

Pacific Northwest Tree Improvement
Research Cooperative
Annual Report
2015-2016

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

Glenn Howe, Oguz Urhan, Scott Kolpak,
Erda Çeler, Keith Jayawickrama, Dominique Bachelet,
Brad St.Clair, Anna Magnuson



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

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



2015-2016

Annual Report

Report editors

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Pacific Northwest Tree Improvement Research Cooperative

About the PNWTIRC

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
Olympic Resource Management
Oregon Department of Forestry
Oregon State University
Port Blakely Tree Farms
Rayonier Forest Products
Roseburg Forest Products
Stimson Lumber Company
Washington State Department of Natural Resources
Weyerhaeuser

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 2015-2016

- Jennifer Kling joined the PNWTIRC as a Research Scientist. Jennifer is also a member of the Crop and Soil Science Department at OSU. She has applied and research experience in crop breeding (e.g., meadowfoam, barley, oats, corn); and teaching a graduate course (on-campus or Ecampus) in biological data analysis (CROP 590: Experimental Design in Agriculture) for the past 15 years.
- We analyzed the Axiom SNP chip for Douglas-fir using a three-generation breeding population. This work was done in collaboration with Keith Jayawickrama and Terrance Ye, and the Northwest Advanced Renewables Alliance (NARA).
- We began genomic selection analyses in Douglas-fir. This work was done in collaboration with Keith Jayawickrama and Terrance Ye, and the Northwest Advanced Renewables Alliance (NARA).
- Lauren Magalska evaluated the effects of climate change on the growth of Douglas-fir plantations.
- Erda Çeler obtained field results from the drought hardiness study in collaboration with Keith Jayawickrama, BLM, Plum Creek, Silver Butte. Erda Çeler is being supported by a scholarship from the Turkish government.
- Scott Kolpak completed transcriptome sequencing in WWP to facilitate SNP discovery in the three regional WWP breeding programs in the western US.
- Oguz Urhan continued to develop breeding strategies for WWP in collaboration with Marc Rust, Richard Sniezko, and others. Oguz Urhan is being supported by a scholarship from the Turkish government.
- We completed a draft of the PNWTIRC Five-year Plan that will help guide future research and extension activities of the cooperative. The five-year plan includes: an overview of the PNWTIRC organization and membership, proposed research projects (core, other, future), and potential technology transfer (e.g., workshops).
- The Seedlot Selection Tool (SST) has been redesigned and launched with the collaboration of Dominique Bachelet and staff at the Conservation Biology Institute.

MESSAGE FROM THE DIRECTOR

Glenn T. Howe, PNWTIRC Director

AGENDA – WEDNESDAY OCTOBER 19, 2016
– ANNUAL MEETING –
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH
COOPERATIVE (PNWTIRC)

START TIME 8:30 AM for coffee; 9:00 AM for presentations
LOCATION David Douglas Room; World Forestry Center, Portland, 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 <ul style="list-style-type: none"> • <i>PNWTIRC accomplishments for 2015-16</i> • <i>PNWTIRC plans for 2016-17</i> 	Glenn Howe
9:20-9:45	Quantitative genetics of blister rust resistance in western white pine (CAFS/STDP)	Oguz Urhan
9:45-10:05	Toward a SNP chip for western white pine (CAFS/STDP)	Scott Kolpak
10:05-10:30	Genetics of drought hardiness in Douglas-fir	Erda Celer
10:30-10:50	Break	
10:50-11:15	Effects of climate change on growth of Douglas-fir plantations (CAFS)	Lauren Magalska
11:15-11:40	Next-generation SNP chip for Douglas-fir	Glenn Howe
11:40-12:00	Validation of SNP data for genomic selection in Douglas-fir	Jennifer Kling
12:00-12:45	Lunch	
12:45-1:15	Genomic selection in Douglas-fir	Glenn Howe
1:15-2:00	Draft Five-Year Plan	Glenn Howe
2:00-2:10	Budget and other business <ul style="list-style-type: none"> • <i>Budget presentation and vote</i> • <i>Elect new Policy/Technical Committee Chair</i> 	Glenn Howe Sara Lipow
2:10-2:30	Break	
2:30-3:00	Seedlot Selection Tool	Glenn Howe
3:00	Wrap-up and adjourn	Glenn Howe

Overview – 2015/2016

By Glenn Howe

Glenn Howe began this year's PNWTIRC annual meeting (2016) by presenting an overview of the personnel, highlights, collaborations, and grants for 2015 – 2016. Current PNWTIRC personnel include: Glenn Howe (Director), Scott Kolpak (Research Coordinator), Jennifer Kling (Research Scientist), Anna Magnuson (Program Manager), Oguz Urhan and Erda Çeler (Graduate students), Lauren Magalska (Faculty Research Assistant), and Sara Lipow (Policy/Technical Committee Chair). Jennifer and Anna are new cooperative staff. Glenn presented an overview of the upcoming presentations: Genetics of western white pine (Oguz Urhan), SNP chip for western white pine (Scott Kolpak), Douglas-fir drought hardiness (Erda Çeler), Effects of climate change on Douglas-fir (Lauren Magalska), Next-generation SNP chip (Glenn Howe), Validation of SNP data (Jennifer Kling), Genomic selection in Douglas-fir (Glenn Howe), Draft Five-Year Plan (Glenn Howe), and the Seedlot Selection Tool (Glenn Howe). The highlights of PNWTIRC research and outreach activities for year were presented. A brief overview of external collaborations and grants that are helping to support PNWTIRC projects and other non-PNWTIRC allied projects was also presented. Glenn led a discussion of the new PNWTIRC Five-year Plan that was adopted at the meeting.

PNWTIRC Annual Meeting 2016

October 19, 2016

Glenn Howe

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



PNWTIRC personnel

2015-2016

- Director – **Glenn Howe**
- Research Coordinator – **Scott Kolpak**
- Research Scientist – **Jennifer Kling**
- Program Manager – **Anna Magnuson**
- Graduate students – **Oguz Urhan, Erda Çeler**
- Faculty Research Assistant – **Lauren Magalska**
- Policy/Technical Committee Chair – **Sara Lipow**

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Genetics of western white pine
Oguz Urhan

Quantitative Genetics of Blister Rust Resistance in Western White Pine
Oguz Urhan
Glenn Howe
Marc Rust
Richard Sniezko
Scott Kolpak

SNP chip for western white pine
Scott Kolpak

Toward a SNP Chip for Western White Pine
Scott Kolpak
Glenn Howe
Brent Kronmiller

Douglas-fir drought hardiness
Erda Çeler

Genetics of Douglas-fir Drought Hardiness
Erda Çeler
Glenn Howe

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Effects of climate change on Douglas-fir
Lauren Magalska

Effects of Climate Change on Growth of Douglas-fir Plantations
Lauren Magalska
Glenn Howe
Doug Maguire
Scott Kolpak

Next-generation SNP chip
Glenn Howe

Next-generation SNP Chip for Douglas-fir
Glenn Howe
Keith Jayawickrama
Scott Kolpak
Stephanie Guida
Sanjuro Jagdeo
Rich Cronn
Callum Bell

Validation of SNP data
Jennifer Kling

Validation of SNP Data for Genomic Selection in Douglas-fir
Jennifer Kling, Matt Trappe
Scott Kolpak, Terrance Ye
Keith Jayawickrama,
Glenn Howe

Tree → DNA sample & SNP data → Phenotype (BLUPs)

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**Genomic selection in Douglas-fir
Glenn Howe**

Genomic Selection in Douglas-fir
Glenn Howe
PNWTIRC
Oregon State University

**Draft Five-Year Plan
Glenn Howe**

**Seedlot Selection Tool
Glenn Howe**

Five-Year Plan
Glenn Howe
PNWTIRC

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PNWTIRC highlights for 2015-2016

- **Jennifer Kling joined the PNWTIRC**
- **We completed the analysis of the Douglas-fir SNP chip**
 - *Collaboration with Keith Jayawickrama and the Northwest Advanced Renewables Alliance (NARA)*
- **We started genomic selection analyses in Douglas-fir**
- **Lauren Magalska evaluated the effects of climate change on the growth of Douglas-fir plantations**

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PNWTIRC highlights for 2015-2016

- **Erda Çeler obtained results from the drought hardiness study**
 - Collaboration with Keith Jayawickrama, BLM, Plum Creek, Silver Butte
 - Erda Çeler is being supported by a scholarship from the Turkish government
- **Oguz Urhan continued to develop breeding strategies for WWP**
 - Collaboration with Marc Rust, Richard Sniezko, and others
 - Oguz Urhan is being supported by a scholarship from the Turkish government
- **Scott Kolpak completed transcriptome sequencing in WWP**
- **We completed the draft Five-Year Plan**
- **We launched the Seedlot Selection Tool**

Highlights of 2015-2016

Presentations

- Lu, H., Howe, G.T., Horvath, D.P., Dharmawardhana, P., Priest, H.D., Mockler, T.C., and Strauss, S.H. 2016. Extensive transcriptome changes during natural onset and release of vegetative bud dormancy in *Populus*. *Abstract in: Plant Dormancy Workshop, Plant & Animal Genome XXIV, January 9-13, 2016, San Diego, CA.*
- Howe, G.T. and Jayawickrama, K.J. 2016. Genomic selection for Douglas-fir tree improvement. *Presentation in: Center for Advanced Forestry Systems Annual Meeting, April 26-28, 2016, Pensacola Beach, Florida.*
- Urhan, O., Rust, M.L., Davis, A., Howe, G.T., Hipkins, V. 2016. Development of genetic markers for western white pine and Douglas-fir. *Presentation in: Center for Advanced Forestry Systems Annual Meeting, April 26-28, 2016, Pensacola Beach, Florida.*
- Howe, G.T. 2016. Douglas-fir breeding and the Pacific Northwest Tree Improvement Research Cooperative. Scion, June 7, 2016, Rotorua, New Zealand.



Highlights of 2015-2016

Presentations

- Pluess, A.R., Frank, A., Rellstab, C., Vendramin, G.G., Howe, G.T., Sperisen, C., Heiri, C., and Oddou-Muratorio, S. 2016. Evidence for local adaptation and potential maladaptation to climate change in *Fagus sylvatica*: Genome-environment and phenotype-environment associations at regional scale. *Abstract in: Genomics and Forest Tree Genetics: A conference jointly organized by the four working in parties of IUFRO Subdivision 2.4 (Genetics)*, May 30-June 3, 2016, Arcachon, France.
- Howe, G.T. 2016. Possibilities for genomics in Douglas-fir breeding. *Presentation in: Douglas-fir Breeding Workshop*, organized by Scion and the Specialty Wood Products (SWP) Research Partnership, June 9, 2016, University of Canterbury, Christchurch, New Zealand.

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Highlights of 2015-2016

Presentations

- Howe, G.T. 2016. Douglas-fir breeding and genealogy, University of Forestry, June 23, 2016, Sofia, Bulgaria.
- Howe, G.T. 2016. Forest genetics from science to management, Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL), June 30, 2016, Zurich, Switzerland.

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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 Forest Health Protection, Special Technology Development Program. Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust.** Howe, G.T., Davis, A., Hipkins, V., Liu, J.-J., Mahalovich, M.F., Rust, M., and Sniezko, R., 2014-2018, \$99,500.
- **University of Idaho and the Inland Empire Tree Improvement Cooperative. Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust.** Howe, G.T., 2013-2016, \$60,000.

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Collaborations and grants

- **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, 2011-2015, \$28,755.
- **U.S. Endowment for Forestry and Communities. Forest health biotechnologies: What are the drivers of public acceptance?** Needham, M.D. and Howe, G.T. 2013-2015, \$100,000.
- **USFS Pacific Northwest Research Station. Meta-analysis of Douglas-fir provenance tests to estimate responses to seed transfer and climate change.** Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2018, \$100,000.
- **USFS Pacific Northwest Research Station. Evaluating assisted migration options for adapting to climate change.** Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2019, \$40,000.

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Five-Year Plan

Glenn Howe

*PNWTIRC
Dept Forest Ecosystems and Society
Oregon State University*

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Plans for 2016-2017

PNWTIRC Five-Year Plan activities

Activity	Deliverable	Target date
Five-Year Plan survey	PNWTIRC report on survey results	Nov 18, 2016
Dues increase	Vote on dues increase	Dec 31, 2016
Affymetrix Axiom array	PNWTIRC report	Dec 31, 2016
Douglas-fir site characterization	PNWTIRC report	Dec 31, 2016
Genomic selection work plan	Approved work plan	Dec 31, 2016
Five-Year Plan	Approved plan	Dec 31, 2016
Drought hardiness study	Master's thesis	Mar 15, 2017
Genomic selection (array design)	PNWTIRC report	June 30, 2017
Facilitated research plan	Work plan or no-go decision	June 30, 2017
Workshop plans for FY2017-2018	Workshop proposal	June 30, 2017

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SAVE THE DATE
May 2-4
CAFS Annual Meeting
Portland, Oregon
go.ncsu.edu/cafs-meeting

Center for Advanced Forestry Systems
2017 Industrial Advisory Board Meeting

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Quantitative Genetics of Blister Rust Resistance in Western White Pine

By Oguz Urhan, Glenn Howe, Marc Rust, Richard Sniezko, and Scott Kolpak

Oguz Urhan (PhD student) is evaluating quantitative genetic and genomic approaches to enhance resistance to the non-native fungal pathogen, *Cronartium ribicola*, in western white pine (WWP). Continued advances in genomic technologies and adoption of new genomic techniques could be used in tandem with classical breeding to enhance disease resistance, or shorten the time to develop improved populations. We are collaborating with the three main resistance breeding programs in North America. Breeding in Idaho is being conducted by Marc Rust of the Inland Empire Tree Improvement Cooperative and Mary Mahalovich from Region 1 of the USFS. Breeding in Oregon is being conducted by Richard Sniezko of the USFS Dorena Genetic Resource Center, and breeding in Canada is being conducted by Nicholas Ukrainetz and John King of the British Columbia Ministry of Forests, Lands, Natural Resource Operations, and Rural Development. Another key contributor is Jun-Jun Liu of the Canadian Forest Service in British Columbia.

