984 (0.54)	94,092 (3.18)	32,964 (1.11)

[*]GS-FLX Titanium is the Roche 454 sequencing platform.

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DF transcriptome assembly

Statistic	Number
Total reads	2,764,549
Assembled reads	2,544,087
Total assembled	2,741,911
Singletons	102,623
Isogroups (genes)	25,002
Isotigs	38,589
One isotig/isogroup	18,774
Mean length of isotig	1,390
N50	1,883
Total consensus nucleotides	72,302,278

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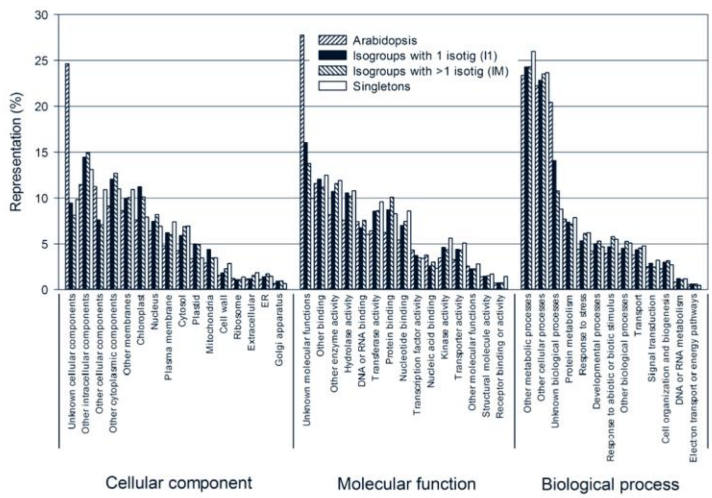
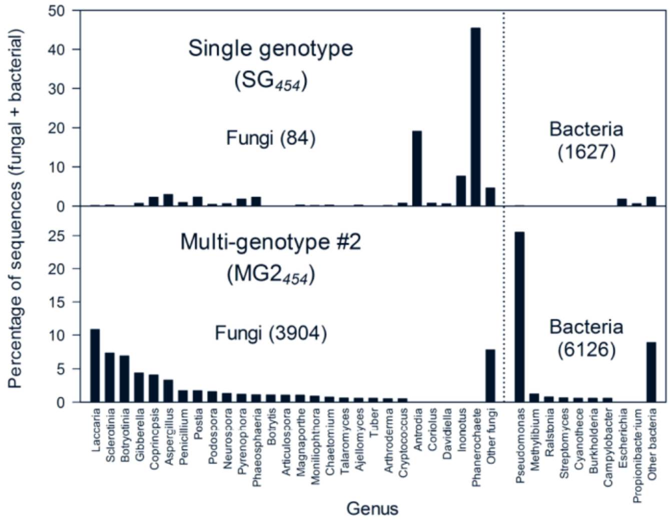
We can infer Douglas-fir gene function



Next-generation sequencing
Can be used to characterize 'ecosystems'



What are SNPs?
How do we find them?

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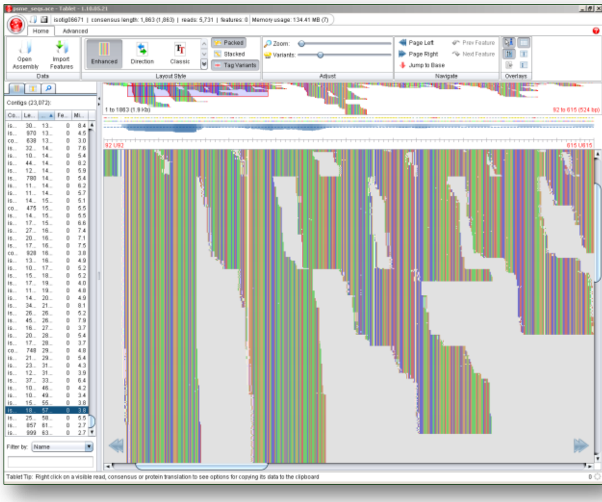
A genome from many short sequences