The objectives of this project are to (1) review and synthesize breeding program strategies for improving genetic resistance in the three main breeding programs (ID, OR, BC); (2) conduct quantitative genetic analyses of blister rust resistance in the three programs; (3) evaluate molecular breeding strategies to improve resistance breeding including breeding-without-breeding (BWB) and genomic selection (GS); (4) synthesize traditional and molecular breeding options for enhancing resistance breeding in western white pine; and (5) position ourselves to implement GS in WWP by developing single nucleotide polymorphic (SNP) genetic markers and conducting simulation studies of GS. Funding comes from the USFS Special Technology Development Program, Center for Advanced Forestry Systems, PNWTIRC, and the Turkish Government.

Oguz presented the overall framework he will use to evaluate the quantitative genetics of blister rust resistance. The analyses include estimating narrow-sense heritabilities for rust resistance traits, evaluating spatial analyses of genetic test plantations to improve estimates of heritabilities, and evaluating genetic correlations between disease resistance and growth traits. Analyses were conducted for four progeny tests from the Idaho breeding programs (Bertha, Cedar, Paradise Valley, and Quartz Creek). The preliminary analyses at these four sites suggest individual-tree narrow-sense heritabilities were higher for resistance traits than for growth traits, and heritabilities were similar when spatial analysis techniques were used. Genetic correlations were high among growth traits, but inconsistent between growth and resistance traits. The genetic correlations were low, ranging from slightly negative to positive among the different sites. Future analyses will include genetic field tests and inoculation trials from all three North American breeding programs.

Quantitative Genetics of Blister Rust Resistance in Western White Pine

Oguz Urhan
Glenn Howe
Marc Rust
Richard Sniezko
Scott Kolpak



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Outline

- Introduction
- Thesis overview
- Quantitative genetics of rust resistance
- Future directions
- Conclusions
- Funding
 - *Turkish government*
 - *NSF Center for Advanced Forestry Systems (CAFS)*
 - *University of Idaho*

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Western white pine (WWP) breeding programs

- WWP is an economically and ecologically important conifer
- White pine blister rust (WPBR) causes heavy mortality
- Three main resistance breeding programs in North America
 - USFS Dorena Genetic Resource Center (DGRC)
 - USFS and Inland Empire Tree Improvement Cooperative (IETIC)
 - BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development (BC FLNRORD)

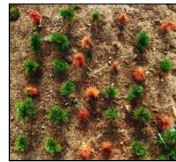


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Resistance breeding programs

- Focus on improving quantitative resistance using recurrent selection
- The main goal is to incorporate disease resistance into improved genotypes and seed orchards
 - Nursery inoculation trials
 - Field performance tests
 - Field progeny tests
- Measurements focus on survival, infection rates, resistance mechanisms, and growth



Susceptible family



Resistant family



Seed orchard



Plantation

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Thesis chapters

- A review of breeding programs and strategies designed to enhance blister rust resistance in western white pine
- Quantitative genetics of blister rust resistance in western white pine
- Molecular breeding strategies to enhance blister rust resistance in western white pine
- A synthesis of traditional and molecular breeding options for enhancing blister rust resistance in western white pine

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Thesis chapters

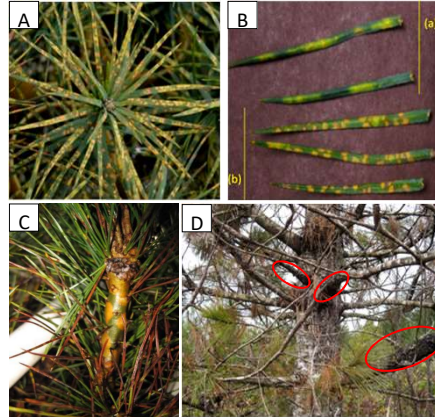
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- Quantitative genetics of blister rust resistance in western white pine
- Molecular breeding strategies to enhance blister rust resistance in western white pine
- A synthesis of traditional and molecular breeding options for enhancing blister rust resistance in western white pine

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Why do we need quantitative resistance?

- Qualitative (single gene) resistance one of the most successful resistance mechanisms
 - Resistance gene (*Cr1*) in sugar pine
 - Resistance gene (*Cr2*) in WWP
 - But pathogen (*Cronartium ribicola*) evolves over time
- Quantitative (multiple gene) or partial resistance is the reduction in symptoms and disease
 - More durable
 - Documented in white pine species
 - Slow canker growth, less stem infection, and higher survival after infection



Sniezko et al. 2014

Quantitative genetics of blister rust resistance

Research questions (today)

- What are the narrow-sense heritabilities for quantitative rust resistance in western white pine?
- Do spatial analyses of genetic test plantations improve estimates of heritability for rust resistance?
- What are the genetic correlations between disease resistance and growth traits?



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Quantitative genetics of blister rust resistance

Plant materials

Table 1. Western white pine breeding programs and tests in the Pacific Northwest, Inland Empire, and British Columbia.

Breeding program	Test type	Number of				Current age	Traits	Measurement	Age
		Tests	Plantations	Families	Mating design				
USFS Dorena	Progeny	2	12	80 - 81	Half-diallel, OP	9 - 16	HT, DBH, RUST traits, SURV, Damage		7,12
	Nursery screening	3	4	60 - 240	Half-diallel, OP	5 - 16	HT, DBH, RUST traits, SURV, Damage		1,2,3,4,5,6
USFS/IETIC	Progeny	5	8	200 - 325	OP	32 - 37	HT, DBH, RUST traits, SURV, Damage		3,5,7,10,15
	Farm-field	5	5	105 - 600	OP	19 - 32	HT, DBH, RUST traits, SURV, Damage		
	Nursery screening	17	17	100 - 318	OP	5 - 32	HT, DBH, RUST traits, SURV, Damage		
	Realized gain	3	6	422 - 462	OP	3 - 10	HT, DBH, RUST traits, SURV, Damage		
	Performance	22	27	7 - 262	OP	2 - 28	HT, DBH, RUST traits, SURV, Damage		
BC Ministry of Forests	Progeny	5	14	49+	Half-diallel, OP	9 - 14	HT, DBH, RUST traits, SURV, Damage		7,10,13

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What are the narrow-sense heritabilities?

Table 2: Individual-tree heritability (h^2) and heritabilities from spatial analysis (h^2_s) from genetic tests in Inland Empire Tree Improvement Cooperative

Traits (age)	Individual-tree heritability (h^2)						Spatial analysis heritability (h^2_s)					
	IETIC-2		IETIC-3		Quartz	Mica	IETIC-2		IETIC-3		Quartz	Mica
	Bertha	Cedar	Paradise Valley	Tired Wolf	Quartz Creek	Mica Creek	Bertha	Cedar	Paradise Valley	Tired Wolf	Quartz Creek	Mica Creek
Rust Index	0.14	0.58	0.04	-	0.10	-	0.14	0.58	?	-	0.10	-
Rust	0.15	0.89	0.06	-	0.15	-	0.16	0.95	?	-	0.16	-
Mortality	0.15	0.18	0.03	-	0.06	-	0.16	0.19	?	-	0.06	-

- Individual-tree narrow-sense heritabilities (h^2) for rust index ranged from 0.04 to 0.58
- Individual-tree narrow-sense heritabilities (h^2) for growth traits ranged from 0.06 to 0.21

Table 3: Range of individual-tree narrow sense heritabilities (h^2) and heritabilities from spatial analysis (h^2_s) from genetic tests in Inland Empire Tree Improvement Cooperative

Traits	Heritability (h^2)	Heritability (h^2_s)
Rust Index	0.04 – 0.58	0.10 – 0.58
Rust	0.06 – 0.89	0.16 – 0.95
Mortality	0.03 – 0.18	0.06 – 0.19
DBH	0.06 – 0.21	0.06 – 0.16
Height	0.07 – 0.19	0.08 – 0.14

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Do spatial analyses improve heritabilities?

Spatial analyses did not substantially improve heritabilities

- Heritabilities from spatial analysis for rust index ranged from 0.10 to 0.58
- Heritabilities from spatial analysis for growth traits ranged from 0.06 to 0.16

Table 3: Range of individual-tree narrow sense heritabilities (h^2) and heritabilities from spatial analysis (h_s^2) from genetic tests in Inland Empire Tree Improvement Cooperative

Traits	Heritability (h^2)	Heritability (h_s^2)
Rust Index	0.04 – 0.58	0.10 – 0.58
Rust	0.06 – 0.89	0.16 – 0.95
Mortality	0.03 – 0.18	0.06 – 0.19
DBH	0.06 – 0.21	0.06 – 0.16
Height	0.07 – 0.19	0.08 – 0.14

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Genetic correlations - disease resistance vs growth

Table 4: Correlation between rust and growth traits from genetic tests in Inland Empire Tree Improvement Cooperative

Traits (age)	Correlations					
	IETIC-2		IETIC-3		Quartz	Mica
	Bertha	Cedar	Paradise Valley	Tired Wolf	Quartz Creek	Mica Creek
	Height (16)	Height (16)	Height (16)	Height (16)	Height (16)	Height (16)
Rust Index	-0.21	-0.21	0.15	-	0.17	-
DBH (16)	0.85	0.85	0.90	-	0.87	-

- Genetic correlations between rust index and height ranged from -0.21 to 0.17
- Genetic correlations between growth traits (height and DBH) ranged from 0.85 to 0.90
- No evidence for defense/growth hypothesis

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Quantitative genetics of blister rust resistance

Future directions

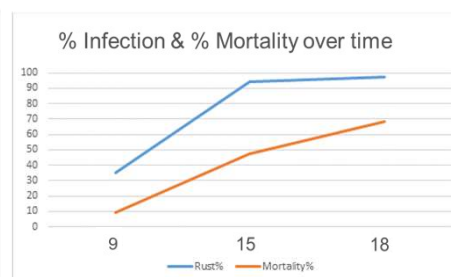
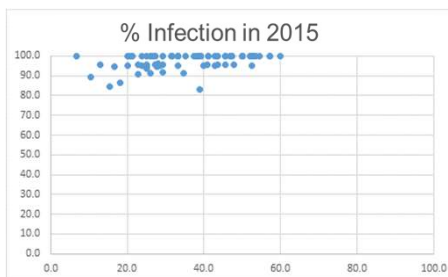
Table 1. Western white pine breeding programs and tests in the Pacific Northwest, Inland Empire, and British Columbia.

Breeding program	Test type	Number of			Mating design	Current age	Measurement	
		Tests	Plantations	Families			Traits	Age
USFS Dorena	Progeny	2	12	80 - 81	Half-diallel, OP	9 - 16	HT, DBH, RUST traits, SURV, Damage	7,12
	Nursery screening	3	4	60 - 240	Half-diallel, OP	5 - 16	HT, DBH, RUST traits, SURV, Damage	1,2,3,4,5,6
USFS/IETC	Progeny	5	8	200 - 325	OP	32 - 37	HT, DBH, RUST traits, SURV, Damage	3,5,7,10,15
	Farm-field	5	5	105 - 600	OP	19 - 32	HT, DBH, RUST traits, SURV, Damage	
	Nursery screening	17	17	100 - 318	OP	5 - 32	HT, DBH, RUST traits, SURV, Damage	
	Realized gain	3	6	422 - 462	OP	3 - 10	HT, DBH, RUST traits, SURV, Damage	
BC Ministry of Forests	Performance	22	27	7 - 262	OP	2 - 28	HT, DBH, RUST traits, SURV, Damage	
	Progeny	5	14	49+	Half-diallel, OP	9 - 14	HT, DBH, RUST traits, SURV, Damage	7,10,13

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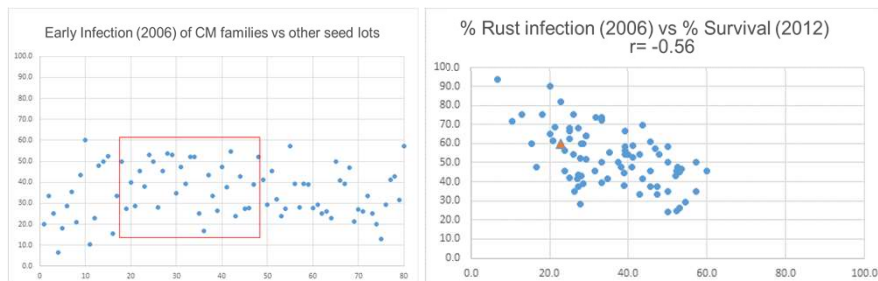
Characteristics of diallel tests from Dorena



Graphs from Richard Sniezko

- Very high overall infection
– 83.3 to 100% infected
- Huge jump in infection from ages 9 to 15, but mortality lagged somewhat

Characteristics of diallel tests from Dorena



Graphs from Richard Sniezko

- Huge difference among the diallels in survival of families
- Virulent strain (*vcr2*) present on this site – and MGR alone doesn't do well
 - *Champion Mine families with Cr2 major gene (MGR) tend to fare worst*
 - *Partial resistance or MGR + partial resistance does better at this site.*
- Bingham F2 doing okay for survival

Quantitative genetics of blister rust resistance

Research questions (future)

- Which levels of disease incidence are best for estimating narrow-sense heritabilities for rust resistance?
- What are the time-trends in heritability of blister rust resistance?
- Is there provenance variation resistance to blister rust?



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Quantitative genetics of blister rust resistance

Research questions (future)

- How well do inoculation trials predict field performance?
- How strong are genotype by environment interactions for disease resistance?
- How can narrow-sense heritability be studied in the presence of major gene resistance?



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Conclusions

- Individual-tree narrow-sense heritabilities (h^2) for rust index ranged from 0.04 to 0.58
- Heritabilities for disease resistance are higher than heritabilities for growth traits.
- Spatial analyses did not substantially improve heritabilities
- Inconsistent correlations between rust index and height among sites, ranging from -0.21 to 0.17
- No evidence for defense/growth hypothesis
- Quantitative resistance or MGR + quantitative resistance show higher survival in Silvermoon site from Dorena

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Acknowledgements

Thanks to....