Next-generation sequencing



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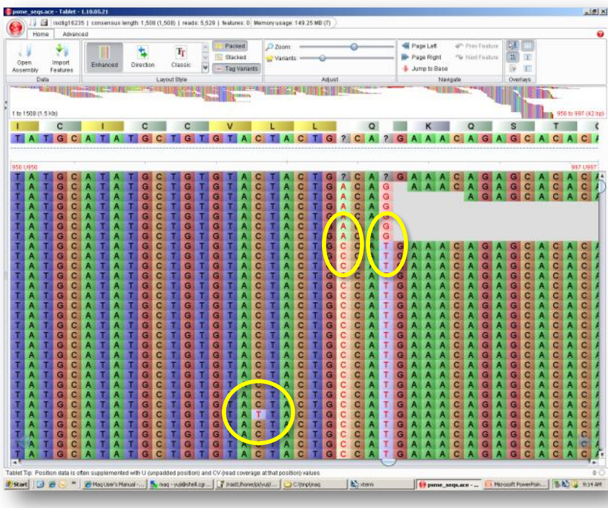
Find SNP markers



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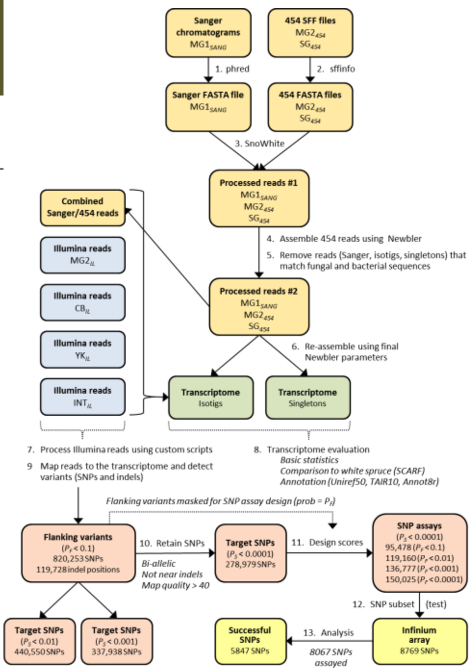
SNP detection

Lots of bioinformatics

- Process raw sequences
- Assemble into a transcriptome
- Map sequences to the transcriptome
- Detect SNPs using various criteria (e.g., probabilities)
- Design primers for SNP assays (SNP chip)
- Analyze resulting SNP data
- Lots of programming needed



Potential SNP markers in Douglas-fir

278,979 SNPS detected in Douglas-fir unigenes

1 isotig/isogroup

Longest isotig/isogroup

Douglas-fir variety	No. of SNPs	No. of genes with SNPs
Coastal	203,231	19,329
Interior	226,124	19,274
Both (in common)	151,014	17,361

Conclusion = lots of SNP markers to choose from!

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A Douglas-fir SNP chip

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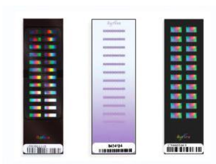
Douglas-fir SNP chip (Illumina Infinium)

Douglas-fir SNP chip is available

- 7256 SNPs can be assessed
- Many more potential SNPs are available



Numbers and percentages of putative Douglas-fir SNPs attempted and assayed with an Illumina Infinium SNP array (n = 260 trees).

SNPs attempted	8769
SNPs assayed by Illumina	8067
Percent of SNPs (assayed/attempted)	**92.0**
SNPs assayed by Illumina	8067
SNPs called (call frequency ≥ 0.85)	7256
Percent of SNPs (called/assayed)	**82.7**
SNPs called (call frequency ≥ 0.85)	7256
SNPs called that are polymorphic (MAF ≥ 0)	5847
Percent SNPs (called MAF > 0/called)	**80.6**
SNPs attempted	8769
SNPs called that are polymorphic (MAF ≥ 0)	5847
Percent SNPs (called MAF > 0/attempted)	**66.7**

MAF = minor allele frequency. MAF > 0 means there's more than 1 allele

SNPs are high quality

Table 8 Characteristics of 5847 successful SNPs based on data from an Illumina Infinium SNP array. Successful SNPs are those with a call frequency > 0.85* and MAF > 0 based on an analysis of 260 trees.

SNP characteristic	Mean	Median	Range
GenTrain score	0.81	0.84	0.35-0.98
GC50 score (median GenCall score)	0.78	0.87	0.15-0.99
Call frequency	0.99	1.00	0.85-1.00
Minor allele frequency (MAF)	0.24	0.24	0.002-0.5
Heterozygosity (observed)	0.33	0.36	0.0-1.0
Heterozygosity (expected)	0.32	0.36	0.004-0.5

Number of SNPs with a significant HWE deviation = 263 (4.5%)†

*Successful calls are those with a GenCall score ≥ 0.15 [79].
† Tested using an exact test of HWE and a probability level of 0.9×10^{-5} (i.e., Bonferroni-corrected P-value of 0.05 based on 5847 SNPs).