- *Lauren Magalska, Oregon State University*
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- *Mary F. Mahalovich, USFS, Northern, Rocky Mountain, Southwestern, and Intermountain Regions*
- *Angelia Kegley, USFS, Dorena Genetic Resource Center*
- *Douglas Savin, USFS, Dorena Genetic Resource Center*
- *John King, British Columbia Ministry of Forests and Range*
- *Nicholas Ukrainetz, British Columbia Ministry of Forests and Range*
- *USDA Forest Service Health Protection–Special Technology Development Program (STDP)*
- *CAFS, Center for Advanced Forestry Systems*
- *MEB, Turkish Ministry of Education*

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Toward a SNP chip for western white pine

By Scott Kolpak, Glenn Howe, and Brent Kronmiller

We are developing single nucleotide polymorphism (SNP) genetic markers for western white pine (WWP). Our long-term goal is to lay the foundation for using genomic selection to enhance resistance to white pine blister rust. We will accomplish this by developing a large number of SNP genetic markers, designing a high-density Axiom genotyping platform, and designing a genomic selection breeding strategy. Then, we will seek additional funds and partnerships needed to conduct a proof-of-concept trial of genomic selection in one or more breeding programs. Our specific objectives are to (1) develop SNP genetic markers for WWP; (2) conduct the bioinformatics analyses needed to design a high-density genotyping array; and (3) design a plant breeding strategy for testing genomic selection in WWP. Ultimately, these SNP markers will be transferred to NFGEL and tree breeders for use in resistance breeding programs.

We extracted RNA from needles, branches, stems, roots, and buds collected from trees in three WWP breeding programs in western North America. These programs are managed by the Inland Empire Tree Improvement Cooperative and USFS in Idaho, the USFS Dorena Genetic Resource Center in Oregon, and the British Columbia Ministry of Forests, Lands, and Natural Resources Operations. To facilitate SNP discovery, we pooled tissues from tens to hundreds of families or genotypes that were collected at different times of the year. A total of 12 RNA samples were pooled, and then two replicate samples (normalized and non-normalized) were sequenced using the Illumina HiSeq 2500 platform. These two samples produced 66–73 million reads of ~250 nt each. These sequences will be used to assemble a WWP reference transcriptome and then used for SNP discovery.




Toward a SNP chip for western white pine

Scott Kolpak¹, Glenn Howe¹, Brent Kronmiller²

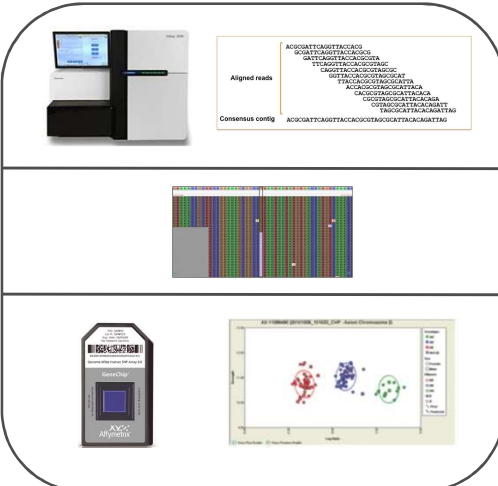
¹ Pacific Northwest Tree Improvement Research Co-op, OSU
² Center for Genome Research and Biocomputing, OSU



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WWP transcriptome and SNP development

- Transcriptome sequencing and assembly
 - Alvaro Hernandez, Carver Biotech. Center, UI
 - Brent Kronmiller, CGRB, OSU
- SNP discovery
 - Similar approach used in Douglas-fir
- SNP array design and manufacture
 - Affymetrix Axiom
 - Seek new funds
- Genomic Selection
 - Seek new funds



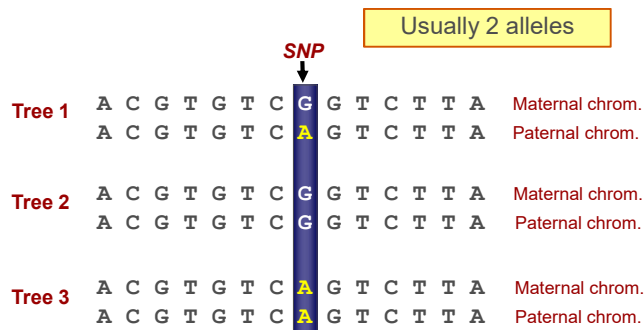
What are SNPs?

What are they good for?

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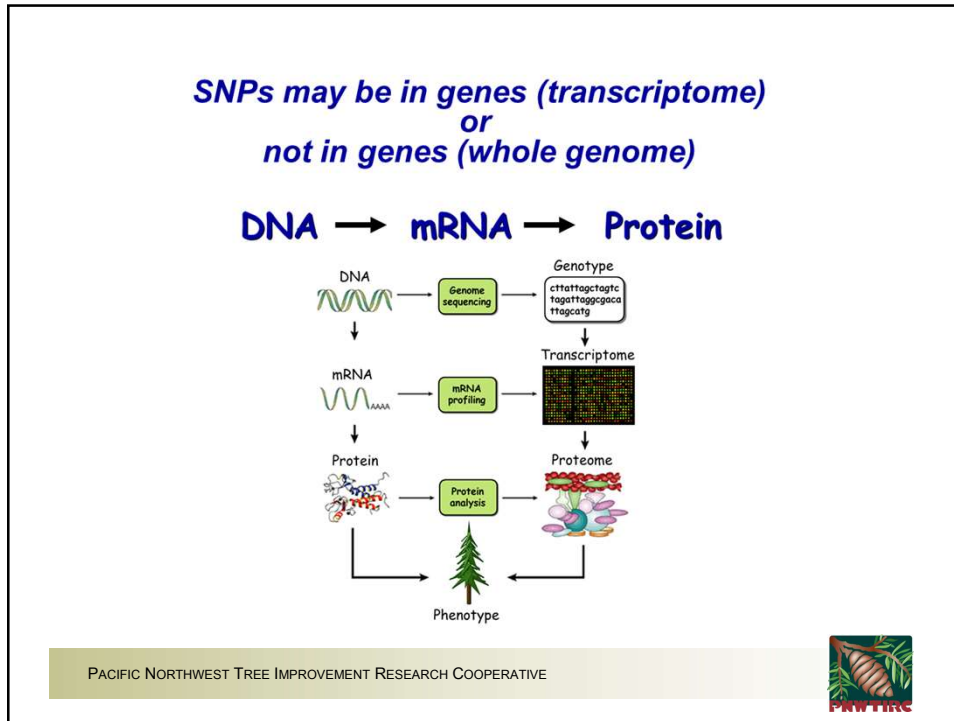
Single nucleotide polymorphism (SNP)



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

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Transcriptome sequencing and assembly

- Tissues included in transcriptome
 - Needles, branches, stems, roots, buds
- Three WWP breeding programs¹
 - USFS DGRC
 - USFS / IETIC
 - BC Ministry of Forests
- Adjusted the final RNA pool
 - Genotype 'diversity'
 - Tissue type

Tissue	Greenhouse			Field		
	November	September	Oct/Nov	Oct/Nov	November	November
Secondary needle	Seedlings Dorena (3)	Saplings Dorena (573+)	Saplings Dorena (9)	Mature trees Bingham (76)	Seedlings Tyrell (230+)	Mature trees BC (31)
Primary needle	—	—	—	—	Seedlings Tyrell (192+)	—
Branch	—	—	Mature trees Dorena (4)	—	—	—
Stem	Seedlings Dorena (3)	—	—	Mature trees Bingham (76)	—	—
Root	Seedlings Dorena (3)	—	—	—	—	—
Bud	—	—	—	Mature trees Bingham (76)	—	—

¹ DGRC = Dorena Genetic Resource Center, IETIC = Inland Empire Tree Improvement Cooperative

Transcriptome sequencing and assembly

RNA sequencing

- Submitted two replicate samples to Carver BioTech.
 - *Non-normalized*
 - *Normalized*
- 250 base pair reads

Illumina HiSeq 2500



Table 2. Western white pine RNAseq libraries and numbers of 250 nt reads.

Sample	Name of fastq file	No. of reads
OSU_WWP_3_15_16	OSU_WWP_3_15_16_ACAGTGAT_L001_R1_001	72,564,364
OSU_WWP_3_15_16	OSU_WWP_3_15_16_ACAGTGAT_L001_R2_001	72,564,364
OSU_WWP_3_15_16_norm	OSU_WWP_3_15_16_norm_GTGAAACG_L001_R1_001	65,941,515
OSU_WWP_3_15_16_norm	OSU_WWP_3_15_16_norm_GTGAAACG_L001_R2_001	65,941,515
		277,011,758



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Transcriptome sequencing and assembly

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OSU_WWP_3_15_16_norm	OSU_WWP_3_15_16_norm_GTGAAACG_L001_R1_001	65,941,515
OSU_WWP_3_15_16_norm	OSU_WWP_3_15_16_norm_GTGAAACG_L001_R2_001	65,941,515
		277,011,758



66 to 73 million reads produced

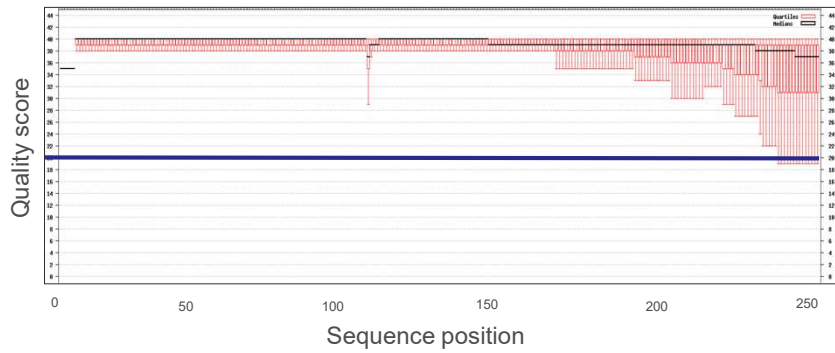
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Transcriptome sequencing and assembly

RNA sequencing

- Read quality
 - Good quality – only the bases near 250 bp limit are questionable



Quality scores > 20 (blue line) are considered high-quality (1% chance of an incorrect base call).

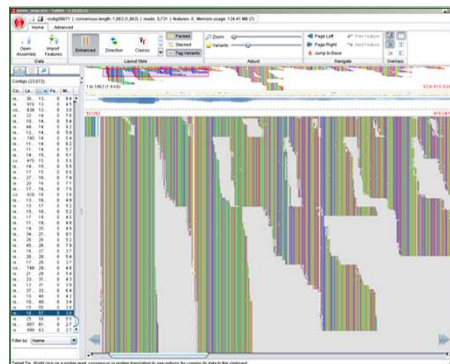
Transcriptome assembly

A transcriptome from many short sequences

Next-generation sequencing



Illumina HiSeq 2500



Transcriptome sequencing and assembly

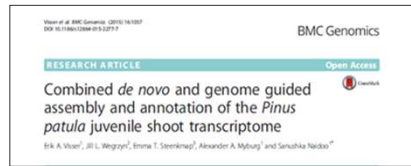
Transcriptome assembly

- Assembly using the Trinity program
 - Brent Kronmiller, *Bioinformatics Analyst, CGRB, OSU*



Preliminary results

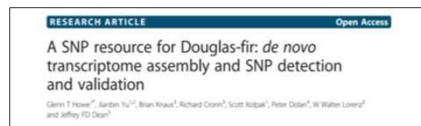
- Non-normalized
 - 528,859 contigs
 - 248,936,300 bp
 - N50 476 bp
- Normalized
 - 784,316 contigs
 - 359,700,673 bp
 - N50 471 bp



Future directions

- Transcriptome sequencing and assembly
 - Brent Kronmiller to refine transcriptome assembly
- SNP discovery
 - Similar approach used in Douglas-fir
- SNP array design and manufacture
 - Affymetrix Axiom (high density)
 - Seek new funds
- Genomic Selection
 - Seek new funds

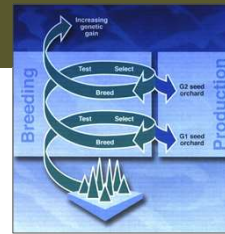
The composite image illustrates various components of transcriptome analysis. At the top left is a sequencing machine. To its right is a diagram showing 'Aligned reads' as multiple overlapping lines of nucleotide sequences, with a 'Consensus contig' shown below as a single line. Below this is a photograph of an Affymetrix Axiom SNP array chip. To the right of the chip is a network graph visualization with nodes and connecting lines.



Future directions (continued)

- Genomic selection (GS)
 - Simulations
 - Proof-of-concept studies

- Apply GS to the three breeding programs in western North America
 - USFS Dorena Genetic Resource Center (DGRC)
 - USFS and Inland Empire Tree Improvement Cooperative (IETIC)
 - BC Ministry of Forests



Acknowledgements

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- Mike Crawford, BLM, Tyrell Seed Orchard
- Alvaro Hernandez, University of Illinois, Roy J. Carver Biotechnology Center
- Angelia Kegley, USFS, Dorena Genetic Resource Center
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- Marc L. Rust, University of Idaho, Inland Empire Tree Improvement Cooperative
- Richard Sniezko, USFS, Dorena Genetic Resource Center
- Nicholas Ukrainetz, British Columbia Ministry of Forests and Range
- Oguz Urhan, Oregon State University, PhD candidate
- USDA Forest Service Health Protection–Special Technology Development Program (STDP)
- CAFS, Center for Advanced Forestry Systems

Genetics of Drought Hardiness in Douglas-fir

By Erda Çeler and Glenn Howe

Breeding programs for Douglas-fir aim to increase growth and wood quality, while maintaining adaptability to frost and drought. However, populations that grow faster are also typically less tolerant of drought and other stresses. To better understand the genetics of drought hardiness in Douglas-fir, a study was initiated by Jeannette Griese of the BLM in 2008-2009, and later implemented cooperatively by the Northwest Tree Improvement Cooperative, Bureau of Land management, Plum Creek Timber Company (now Weyerhaeuser), Silver Butte Timber Company, and Washington Department of Natural Resources.