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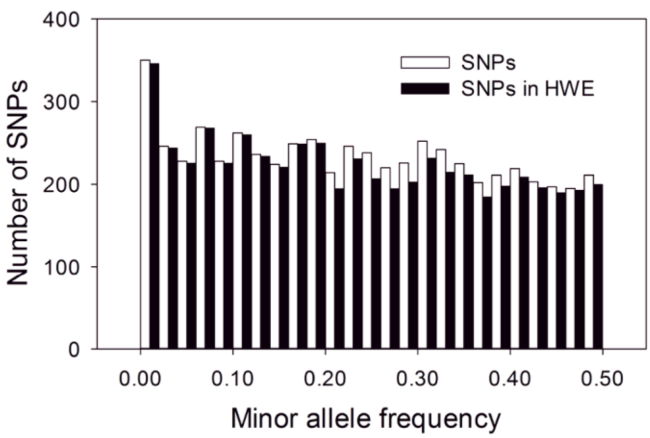
A wide range of minor allele frequencies

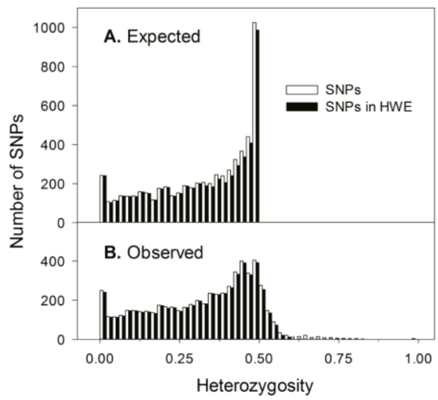


Two alleles ($p + q$) = a frequency of 1.0
If $p = 0.64$, then $q = 0.36$
q is the 'minor' allele and the minor allele frequency = 0.36

Most SNPs are in Hardy-Weinberg Equilibrium

Hardy-Weinberg

- Given allele frequencies of p and q for alleles P and Q
- Expected genotype frequencies are:
  - $P/P = p^2$
  - $P/Q = 2pq$
  - $Q/Q = q^2$
- If not, something 'funny' is going on = be careful



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Markers can be integrated into the tree breeding cycle

- Tree improvement uses one or more cycles of …
  - *Selection*
  - *Breeding*
  - *Testing*
  - *Production*
- Most breeding programs use 'recurrent selection' — repeated cycles of selection, breeding, and testing

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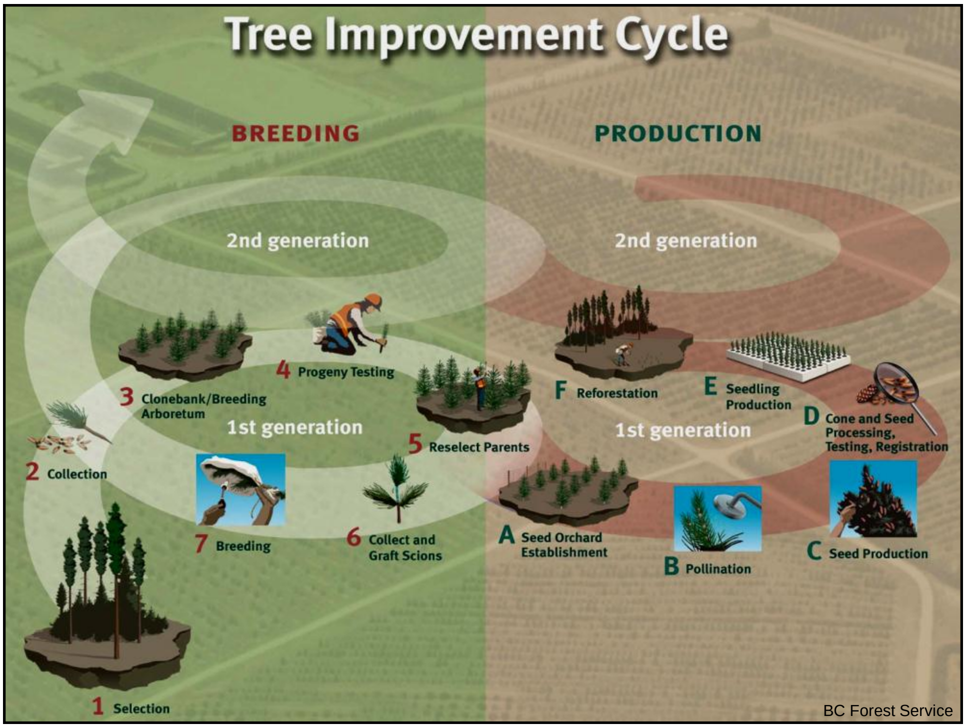
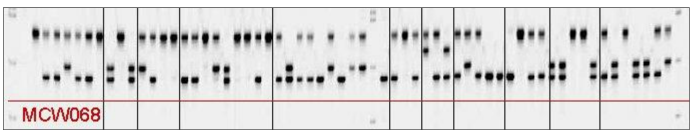