In 2015, Erda Çeler, began using this experiment for her M.S. thesis research. The objectives of her research are to (1) obtain baseline measurements and climate data to help in the analysis and interpretation of future measurements in the Drought Hardiness Study; (2) characterize the quantitative genetics of drought adaptation traits; and (3) determine whether drought adaptation traits are associated with the climatic origin of Douglas-fir seedlings.

The complete experiment contains more than 18,000 Douglas-fir seedlings from more than 429 parents from western Oregon and Washington that were planted at three sites in southern Oregon. For the M.S. thesis research, drought adaptation traits and climate data were collected from two of the sites (Sprague and Lost Creek) between 2015 and 2016. Climate data included weather station data from both sites and a range of climate variables (1961-1990 normals) from the female parent source locations. Measured drought adaptation traits included height, second flushing, spring bud flush, damage (foliage, stems, and leaders), and survival.

In the first growing season, heritabilities and genetic variances differed widely among traits. Estimated genetic gains were large for flushing (Flush), second flushing (SFlush), and height increment (Htinc). Primarily because of the large number of families tested (i.e., high selection differentials), low genetic correlations were found between growth in the greenhouse and other drought adaptation traits, flushing versus field height growth, and flushing versus mortality.

Drought adaptation traits were significantly correlated with some parental climate variables. Large and significant correlations were found between growth in the greenhouse and parent source climates. In addition, some climate variables were moderately correlated with spring bud flush, and low correlations between other drought adaptation traits. For instance, early bud flush was associated with warmer and drier climates, suggesting that early bud flush is a drought avoidance strategy.

These early results increase our understanding about the importance of climatic-driven genetic differences for drought adaptation traits in Douglas-fir. Future measurements and analyses will benefit from these early measurements by understanding causes of early mortality at the sites and utilizing the measured heights as “initial height” to help remove the confounding effects of family height variation resulting from early seedling growth in the greenhouse. Later analyses of the Drought Hardiness Study will provide useful information for understanding drought, enhancing breeding programs, and potentially adjusting forest management to climate change impacts.

Genetics of Drought Hardiness in Douglas-fir

Erda Çeler and Glenn T. Howe

*Department of Forest Ecosystems and Society
Oregon State University*

In collaboration with the Bureau of Land Management, Plum Creek Timber Company (now Weyerhaeuser), and Silver Butte Timber Company

***PNWTIRC Annual Meeting
October 19, 2016***

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Introduction

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Overview

- Drought adversely affects natural and artificial regeneration of Douglas-fir
- Climate change is expected to adversely affect the adaptability of Douglas-fir trees
- Assisted migration may become a necessary tool to mitigate the impacts of climate change

It is important to incorporate drought hardiness into Douglas-fir breeding programs

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Drought hardiness research by the PNWTIRC

Genetic variation in drought hardiness of coastal Douglas-fir seedlings from British Columbia¹

T.S. Anekonda, M.C. Lomas, W.T. Adams, K.L. Kavanagh, and S.N. Aitken

- 39 full-sib families from British Columbia measured in raised nursery beds
- Foliage damage and xylem cavitation increased - xylem hydraulic conductivity decreased under drought stress
- Lots of environmental variability: Heritabilities averaged only 0.19
- Growth in moist conditions nearly uncorrelated with drought hardiness
- Early testing for drought hardiness is possible
- Did not study the effects of seedling origin on drought hardiness

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Drought hardiness research by the PNWTIRC

Response of annual growth ring components to soil moisture deficit in young, plantation-grown Douglas-fir in coastal British Columbia

Andrew D. Bower, W. Thomas Adams, David Birkes, and Darek Nalle

- Can growth ring components be used to evaluate the genetics of drought hardiness?
- 10 growth ring variables measured in 6 progeny tests (X-ray densitometry)
- Drought response coefficient (DRC) may be useful for identifying drought hardy genotypes
- Growth ring approach is challenging – substantial drought is needed – new studies would need to impose substantial drought stress

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Drought Hardiness Study

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About this experiment

- The project was initiated by Jeannette Griese at the BLM in 2008-2009
- Currently managed as a collaboration among:
 - *Northwest Tree Improvement Cooperative (Keith Jayawickrama)*
 - *Bureau of Land Management (Mike Crawford, Jeannette Griese and George McFadden)*
 - *Plum Creek Timber Company (now Weyerhaeuser) (Jim Smith)*
 - *Silver Butte Timber Company (Darin McMichael)*
 - *Washington DNR (Jeffrey DeBell)*

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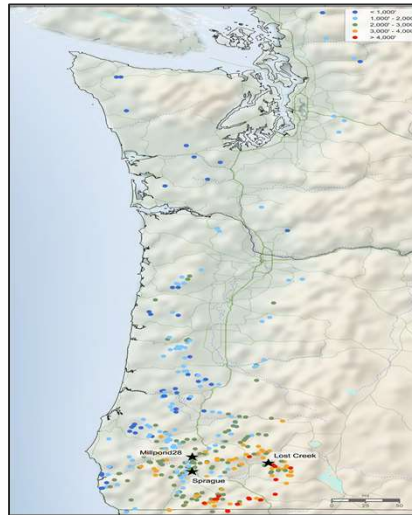
Source of germplasm

- The genetics of drought hardiness is being investigated using Douglas-fir seedlings from 429 elite families in western Oregon and Washington planted at three sites: Lost Creek, Sprague, and Millpond
- Seedlots
 - *Most are OP seed from first-generation parents in orchards*
 - *Some are half-sib families created by pooling full-sib families*
 - *Two are woods-run seedlots from southern Oregon*
- Seedlings were grown in the BLM Sprague greenhouse

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Sites of collection



Seed Orchards:

- Horning
- Tyrrell
- Schroeder
- Provolt
- DNR Meridian
- Plum Creek
- Stimson

Figure 1. Location of test sites and origin of parents (Jayawickrama and Crawford 2016)

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Experimental methods

- After 2 years in the greenhouse, the seedlings were planted at three sites in southern Oregon
- Each site has a weather station that was installed for the experiment
- We measured about 10,000 seedlings on two sites: Sprague and Lost Creek
- A number of variables including growth traits (height), second flushing, spring bud flush, damage (foliage, sun scald, and leaders), and survival were measured

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Goals and Objectives

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Main goals

- Understand the impacts of drought on the growth and survival of Douglas-fir seedlings
- Enhance approaches for genetically improving drought hardiness
- Enhance approaches for appropriately deploying genotypes from breeding programs
- Understand the potential effects of climate change and provide information for practicing effective assisted migration

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Specific objectives

- Obtain baseline measurements to help in the analysis and interpretation of future measurements in the drought hardiness study
- Characterize the quantitative genetics of drought adaptation traits
- Determine whether drought adaptation traits are associated with the climatic origin of Douglas-fir seedlings
- Examine the relationships between seedling traits at the time of planting and drought adaptation traits
- Develop recommendations for deploying Douglas-fir genotypes and practicing assisted migration

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Research Questions

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Research questions

- Is there genetic variation in drought adaptation traits among Douglas-fir families?
- Are drought adaptation traits associated with the climatic origin of the Douglas-fir families?
- Is there an association between drought adaptation traits and seedling characteristics at the time of planting?
- Is early bud flush associated with other drought adaptation traits?

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Sites

Seedlings were planted in 2015 on two sites in southern Oregon



Figure 2. Lost Creek site



Figure 3. Sprague site

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Locations

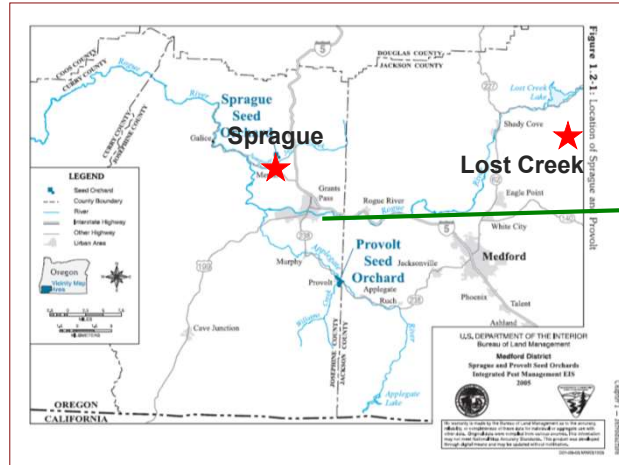
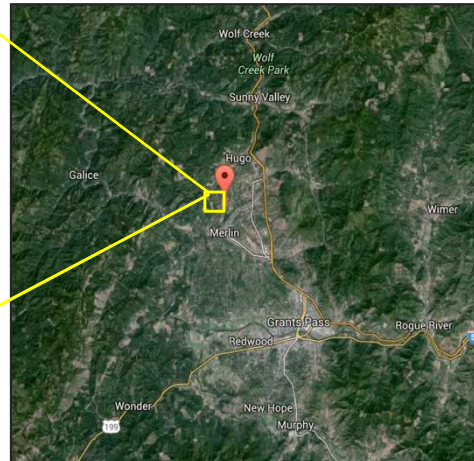
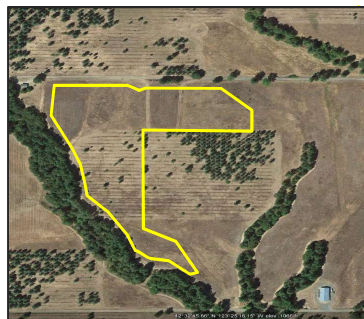


Figure 4. Locations of test sites

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Sprague site



- 6480 Douglas-fir seedlings
- 427 families
- RBD with 22 blocks
- Single-tree plots

Figure 5. Satellite imagery of Sprague site

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Lost Creek site



- 3449 Douglas-fir seedlings
- 293 families
- RBD with 17 blocks
- Single-tree plots

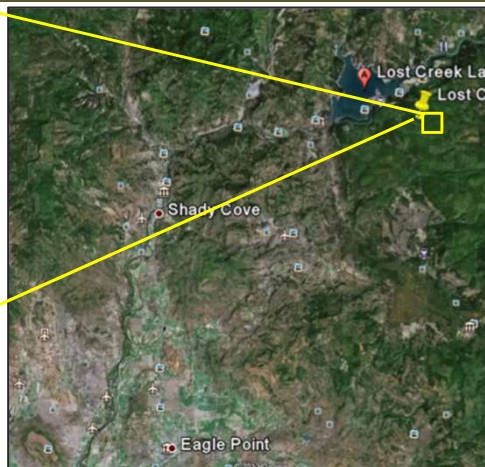


Figure 6. Satellite imagery of Lost Creek site

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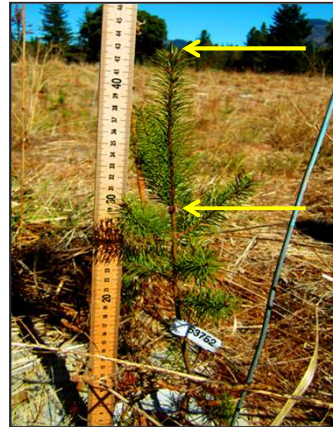
Methodology

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Trait measurements

- **Height**
 - *Ht14* is total growth in the greenhouse at year 2014
 - *Ht15* is height in the field at year 2015
 - *Htinc* growth in the field (2014-2015)
- **Second flushing (Sflush)**
 - Presence/absence



Height measurements

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Trait measurements (Cont'd)

- **Bud flush (Flush)**

Five categories to classify timing of bud flush

 - 1 = the bud was closed, tight, and dark
 - 2 = the bud was closed, swollen, light colored
 - 3 = the bud was just beginning to burst through tip
 - 4 = the bud was open, needles around 1 cm long
 - 5 = the bud was fully open, needles fully elongated
- **Foliage damage (FD)**
 - Percentage of dead foliage



Buds were fully open

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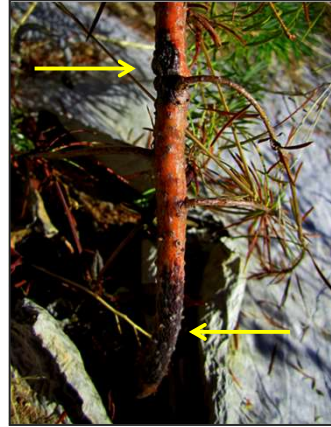


Trait measurements (Cont'd)

- **Stem damage (SD)**
– *Percentage of stem damage by sunscald*

- **Leader damage (LD)**
– *Presence/absence*

- **Mortality (Mort)**
– *Dead/alive*



Stem with sun-scald

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Preliminary Results Height Growth

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Statistics for traits measured on families

- Large height differences from the greenhouse persist in the field
- Modest differences in height growth among families in the field

Sprague and Lost Creek					
	Overall Mean	Min	Max	Range	h^2
Ht14 (cm)	41.34	10	74	64	0.95
Ht15 (cm)	50.81	20	83	63	0.95
Htinc (cm)	9.47	0	20	20	0.17
Flush	2.15	1	4	3	0.73
Sflush	0.22	0	1	1	0.09
FD	22.54	0	100	100	0.09
SD	1.02	0	10	10	0.03
LD	0.12	0	2	2	0.07
Mort	0.21	0	1	1	0.10

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Genetic correlations among drought traits

- Low correlation between greenhouse and field growth

	Ht14	Ht15	Htinc	Flush	Sflush	FD	SD	LD	Mort
Ht14		0.97	0.06	-0.13	-0.18	0.06	0.08	-0.21	0.11
Ht15	0.97		0.28	-0.20	-0.18	0.03	0.08	-0.26	0.09
Htinc	0.23	0.45		-0.31	-0.05	-0.14	0.02	-0.23	-0.10
Flush	-0.20	-0.17	0.04		0.29	-0.16	-0.04	0.41	-0.18
Sflush	0.34	0.41	0.42	0.05		-0.19	-0.04	0.26	-0.20
FD	-0.05	-0.20	-0.59	-0.02	-0.28		0.04	-0.05	0.96
SD	0.21	0.21	0.11	-0.14	0.03	-0.03		-0.03	0.04
LD	0.01	0.01	0.00	0.25	0.04	0.00	-0.03		-0.11
Mort	-0.07	-0.21	-0.57	-0.02	-0.29	0.94	-0.01	-0.05	

Sprague correlations are above the diagonal, and Lost Creek correlations are below.