Markers in tree improvement

- Check the identity of genotypes (fingerprinting)
- Seed orchard management (parental analysis)
- Measure relatedness (pedigree reconstruction)
- Management of genetic diversity including inbreeding
- Marker assisted selection (MAS) and breeding (MAB)



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Breeding without crossing
Open-pollinated mating designs

'Breeding without breeding' (El-Kassaby et al 2007)

- Field test open-pollinated seed from seed orchard parents
- Use SNPs to fingerprint the top individuals in the best OP families to identify the fathers
- Saves time and the cost of full-sib crossing, and may increase gain
- Might be particularly valuable for identifying both parents of blister rust resistant trees in operational plantations?

**El-Kassaby,Y.A., M. Lstiburek, C. Liewlaksaneeyanawin, G.T. Slavov and G.T. Howe. 2007. Breeding without breeding: approach, example, and proof of concept. In: Proc. IUFRO, Low input breeding and genetic conservation of forest tree species. Antalya, Turkey. pp. 43-54.*

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Genomic selection

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The promise of genomic selection

Paradigm shift in perspective

- Forget about finding individual markers associated with desirable traits
- Explain desirable traits by using many, many markers at the same time
- Now possible because we can genotype many SNP markers at modest cost
  - *SNP, single nucleotide polymorphism, = changes between A, G, C, T*
- Why might it be useful?

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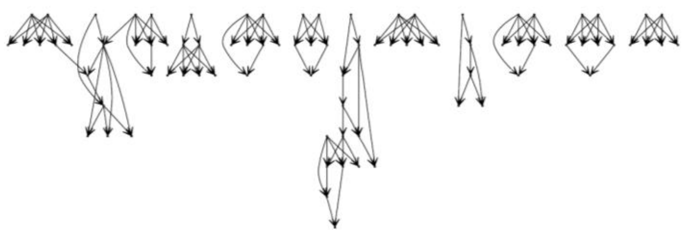
Pedigree reconstruction

What is pedigree reconstruction?

- Determine who's related to whom in a population of individuals
- You may have no specific expectations about the relationships you seek to uncover
- Markers must have excellent genetic resolving power (SSRs, SNPs)



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The promise of genomic selection

Parent 1 **x** ***Parent 2***

offspring 1
offspring 2
offspring 3
etc

- All offspring have the same expected phenotype (= parental average)
- Field testing is used to find which offspring are superior
- May be able find superior trees using many markers
- May be able to use early culling to make field tests much smaller
- May be able to skip testing and quickly move to the next generation

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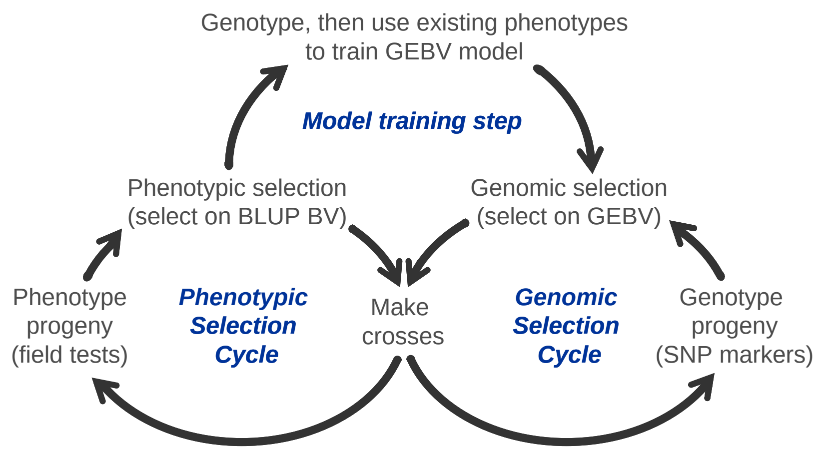
The promise of genomic selection