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Correlations between BLUPs and climate

- Height in the greenhouse is positively associated with temperature variables

	MAT	MSP	SHM	NFFD	EMT	EXT	EREF
Ht14	0.42	0.05	0.16	0.36	0.25	0.39	0.43
Ht15	0.45	0.10	0.10	0.37	0.24	0.45	0.48
Htinc	0.11	0.07	0.00	0.05	0.01	0.15	0.15
Flush	0.07	-0.29	0.27	0.11	0.21	-0.02	-0.03
Sflush	0.27	0.07	0.07	0.24	0.20	0.24	0.25
FD	0.01	-0.02	0.01	0.05	0.03	-0.05	-0.02
SD	0.08	0.02	0.00	0.09	0.05	0.07	0.07
LD	0.01	-0.10	0.08	0.03	0.07	-0.03	-0.02
Mort	0.01	-0.04	0.02	0.06	0.04	-0.06	-0.03

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Correlations between BLUPs and climate

- Correlations are much lower for growth in the field

	MAT	MSP	SHM	NFFD	EMT	EXT	EREF
Ht14	0.42	0.05	0.16	0.36	0.25	0.39	0.43
Ht15	0.45	0.10	0.10	0.37	0.24	0.45	0.48
Htinc	0.11	0.07	0.00	0.05	0.01	0.15	0.15
Flush	0.07	-0.29	0.27	0.11	0.21	-0.02	-0.03
Sflush	0.27	0.07	0.07	0.24	0.20	0.24	0.25
FD	0.01	-0.02	0.01	0.05	0.03	-0.05	-0.02
SD	0.08	0.02	0.00	0.09	0.05	0.07	0.07
LD	0.01	-0.10	0.08	0.03	0.07	-0.03	-0.02
Mort	0.01	-0.04	0.02	0.06	0.04	-0.06	-0.03

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Preliminary Results Bud Flush

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Statistics for traits measured on families

- High heritability for bud flush

	Sprague and Lost Creek				h^2
	Overall Mean	Min	Max	Range	
Ht14	41.34	10	74	64	0.95
Ht15	50.81	20	83	63	0.95
Htinc	9.47	0	20	20	0.17
Flush (1-5)	2.15	1	4	3	0.73
Sflush	0.22	0	1	1	0.09
FD	22.54	0	100	100	0.09
SD	1.02	0	10	10	0.03
LD	0.12	0	2	2	0.07
Mort	0.21	0	1	1	0.10

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Genetic correlations among drought traits

- Relationship between height growth and flushing differs between plantations

	Ht14	Ht15	Htinc	Flush	Sflush	FD	SD	LD	Mort
Ht14		0.97	0.06	-0.13	-0.18	0.06	0.08	-0.21	0.11
Ht15	0.97		0.28	-0.20	-0.18	0.03	0.08	-0.26	0.09
Htinc	0.23	0.45		-0.31	-0.05	-0.14	0.02	-0.23	-0.10
Flush	-0.20	-0.17	0.04		0.29	-0.16	-0.04	0.41	-0.18
Sflush	0.34	0.41	0.42	0.05		-0.19	-0.04	0.26	-0.20
FD	-0.05	-0.20	-0.59	-0.02	-0.28		0.04	-0.05	0.96
SD	0.21	0.21	0.11	-0.14	0.03	-0.03		-0.03	0.04
LD	0.01	0.01	0.00	0.25	0.04	0.00	-0.03		-0.11
Mort	-0.07	-0.21	-0.57	-0.02	-0.29	0.94	-0.01	-0.05	

Sprague correlations are above the diagonal, and Lost Creek correlations are below.

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Preliminary conclusions

- There were large differences in height among families in the greenhouse ($h^2=0.95$)
- Genetic variation for growth in the field was modest
- Variation in height after one year in the field largely reflected differences in growth that occurred in the greenhouse
- Initial measurements can be used as covariates in later analyses

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Preliminary conclusions

- The genetic correlation between greenhouse growth and field growth was low
- Temperature variables from the sites of origin were positively associated with growth in the greenhouse, but showed little relationship to growth in the field
- Bud flush was highly heritable ($h^2=0.75$)
- Relationships between bud flush and field growth differed between sites

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- PNWTIRC (Measurement Crew)

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Thank you!!

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PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Effects of climate change on growth of Douglas-fir plantations

By Lauren Magalska

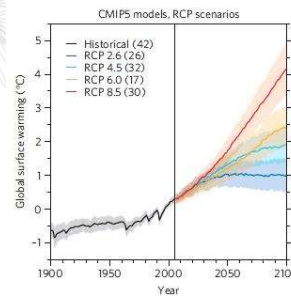
Our ability to project the value of Douglas-fir plantations is limited by our understanding of (1) how stand growth, stem quality, and adaptability are influenced by site characteristics such as climate, weather, topography, and soils; (2) the mechanistic basis of genotype by site interactions; and (3) the effects of seed source and genotype transfer among sites. An extensive network of Douglas-fir progeny tests in the Pacific Northwest (PNW) allows us to use existing data on growth, stem quality, and adaptive traits to (1) remove the confounding effects of genetics when predicting stand-level performance from site characteristics, and (2) better understand and quantify genotype by site interactions. The progeny tests used in this study are part of the Northwest Tree Improvement Cooperative (NWTIC). Previous analysis of site characteristics influencing stem defects was reported in Magalska & Howe (2014).

We finalized the modeling of site characteristics influence on Douglas-fir productivity (e.g., height diameter, volume growth) and survival. We used progeny test data from 32 breeding programs and 348 NWTIC test sites in the Pacific Northwest to derive average annual growth and percent survival among different measurement periods corresponding to the progeny test measurements recorded 5-yr, 10-yr, and 15-yr after planting. Data from 90 site characteristics were derived from the ClimateWNA model and digital terrain models. Initial variable selection removed 48 site characteristics were highly correlated many other variables for range of response variables. Random Forest analyses on PC1 and PC2 using the remaining 40 site characteristics resulted in final set of 19 site characteristics for detailed modeling using individual and multivariate Random Forest and Lasso Regression analyses. The final modeling

Effects of Climate Change on Growth of Douglas-fir Plantations



Lauren Magalska
 Glenn Howe
 Doug Maguire
 Scott Kolpak



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Introduction

Site productivity and stem form are directly related to:

- Profitability of owning forestland
- Return on silvicultural investment

Current methods of assessing site productivity have limitations

Douglas-fir response to near-term climate change needs to be better understood

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Forest productivity models

Empirical growth and yield models



Mechanistic Models



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Climate change

- Projections
 - *Uncertain changes to precipitation*
 - *Summer warming, more pronounced inland*
 - *Greater winter warming than summer warming in the western Cascades*
 - *Increased CO₂*
- How will Douglas-fir respond?
- Which climate and site characteristics should be investigated in detail?

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Why use progeny tests?

Differences in site characteristics and productivity across the landscape typically involve environmental and genetic differences (they are confounded)

but.....

We're interested in the environmental differences

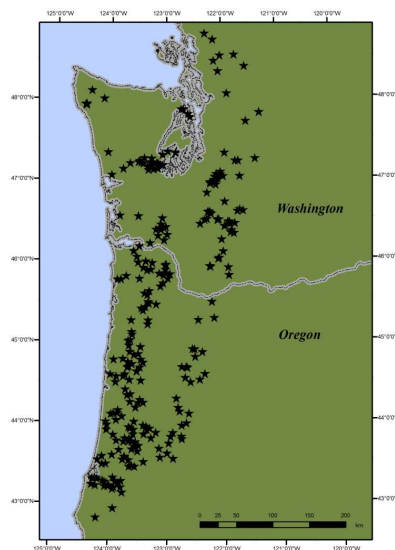
We can use progeny tests within breeding programs to remove genetic differences

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Site locations

- 348 NWTIC progeny test sites in 32 breeding programs
- Oregon and Washington
- West of Cascade crest
- Measured between 1967 and 2005



Site characteristics – Thesis approach

Climate

- ClimateWNA
- 35 climate site characteristics

Soils

- NRCS SSURGO
- 3 soils site characteristics

Topography

- USGS DEM
- 2 topographic site characteristics

Total of 40 site characteristics

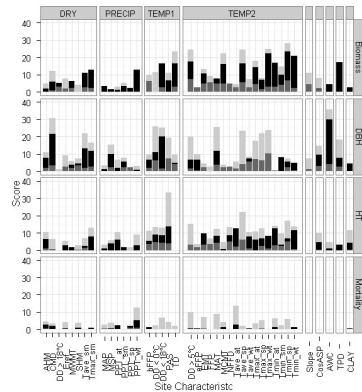


Site productivity analyses - Thesis

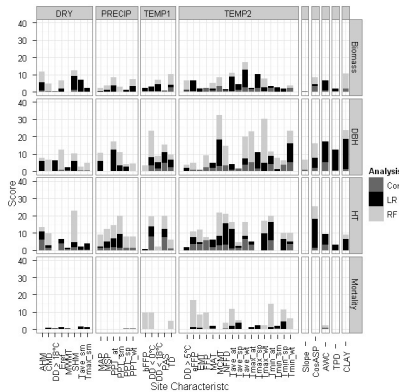
- Across-program and within-program analyses
- Hierarchical clustering of site characteristics into site characteristic groups (SCG)
- Three-pronged variable selection
 - Simple correlation (*Corr*)
 - Linear regression (*LR*)
 - Random forest (*RF*)
- A total importance score was calculated for each site characteristic in both the across-program and within-program data

Site productivity results - Thesis

Across-program



Within-program



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Site productivity conclusions - Thesis

Cold season temperatures and available water capacity explain variation in site productivity

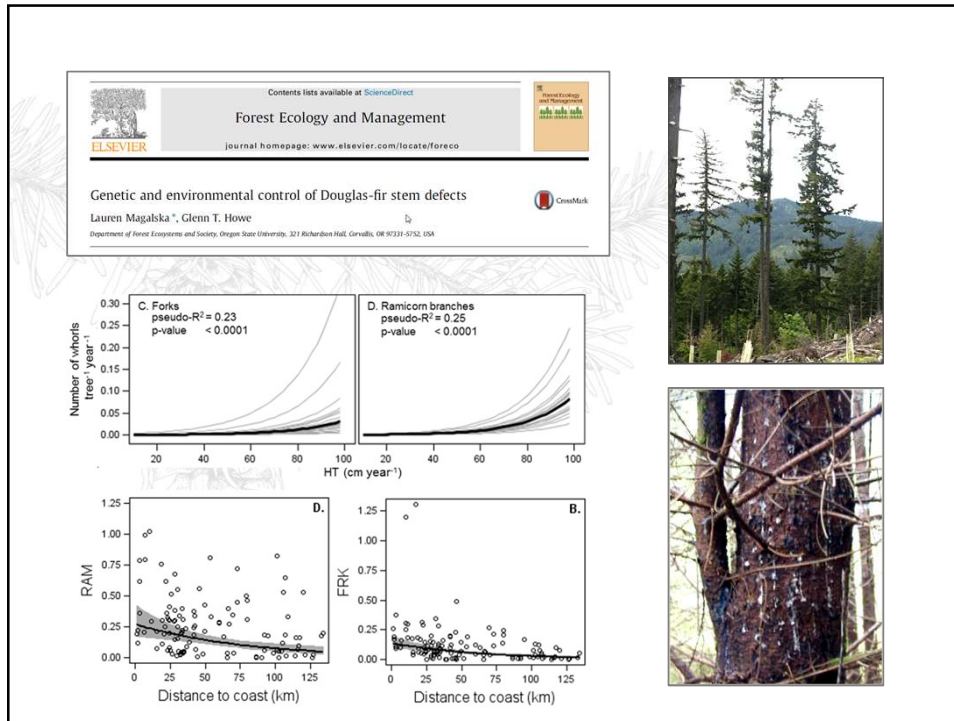
General relationships have been identified, but there was ample opportunity for continued work

Continue to refine the analytical approach

Define approaches for reducing the number of independent variables

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What's new?

- Substantially increased the number of sites
- Increased number of climate variables
 - *ClimateWNA was expanded*
- Added geomorphometric variables
- Removed soils site characteristics
- Improved the Random Forests approach
 - *Sabatia and Burkhart 2014*
- Improved the linear regression approach
 - *Lasso regression*

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Predicting site index of plantation loblolly pine from biophysical variables

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ABSTRACT

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Site measurements

Response group and variable ^a	Units	All plantations ^b						Within-programs ^c
		N	Median	Mean	Min	Max	Range	Range
<i>Individual tree variables</i>								
<i>Height</i>								
HT ₀₋₁	cm year ⁻¹	287	21.5	23.1	5.2	53.7	48.5	15.4
HT ₀₋₂	cm year ⁻¹	321	45.1	45.4	7.4	74.4	67.0	29.8
HT ₀₋₃	cm year ⁻¹	223	60.1	59.1	16.4	85.6	69.2	25.2
HT ₁₋₂	cm year ⁻¹	278	72.8	71.9	0.3	139.1	138.8	46.9
HT ₂₋₃	cm year ⁻¹	217	93.9	92.7	27.1	159.8	132.7	32.3
HT ₁₋₃	cm year ⁻¹	197	82.9	81.3	31.9	112.7	80.8	33.3
<i>DBH</i>								
DBH ₀₋₁	mm year ⁻¹	55	3.6	3.8	1.4	8.6	7.3	3.9
DBH ₀₋₂	mm year ⁻¹	164	5.6	5.6	1.0	17.6	16.6	4.3
DBH ₀₋₃	mm year ⁻¹	247	8.0	7.9	2.0	12.7	10.7	3.5
DBH ₁₋₂	mm year ⁻¹	13	5.5	8.1	3.4	23.5	20.1	7.9
DBH ₂₋₃	mm year ⁻¹	105	13.2	13.3	6.8	23.7	16.9	3.4
DBH ₁₋₃	mm year ⁻¹	47	10.9	11.4	5.6	22.8	17.2	6.2
<i>Volume</i>								
VOL ₀₋₁	dm ³ year ⁻¹	47	0.0	0.2	0.0	1.3	1.4	0.4
VOL ₀₋₂	dm ³ year ⁻¹	162	0.7	0.9	-0.1	4.1	4.2	1.5
VOL ₀₋₃	dm ³ year ⁻¹	208	3.2	3.5	0.1	9.6	9.4	3.4
VOL ₁₋₂	dm ³ year ⁻¹	13	1.3	1.9	0.4	7.6	7.2	2.7
VOL ₂₋₃	dm ³ year ⁻¹	77	8.1	8.1	0.9	22.6	21.7	5.9
VOL ₁₋₃	dm ³ year ⁻¹	39	6.0	6.2	1.4	16.1	14.7	6.4

^a HT is mean annual height growth. DBH is mean annual diameter growth. VOL is mean annual volume growth (per tree). The response variable subscripts indicate the growth period: 0-1=sowing to age at measurement cycle 1, 0-2=sowing to age at measurement cycle 2, 0-3=sowing to age at measurement cycle 3, 1-2=age between measurement cycles 2 and 3, 1-3=age between measurement cycles 1 and 3.

^b Summary statistics from all plantations (n ≤ 348).

^c Average range from plantations within programs (n = 32 programs).

Site measurements – cont.

Response group and variable ^a	Units	All plantations ^b						Within-programs ^c
		N	Median	Mean	Min	Max	Range	Range
<i>Stand variables</i>								
<i>Survival</i>								
SURV ₀₋₁	%	348	97	94	29	100	71	15
SURV ₀₋₂	%	348	95	92	29	100	71	18
SURV ₀₋₃	%	348	93	87	0	100	100	22
<i>Stand volume</i>								
SVOL ₀₋₁	m ³ ha ⁻¹ year ⁻¹	40	0.0	0.1	0.0	0.7	0.7	0.3
SVOL ₀₋₂	m ³ ha ⁻¹ year ⁻¹	151	0.9	1.5	-0.1	6.8	6.9	2.4
SVOL ₀₋₃	m ³ ha ⁻¹ year ⁻¹	194	4.6	4.9	0.2	16.1	15.9	5.7
SVOL ₁₋₂	m ³ ha ⁻¹ year ⁻¹	13	1.1	1.8	0.3	6.4	6.1	2.4
SVOL ₂₋₃	m ³ ha ⁻¹ year ⁻¹	72	9.6	10.2	1.3	22.9	21.6	8.2
SVOL ₁₋₃	m ³ ha ⁻¹ year ⁻¹	39	7.5	7.4	1.5	15.8	14.3	6.7

^a SURV is the mean percent of trees surviving to a given measurement. SVOL is the mean annual volume growth in stands. The response variable subscripts indicate the growth period: 0-1=sowing to age at measurement cycle 1, 0-2=sowing to age at measurement cycle 2, 0-3=sowing to age at measurement cycle 3, 1-2=age between measurement cycles 1 and 2, 2-3=age between measurement cycles 2 and 3, 1-3=age between measurement cycles 1 and 3.

^b Summary statistics from all plantations (n ≤ 348).

^c Average range from plantations within programs (n = 32 programs).



Site characteristics

Climate

- ClimateWNA
- 69 climate site characteristics
- 6 periodic growth climates

Geomorphometry

- USGS DEM
- TNC geomorphometry toolkit for ArcGIS
- 21 geomorphometry site characteristics

Total of 90 site characteristics

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Variable selection

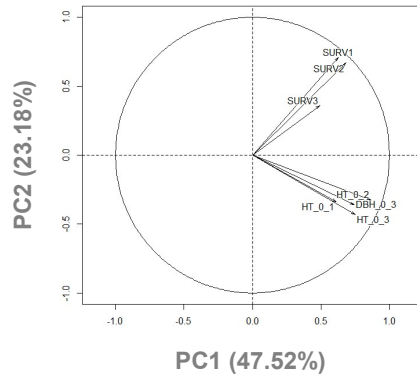
Reduced the number of site characteristics

- Removed site characteristics that were highly correlated with many others
- Conducted principle components analysis on growth and survival traits

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Variables factor map



Two factors explain 71% of the variation among sites

PC1 represents growth

PC2 represents survival

Both used as responses in random forest analysis

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Variable selection

Identified site characteristics in both models

Included *a priori* site characteristics to facilitate comparison to other studies

List of 19 candidate site characteristics

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Variable selection

Table 3. Selected site characteristics of Douglas-fir progeny test sites.

Biophysical variable, definition, and datasource ^a	units	All plantations ^b					Within-	
		Min	Median	Mean	Max	Range	program ^c	
<i>Climate/WNA</i>								
AFM	Annual <u>heat moisture</u> index	°C m ⁻¹	5	12	12	28	24	6
EMT	Extreme minimum temperature (30 years)	°C	-30	-19	-19	-11	19	4
Eref _{AT}	Hargreaves reference evaporation - Autumn	mm d ⁻¹	111	157	158	201	90	22
MAP	Mean annual precipitation	mm	740	1709	1800	3815	3075	869
MAT	Mean annual temperature	°C	6	11	10	13	7	2
MCMT	Mean coldest month temperature	°C	-4	3	3	7	11	3
MSP	Mean summer (May to Sept.) precipitation	mm	127	267	275	633	506	124
MWMT	Mean warmest month temperature	°C	15	18	18	21	6	2
NFFD _{AT}	Number of frost-free days - Autumn	days	51	78	77	87	36	8
NFFD _{SM}	Number of frost-free days - Summer	days	87	91	91	92	5	1
NFFD _{SP}	Number of frost-free days - Spring	days	44	74	73	85	41	13
NFFD _{WT}	Number of frost-free days - Winter	days	16	49	50	74	58	17
PAS	Precipitation as snow	mm	13	54	78	599	586	107
PPT _{SM}	Summer precipitation	mm	48	114	120	292	244	59
Tave _{AT}	Mean temperature - Autumn	°C	6	11	11	14	8	2
Tave _{SP}	Mean temperature - Spring	°C	4	10	9	12	8	2
TD	Temperature difference between MWMT and MCMT	°C	9	16	15	20	11	3
<i>Nature Conservancy Geomorphometry Toolkit</i>								
DISS	<u>Martonne's</u> modified dissection, measure of <u>rugosity</u>		0.43	0.51	0.51	0.61	0.18	0.07
SLOPE	Mean slope	°	0.2	8.5	9.3	28.4	28.2	11.7

Variable selection

- Random forest
- Lasso regression
- Multiple imputation and multivariate regression trees and lasso regression

Variable selection

Final model selection

- Growth
 - 2 or more multivariate models or
 - 1 multivariate model and 3 of the individual trait models or
 - 4 individual trait models
- Survival
 - 2 or more multivariate models
 - 1 multivariate model and 3 of the individual trait models or
 - 2 individual trait models

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Variable selection

Within trait	Across trait	Growth												Survival										
		RF				MLR				MLR				RF		MLR								
		PC1	HT ₁	HT ₂	HT ₃	DBH ₃	MV	PC1	HT ₁	HT ₂	HT ₃	DBH ₃	MV	PC2	SURV ₁	SURV ₂	SURV ₃	MV	PC2	SURV ₁	SURV ₂	SURV ₃	MV	
AHM	AHM	X	X	X	X	X	X																	
Dissection	Dissection	X	X	X	X	X	X																	
EMT	EMT	X	X	X	X	X	X																	
Eref _{AT}	Eref _{AT}	X	X	X	X	X	X																	
MAT	MAT																							
MCMT	MCMT																							
Mean_SP	Mean_SP																							
MWMT	MWMT	X	X	X	X	X	X																	
NFFD	NFFD																							
NFFD _{AT}	NFFD _{AT}	X	X	X	X	X	X																	
NFFD _{SP}	NFFD _{SP}	X	X	X	X	X	X																	
NFFD _{SP}	NFFD _{SP}	X	X	X	X	X	X																	
NFFD _{WT}	NFFD _{WT}	X	X	X	X	X	X																	
PAS	PAS	X	X	X	X	X	X																	
PPT _{SP}	PPT _{SP}																							
Tave _{AT}	Tave _{AT}																							
Tave _{SP}	Tave _{SP}	X	X	X	X	X	X																	
TD	TD	X	X	X	X	X	X																	
Total # in model	Total # in model	9	14	10	14	11	10	0	6	5	11	0	10	10	7	5	9	9	0	0	0	0	0	0

- NFFD verses all seasonal NFFD variables were compared
- TaveSP was compared to MAT
- EMT was compared to MCMT
- TD was added to the model
- Eref_{AT} was compared to Eref

AHM + Dissection + EMT + Eref + MWMT + NFFD + PAS + MAT + TD

Random forest analyses

Table 4. Performance of the final model and best model for key growth and survival traits. The final model included the same 9 variables for each trait, whereas the best model included 0 to 16 variables specific to each trait.

Model statistic	Within programs					Across programs				
	HT ₃	DBH ₃	VOL ₃	SURV ₃	VOL ₃ /Ha	HT ₃	DBH ₃	VOL ₃	SURV ₃	VOL ₃ /Ha
N	222	246	207	278	194	222	246	207	278	194
Mean	59.1	7.9	3.5	87	4.9	59.1	7.9	3.5	87	4.9
<i>RF best</i>										
No. of variables	16	9	6	6	7	14	11	10	9	12
RMSE	7.20	1.10	1.08	0.08	1.65	9.50	1.56	1.55	0.10	2.33
RMSE (%)	0.12	0.14	0.31	0.00	0.34	0.16	0.20	0.44	0.00	0.48
Pseudo-R ²	0.29	0.32	0.29	0.17	0.19	0.41	0.36	0.33	0.22	0.37
<i>RF final</i>										
No. of variables	9	9	9	9	9	9	9	9	9	9
RMSE	7.27	1.15	1.15	0.08	1.71	10.01	1.61	1.61	0.10	2.40
RMSE (%)	0.12	0.15	0.33	0.00	0.35	0.17	0.20	0.46	0.00	0.49
Pseudo-R ²	0.28	0.27	0.18	0.15	0.14	0.34	0.32	0.28	0.19	0.34

RMSE is root mean square error.
RMSE (%) is [RMSE/mean]*100.

Random forest analyses

The final RF models perform well for individual traits when compared to the respective best models

The final RF models perform well both across and within groups (i.e., regionally and locally)

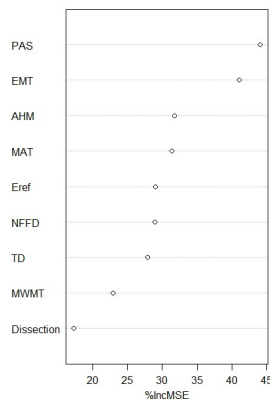
Random forest analyses – Final model

AHM + Dissection + EMT + Eref + MWMT + NFFD + PAS + MAT + TD

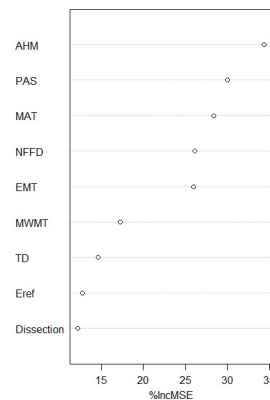
- Annual variables performed as well as seasonal variables (i.e. Eref_{AT} vs Eref)
- Interactions between temperature and precipitation are important (AHM and Eref)
- Temperature extremes are important (EMT and MWMT)
- Mean temp and growing season length are important (MAT, NFFD)
- PAS is important – water availability or temperature indicator?
- Terrain (Dissection) influences interaction of temp and precipitation

Random forest analyses

Across VI VOL₃/HA



Within VI VOL₃/HA



Productivity under future climates

ClimateWNA future climate predictions for three years

– 2025, 2055 and 2085

Ensemble climate models

– 15 different climate models combined

Two emissions scenarios

– RCP4.5 and RCP8.5

Predicted Vol₃/Ha at plantation and regional levels

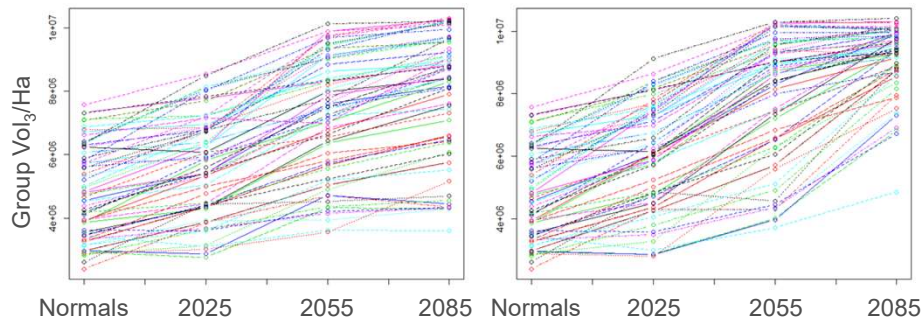
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Predicted Vol₃/Ha across groups