GEBV = genomic estimated breeding value

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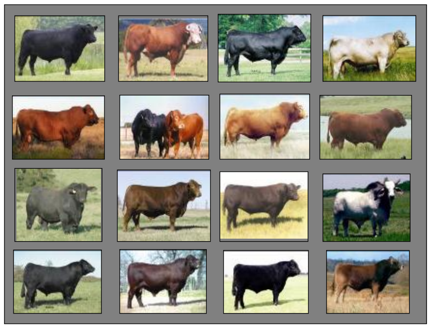
Livestock breeders are leading the way


Technical Note: DNA Analysis
illumina
Genomic Selection–A Paradigm Shift in Animal Breeding
Illumina next-generation sequencing and genotyping technologies are revolutionizing animal breeding.



"It is already widely used in dairy cattle breeding (Dalton, 2009) and is expected to revolutionize all livestock genetic improvement programmes and can be extended to plants"

Goddard et al. 2010. Genomic selection in livestock populations. Genet. Res. 92:413-421.

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Genotyped Holsteins

**Traditional evaluation*
***No traditional evaluation*

Date	Bulls*	Cows*	Young animals**		All animals
			Bulls	Heifers	
04-10	9,770	7,415	16,007	8,630	41,822
08-10	10,430	9,372	18,652	11,021	49,475
12-10	11,293	12,825	21,161	18,336	63,615
01-11	11,194	13,582	22,567	22,999	70,342
02-11	11,196	13,935	23,330	26,270	74,731
03-11	11,713	14,382	24,505	29,929	80,529
04-11	12,152	11,224	25,202	36,545	85,123
05-11	12,429	11,834	26,139	40,996	91,398
06-11	15,379	12,098	27,508	45,632	100,617
07-11	15,386	12,219	28,456	50,179	106,240
08-11	16,519	14,380	29,090	52,053	112,042
09-11	16,812	14,415	30,185	56,559	117,971

DNA** LandMarks **User Group Meeting- Oct, 2011(34)

G.R. Wiggans

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Implications

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Future

Genomic selection looks promising in many species

- Could revolutionize tree breeding
  - *Replace some field testing*
  - *Shorten breeding cycles*
- Strong potential to be combined with other approaches:
  - *Polymix or OP breeding with paternity testing*
  - *Early testing of trees before going to the field*
- Will be facilitated by small breeding populations
  - *Subline breeding*
- Need more markers = only constraint is money
- Keep clonal archives to be able to obtain parental genotypes

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Next steps

PNWTIRC subcommittee

- What is the relationship between research and the operational use of SNP markers in breeding programs? What are PNWTIRC objectives?
- Should future breeding be designed to accommodate genomic selection?
  - *Breeding population size is critical*
- What is the relationship between genomic selection and other potential approaches to breeding and testing?
  - *Breeding w/out breeding or early testing*
- How to cover the costs of SNP genotyping?

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

PNWTIRC

APPENDIX I

Literature Cited

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APPENDIX II

Publications

by PNWTIRC personnel 2011-2012

Howe, G.T., Yu, J., Knaus, B., Cronn, R., Kolpak, S., Dolan, P., Lorenz, W.W., and Dean, J.F.D. Submitted. A SNP resource for Douglas-fir: *De novo* transcriptome assembly and SNP detection and validation. Submitted to BMC Genomics.

Lorenz, W. W., Ayyampalayam, S., Bordeaux, J.M., Howe, G.T., Jermstad, K.D., Neale, D.B., Rogers, D.L., and Dean, J.F.D. 2012. Conifer DBMagic: A database housing multiple *de novo* transcriptome assemblies for twelve diverse conifer species. Tree Genetics and Genomes 8:1477-1485.