RCP4.5

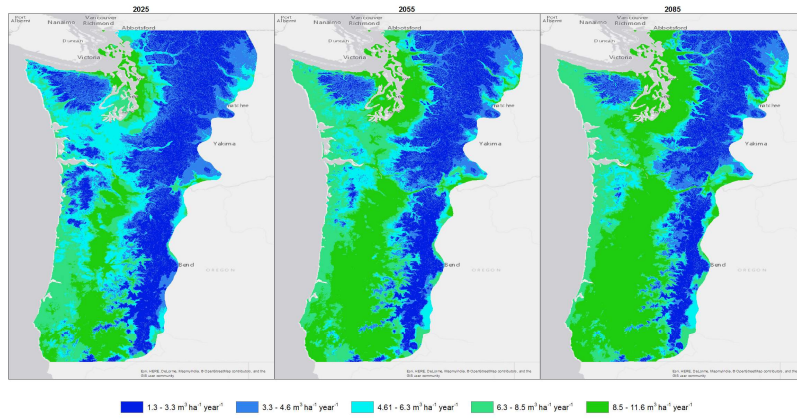
RCP8.5



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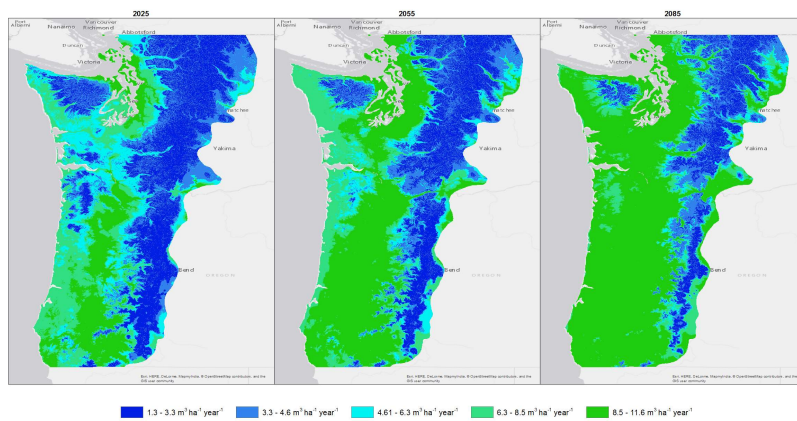
Regional Vol₃/Ha RCP4.5



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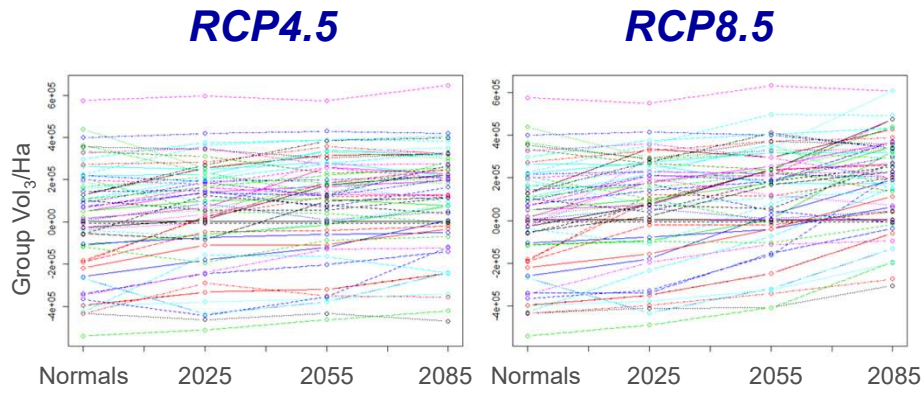
Regional Vol₃/Ha RCP8.5



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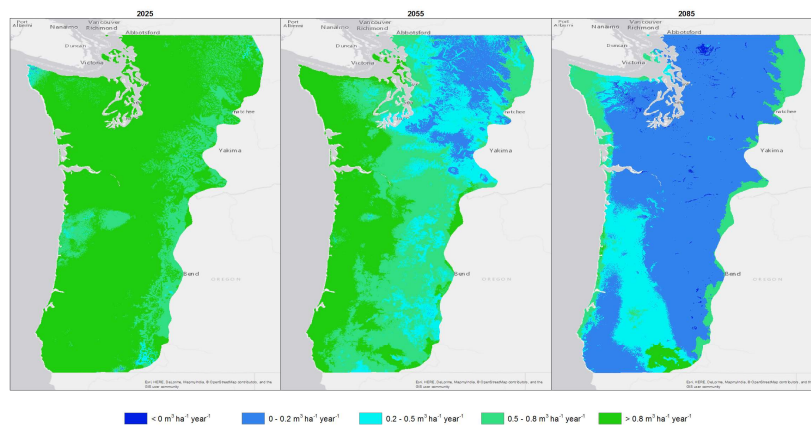
Predicted Vol₃/Ha within groups



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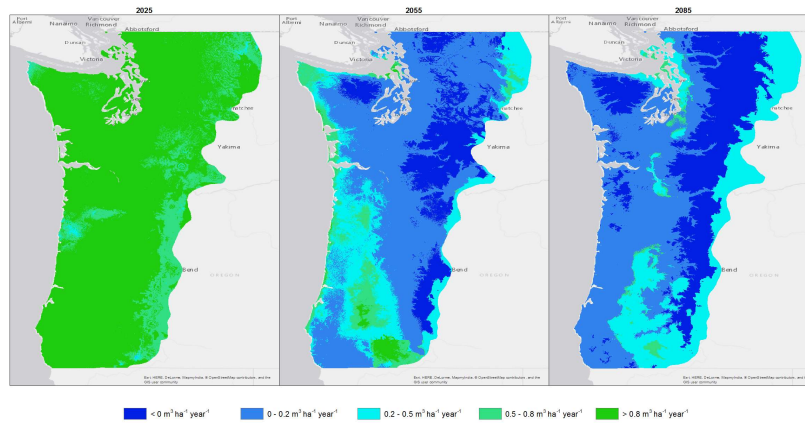
Regional Vol₃/Ha within RCP4.5



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Regional Vol₃/Ha within RCP8.5



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Conclusions

- Relationships between site characteristics and productivity are subtle for areas occupied by Douglas-fir plantations
- PAS and AHM are important for explaining variation in site productivity
- Models predict increases in productivity from climate change, but...
- Projections based on the across program analyses may be biased upward because of genetic differences among programs
- Projections assume small changes in precipitation

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Future work – Addressed in Five-Year Plan

Environmental transfer distances

- GxE
- Adaptability
- Seed transfer guidelines

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Thanks!

Acknowledgements

- Keith Jayawickrama, NWTIC, CAFS

References

- Sabatia, Charles O., and Harold E. Burkhart. "Predicting Site Index of Plantation Loblolly Pine from Biophysical Variables." *Forest Ecology and Management* 326 (August 15, 2014): 142-56. doi:10.1016/j.foreco.2014.04.019.



Next-generation SNP Chip for Douglas-fir

By Glenn Howe, Keith Jayawickrama, Scott Kolpak, Stephanie Guida, Sanjuro Jogdeo, Rich Cronn, and Callum Bell

The goal of this research is to develop procedures and technology for using Single Nucleotide Polymorphism (SNP) genetic markers to enhance existing operational tree improvement programs in Douglas-fir. This includes transitioning tree breeders from using previously developed sequence repeat markers (Slavov et al 2005) for routine breeding program management (e.g., identifying mislabeled genotypes) and pursuing advanced genomic techniques such as genomic selection. The key milestones are to develop and test (1) a low-cost, low-density genotyping array for Douglas-fir (~50 SNPs); and (2) a next-generation high-density (~50K SNPs) genotyping array for Douglas-fir.

We have completed SNP discovery using transcriptome sequencing (completed as part of PNWTIRC/CTGN research; Howe et al 2013). We have chosen the best set of SNPs to include on a low-density SNP genotyping array using the Sequenom genotyping platform. The Sequenom array is suitable for the routine breeding program management activities. We also developed two high-density genotyping arrays that will allow us to practice genomic selection in Douglas-fir. We first developed a high-density Illumina Infinium genotyping array and tested it on almost 2,000 trees (Howe et al 2013). The cost of automated SNP genotyping has declined because of advances in genotyping platforms and competition among service providers. Therefore, another high-density genotyping array (Affymetrix Axiom) is now significantly more affordable than the Illumina Infinium array. Thus, we included the best SNPs from previous genotyping efforts, along with new SNPs from additional transcriptome sequencing (Mueller et al 2012), into the Affymetrix Axiom genotyping array (completed as part of PNWTIRC/NARA research; collaborator Keith Jayawickrama). We have tested the Axiom Array on the genomic selection study trees from the NARA project (~1,900 trees). Future activities will include characterizing the SNPs on the Axiom array (i.e., minor allele frequency, observed heterozygosity, call rate, Hardy-Weinberg Equilibrium).

Next-generation SNP Chip for Douglas-fir

Glenn Howe
Keith Jayawickrama
Scott Kolpak
Stephanie Guida
Sanjuro Jogdeo
Rich Cronn
Callum Bell

Single nucleotide polymorphism (SNP)

	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*

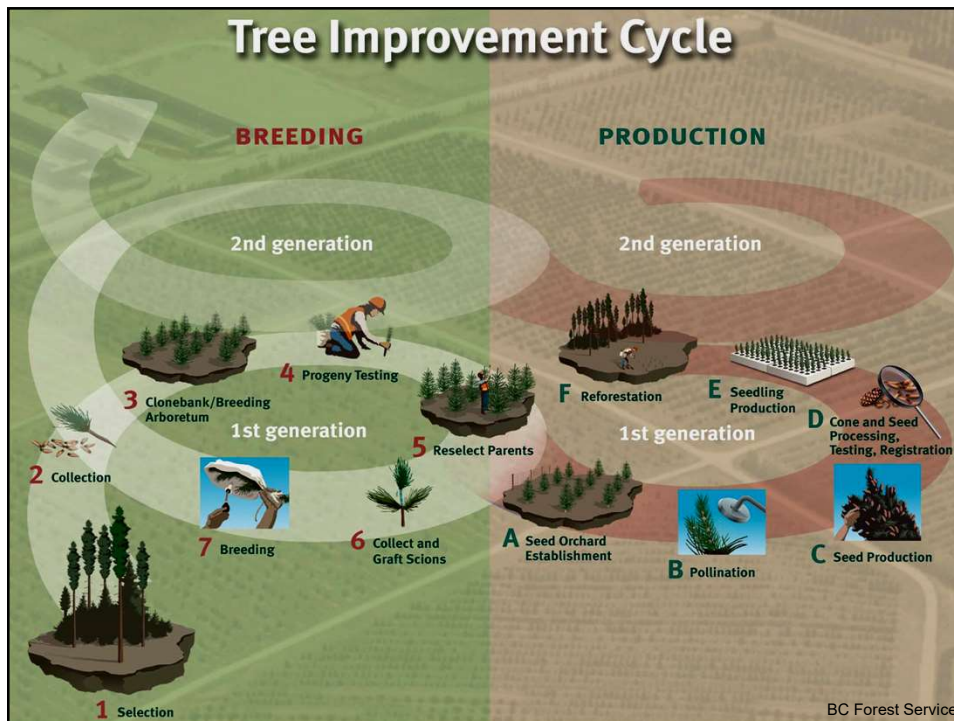
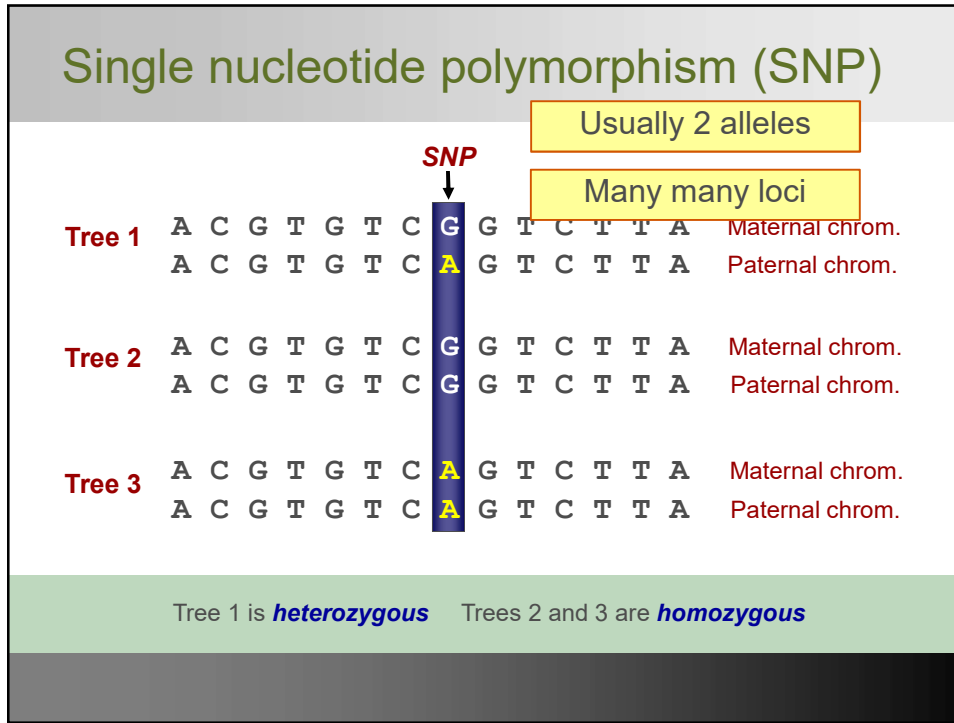


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What are SNPs?

What are they good for?