Magalska, L.E. 2011. Identifying site characteristics that explain variation in Douglas-fir site productivity and stem form. M.S. Thesis, Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR. 161pp.

APPENDIX III

Workshops, Presentations, and Abstracts

by PNWTIRC personnel 2011-2012

Colin, A.-L. Howe, G.T., St.Clair, J.B., and Maguire, D.A. 2012. Mechanistic growth models: Decomposing phenotypic models into their genetic and environmental components. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.

Howe, G.T. 2012. Genomics, wood properties, and climate change: Technological advances and challenges for western tree improvement. Keynote address, Annual Meeting of the Inland Empire Tree Improvement Cooperative, February 29, 2012, Coeur d'Alene, ID.

Howe, G.T. 2012. Plant conservation and climate change: Hitting a moving target. Invited talk and abstract In: Proceedings of the Second International Symposium on Biology of Rare and Endemic Plan Species, 23-27 April 2012 Fethiye, Turkey, p2.

Howe, G.T., Kolpak, S., Urhan. O., Cress, D., Jayawickrama, K., and Ye, T. 2012. Early genetic selection for wood stiffness in Douglas-fir and western hemlock. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.

Magalska, L.E., Howe, G.T., Maguire, D.A. 2012. Site characteristics of Douglas-fir productivity and stem form. Poster presentation, Northwest Forest Soils Council Winter Meeting, 28 February 2012, Gifford Pinchot National Forest Headquarters, Vancouver, WA.

Magalska, L.E., Howe, G.T., and Maguire, D.A. 2012. Site characteristics of Douglas-fir productivity and stem form. Poster presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.

Rust, M.L. and Howe, G.T. 2012. Development of genetic markers for western white pine and Douglas-fir. Oral presentation, Center for Advanced Forestry Systems Annual Meeting, 26-28 June 2012, Bangor, ME.

Urhan, O.S. 2012. Genetic improvement of wood stiffness in young Douglas-fir and western hemlock. Oral presentation at the Forest Ecosystems and Society Graduate Student Symposium, 21 May 2012, Corvallis, OR.

APPENDIX IV

Collaborations and Grants

2011-2012

CAFS Center for Advanced Forestry Systems – Phase II. Howe, G.T., Maguire, D.A., and Strauss, S.H. National Science Foundation Industry/University Cooperative Research Center Program, 2012-2017, $300,000 (OSU).

USFS Rocky Mountain Research Station. Developing a SNP panel for interior Douglas fir. Howe, G.T., and Cushman, S. USDA-Forest Service Joint Venture Agreement, $28,755 (2011-2013).

USDA AFRI. Western conifer forest systems: Strategies for climate change adaptation and mitigation. Howe, Glenn; Abatzoglou, John; Adams, Darius; Bentz, Barbara; Coleman, Mark; Crookston, Nick; Daley-Laurson, Steven; Ettl, Gregory; Fischer, Alexandra Paige; Gosz, James; Gray, Andy; Huang, Ching-Hsun; Johnson, James; Krankina, Olga; Lettenmaier, Dennis; Littell, Jeremy; Maguire, Doug; Mote, Philip; Oniel, Elaine; Robinson, Donald; Turner, Dave; Wang, Tongli; Waring, Richard. Submitted to the USDA National Institute of Food and Agriculture (NIFA) Program entitled Regional Approaches for Adaptation to and Mitigation of Climate Variability and Change in 2012 ($10M; declined).

APPENDIX V

Annual Meeting Minutes

December 6, 2012, Aurora, OR

I. ATTENDEES.

Mike Albrecht – Roseburg Forest Products
Margaret Banks – Stimson Lumber Co.
Dan Cress – Olympic Resource Management
Jeff DeBell – Wash. State Dept. Nat. Res.
Randall Greggs – Green Diamond Resource Co.
Glenn Howe – PNWTIRC, OSU
Keith Jayawickrama – NWTIC, OSU
Francis Kilkenny – Forest Service, PNWRS
Scott Kolpak – PNWTIRC, OSU
Sara Lipow – Roseburg Forest Products
Lauren Magalska – PNWTIRC, OSU
Doug Maguire – CIPS, OSU
Bill Marshall – Cascade Timber Consulting
Larry Miller – Oregon Dept. of Forestry
Bryan Nelson – Lone Rock Timber Mgmt.
Jim Smith – Plum Creek Timberlands
Brad St.Clair – Forest Service, PNWRS
Dean Stuck – Hancock Forest Management
Oguz Urhan – PNWTIRC, OSU
Mike Warjone – Port Blakely Tree Farms
Terrance Ye – NWTIC, OSU

II. WELCOME.

Sara Lipow, PNWTIRC Policy/Technical Chair, called the meeting to order at 9:00 am.

III. PNWTIRC HIGHLIGHTS FOR 20011-12.

Glenn Howe presented an overview of major accomplishments for 2011-12.

1. *PNWTIRC administration*
   Director – Glenn Howe
   Research Coordinator – Scott Kolpak
   Program Managers – Liz Etherington, Kori Ault
   Graduate students – Lauren Magalska, Oguz Urhan
   Faculty Research Assistant – Lauren Magalska
   Policy/Technical Committee Chair – Sara Lipow
2. *Research*
3. *Publications by PNWTIRC personnel during 2011-12*
4. *Presentations by PNWTIRC personnel during 2011-12*
5. *Collaborations and grants during 2011-12*

IV. PNWTIRC PLANS FOR 2012-13.

Glenn Howe presented an overview of plans for 2012-13.

- Oguz Urhan will defend his thesis and graduate with an M.S.
- The wood stiffness study of young Douglas-fir and western hemlock will be completed. A manuscript will be submitted for publication.
- The miniaturized seed orchard study will be completed, and a manuscript will be submitted for publication.
- The site characterization data will be reanalyzed using all available sites and new NRCS soils data.
- The analyses of mechanistic growth models will be completed with CIPS.
- The western white pine/Douglas-fir SNP marker study (CAFS) will begin.
- A subcommittee to guide new research on SNP marker-assigned selection will be formed. A research proposal will be developed, and new SNP genotyping will begin to be developed.

V. PNWTIRC RESEARCH PRESENTATIONS

- *Miniaturized Seed Orchard Study.* Scott Kolpak, Jim Smith, Sara Lipow, Mike Albrecht, Jeff DeBell, Glenn Howe.
- *Center for Advanced Forestry Systems (CAFS) Phase Two Project Proposal.* Genetic Markers for Western White Pine and Douglas-fir. Glenn Howe, Marc Rust, Anthony Davis.
- *Mechanistic Growth Models: Decomposing Phenotypic Models into Their Genetic and Environmental Components.* Anne-Laure Colin, Glenn T. Howe, J. Bradley, StClair, Douglas A. Maguire.
- *Early Genetic Selection for Wood Stiffness in Juvenile Douglas-fir and Western Hemlock.* Oguz Urhan, Scott Kolpak, Glenn Howe.
- *Genetic and Environmental Control of Douglas-fir Stem Form.* Lauren Magalska.
- *Development and Application of SNP Markers in Douglas-fir.* Glenn Howe.

VI. BUDGET AND OTHER BUSINESS.

Glenn Howe presented the budget for FY 2011-012, and the proposed budget for FY 2012-13. Income was $110K for 2011-12, and the 2012-13 income is expected to be the same. CAFS funds were used to pay some salaries. Although we did not begin substantial SNP marker-assisted selection work this year, we will transition into a new PNWTIRC project on SNP-based marker-assisted selection. A motion to approve the budget for 2011-12 and the proposed budget for 2012-13 was offered, seconded, and approved by unanimous voice vote.

VII. POLICY/TECHNICAL COMMITTEE CHAIR.

Sara Lipow was thanked for filling this Chair for the past two years. Randall Greggs was nominated as the new Policy/Technical Committee Chair and approved by unanimous voice vote. Randall Greggs is the Policy/Technical Committee Chair for FY 2012-13, and well as the PNWTIRC representative to CAFS.

VIII. MEETING ADJOURNED.

The meeting adjourned about 3 pm.

APPENDIX VI

Financial Statement
2011-2012

PNWTIRC Financial Support for Fiscal Year 2011-2012

Regular members[1]	$104,000
Associate members[1]	4,000
Contracts	2,000
Forest Research Laboratory, Oregon State University[2]	128,002
Total	**238,002**

[1] Each Regular Member contributed $8,000 and each Associate Member contributed $4,000 excluding in-kind contributions of labor, supplies, etc.

[2] The contribution from Oregon State University includes salaries, facility costs, and administrative support.