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)



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Douglas-fir SSR markers

Theor Appl Genet (2004) 108:873–880
DOI 10.1007/s00122-003-1490-y

ORIGINAL PAPER

G. T. Slavov · G. T. Howe · I. Yakovlev ·
K. J. Edwards · K. V. Krutovskii · G. A. Tuskan ·
J. E. Carlson · S. H. Strauss · W. T. Adams

**Highly variable SSR markers in Douglas-fir:
Mendelian inheritance and map locations**

1992

**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

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Transfer tested SNP markers to NFGEL



A Douglas-fir transcriptome

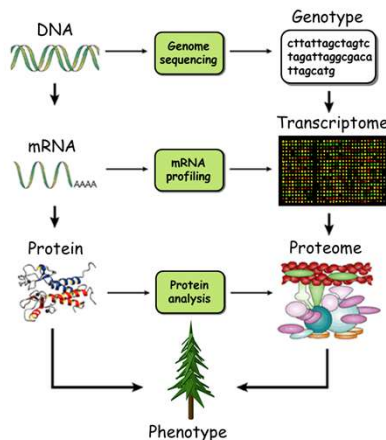
Conifer Translational Genomics Network

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**SNPs may be in genes (transcriptome)
or
not in genes (whole genome)**

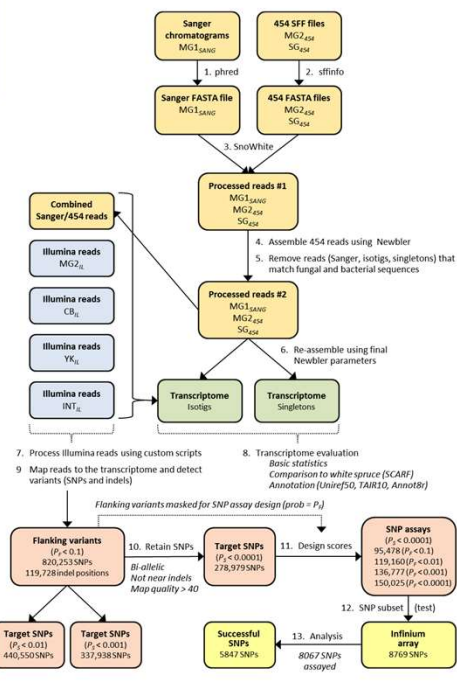
DNA → mRNA → Protein



SNP detection

Lots of bioinformatics

- Process raw sequences
- Assemble into a transcriptome
- Map sequences to the transcriptome
- Detect SNPs
- Design primers for SNP assays (SNP chip)
- Analyze resulting SNP data
- Lots of programming needed



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



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

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

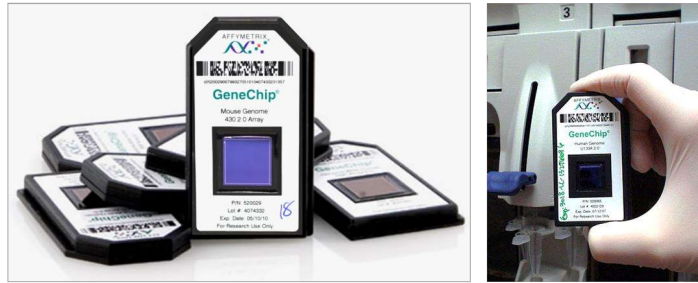
Low-cost / low-density genotyping arrays

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



Affymetrix Axiom array is cheaper


Large-scale genotyping service from GeneSeek



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Axiom Genotyping Array






Northwest Advanced Renewables Alliance

Keith Jayawickrama

Northwest Advanced Renewables Alliance

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Resources for genomic selection

Howe et al. *BMC Genomics* 2011, 14:1217
<http://www.biomedcentral.com/1471-2164/14/1217>

RESEARCH ARTICLE Open Access

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

Glenn T Howe^{1*}, Jianbin Yu^{2,3}, Brian Kraus⁴, Richard Cronn⁵, Scott Kolpak⁶, Peter Dodan⁷, W Walter Lorenz⁸ and Jeffrey FD Dean⁹

"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"

Müller et al. *BMC Genomics* 2012, 13:875
<http://www.biomedcentral.com/1471-2164/13/875>


RESEARCH ARTICLE Open Access

A catalogue of putative unique transcripts from Douglas-fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings

Thomas Müller¹, Ingo Ensminger^{2,3*} and Karl J Schmid⁴

"A total number of 187,653 single nucleotide polymorphisms (SNPs) were detected by three SNP detection tools"

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Design array with Affymetrix

Designing arrays for your markers

- Select Gene, Region, Sequence, SNP type
- Provide information on species and SNP list

Axiom® BioF_x Services
~ 3-5 days, initial report

Design Report

Evaluate

Finalize content and design array

Confirm order with Affymetrix

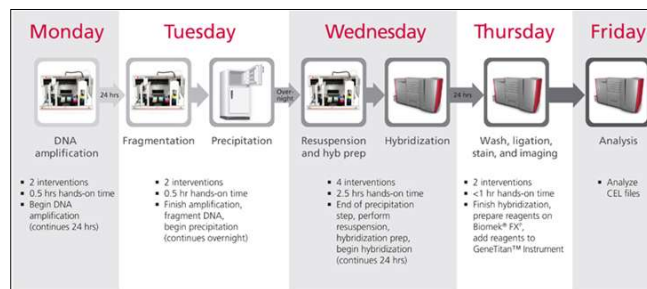
Consultation with Affymetrix' bioinformatics to add/modify markers if necessary

- Start your study in as few as 6 weeks after finalizing array content
- Use in-silico design scores to maximize the number of markers that will genotype for your species
- Develop an array for your consortium with Affymetrix' Community Array Program

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Genotyping by GeneSeek



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Axiom 96-format and 384-format Arrays

SNP discovery
Whole-genome sequencing

SNP validation
650,000–2 million markers

High-volume genotyping
500–650,000 markers

Cattle

Farm animals

Aquaculture

Plants

- **Selection of markers using *insilico* design**

- **Validate discovery across multiple samples**

- **Sub-select makers**
- **Add new markers**
- **Select markers from multiple breeds**

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Axiom Analysis Suite

Simple, Easy, Integrated

Simplified workflow

Advanced visualization features

Designed to handle large data sets

Load data

→

→

Get genotype results

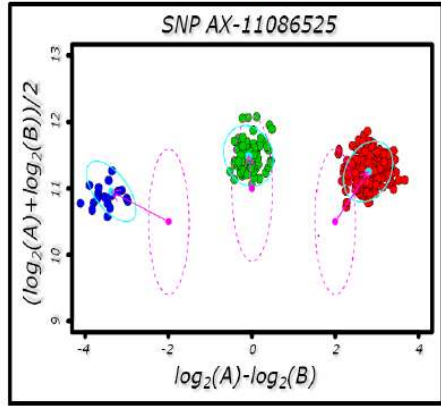
Simple, Easy, Integrated

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Axiom GT1 Genotype Calling Algorithm

Axiom GT1 Adapt to variable three cluster patterns



A and B are summarized intensities produced by A and B alleles in sample

- Axiom GT1 uses a Bayesian procedure
1. Combine a prior for that SNP (dashed oval)
 2. with the data observed (points)
 3. to obtain a posterior estimate of cluster centers (cyan ovals)
 4. The posterior estimate is what is then used to call genotypes*.

Blue=BB

Green= Het,

Red=AA

Grey=NoCalls

= Location of the genotype cluster determined by the Axiom GT algorithm

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Affymetrix Axiom Analysis Suite

affymetrix Axiom Analysis Suite - QC1

Mode: Best Practices Workflow Array Type: Axiom_OSU_DF1L1

CEL Files: 1920

File Name: 1.A01_425, 1.A02_424, 1.A03_423, 1.A04_421, 1.A05_422, 1.A06_476, 1.A07_475, 1.A08_474, 1.A09_512, 1.A10_472, 1.A11_471, 1.A12_473, 1.B01_470, 1.B02_457, 1.B03_518, 1.B04_520, 1.B05_461, 1.B06_515, 1.B07_522, 1.B08_514, 1.B09_378, 1.B10_19, 1.B11_519, 1.B12_521, 1.C01_535, 1.C02_527, 1.C03_530

Analysis Settings

Select Analysis Configurations: Axiom_OSU_DF1L1 (Default)

Sample QC

Analysis File: Axiom_OSU_DF1L1apt-axiom-genotype.AxiomGT1apt2

Prior Model File: Axiom_OSU_DF1L1.genetic_prior

SNP List File: Axiom_OSU_DF1L1.step1

Gender File (optional):

Hints/Inbred File (optional): Inbred Hints

Genotyping

Analysis File: Axiom_OSU_DF1L1apt-axiom-genotype.AxiomGT1apt2

Prior Model File: Axiom_OSU_DF1L1.genetic_prior

SNP List File:

Gender File (optional):

Hints/Inbred File (optional):

Threshold Settings

Select Threshold Configurations: QC2

Sample QC

Name	Settings
DQC	0.5
QC call rate	98
Average call rate for pass...	98

SNP QC

Name	Settings
species-type	Diploid
ci-cutoff	97
fid-cutoff	3.6
het-so-cutoff	-0.1
het-so-ov-cutoff	-0.2
hom-ro-1-cutoff	0.6
hom-ro-2-cutoff	0.3
hom-ro-3-cutoff	-0.9
hom-ro	true

Output Folder: T:\Group\PNWTIRC\AF\AUSF\DRAPFY_20150730\QC2_Crom_20150730

Batch Name: Run Analysis

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Analytical Procedures Needed for Conifers



OSU_DouglasFir

Advanced Filters

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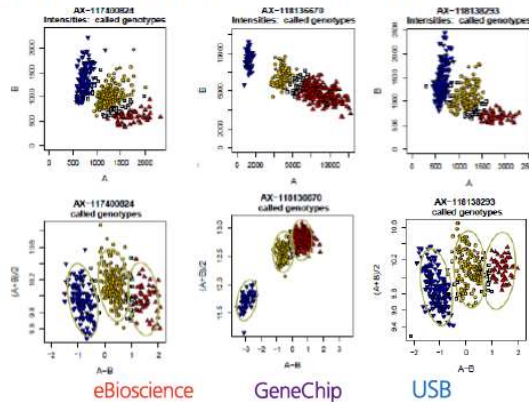
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Results – 3 cluster rescue

- Performing the probeset rescue operation on the combined 'Other' and 'CallRateBelowThreshold' probesets results in **5802** probesets passing the advanced filter thresholds.
 - These probesets are lower resolution (clusters are closer together)
 - SNPs are still likely polymorphic and probesets are providing accurate calls
 - Must use Ps_CallAdjust-ed calls table to increase stringency on call confidence



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Example R code (You will need to change paths) 1 of 3

```
## read in Ps.performance.txt table from default Best Practice Workflow
perf <- read.table("../results/step2/SNPolisher/Ps.performance.txt", sep="t", header=T, stringsAsFactors=F)

## Create combined PS list with Other and CRBT
perf.other <- perf[perf$ConversionType == "Other",]
perf.crbt <- perf[perf$ConversionType == "CallRateBelowThreshold",]
ps.other.crbt <- append(perf.other[,1], perf.crbt[,1])

write.table(ps.other.crbt, "../Final_Workflow/other_crbt.ps", sep="t", quote=F, row.names=F, col.names="probeset_id")

## Execute Ps_CallAdjust and Ps_Metrics

library("SNPolisher")

Ps_CallAdjust(
  pidFile="../Final_Workflow/other_crbt.ps",
  callFile="../results/step2/AxiomGT1.calls.txt",
  confidenceFile="../results/step2/AxiomGT1.confidences.txt",
  threshold=0.1,
  outputFile="../Final_Workflow/CallAdjust_0.1_other_crbt.txt"
)

Ps_Metrics(
  pidFile="../Final_Workflow/other_crbt.ps",
  posteriorFile="../results/step2/AxiomGT1.snp-posteriors.txt",
  callFile="../Final_Workflow/CallAdjust_0.1_other_crbt.txt",
  output.metricsFile="../Final_Workflow/metrics_CallAdjust_0.1_other_crbt.txt"
)
```

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Axiom array – Numbers of SNPs

SNP classification	Call rate = 80%; Dish QC = 0.5	
	Phase 1	Phase 2
Polymorphic high resolution	16,673	16,673
No minor homozygote	9,702	9,702
Monomorphic high resolution	5,485	5,485
Rescued	–	4,978
Converted	31,860	36,838
Off-target variant	1,296	1,296
Other	19,154	16,933
Call rate below threshold	3,456	699
Not converted	23,906	18,928
Total	55,766	55,776

Axiom array – SNP characteristics (n = 164)

55,766 SNPs attempted

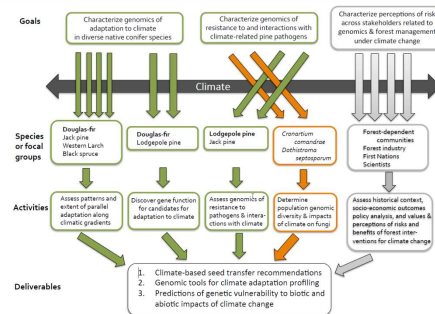
21,651 SNPs polymorphic and ‘called’

21,010 SNPs = polymorphic, ‘called’, HWE

Statistic	Mean	Median	Min	Max
Call rate (%)	0.962	0.988	0.689	1.000
Polymorphic information content	0.261	0.285	0.006	0.375
Heterozygosity	0.324	0.342	0.006	0.702
Minor allele frequency	0.236	0.220	0.003	0.500

Potential collaborations

Genome Canada LSARP Proposal: CoAdapTree: Healthy Trees for New Climates
 Project Leaders: Sally Aitken, UBC; Sam Yeaman, University of Calgary;
 and Richard Hamelin, Laval University and UBC



Multi-species genotyping arrays

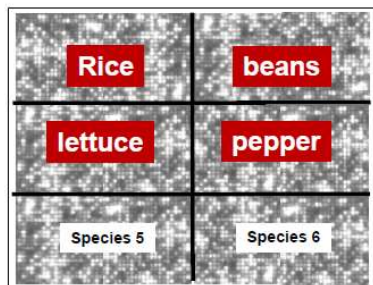
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Multi-species arrays

Single platform for all species

- A technology that can genotype multiple species.



Solution: Multi-species arrays

- Axiom platform enable multi-species arrays
 - Control of feature location allows species-specific array segmenting
 - Species-specific genotyping analysis enabled through separate analysis file
- Advantages :
 - Consolidated inventory management
 - Faster time to result & low sample batching



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Third-generation SNP chip

Lower the costs of SNP genotyping

- Only include successful SNPs on the array (20K instead of 50K)
- Use lower-cost, low-density SNP arrays
 - *Sequenom*
 - *Smaller Axiom arrays*
- Combine low-density and high-density arrays for genomic selection

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Validation of SNP data for genomic selection in Douglas-fir

By Jennifer Kling

In traditional breeding programs, the importance of maintaining accurate records of pedigrees, trees, and phenotypes is well understood. For genomic selection, we also need to ensure that molecular marker data are correctly assigned to trees and phenotypes. In this presentation, we discussed potential errors that can occur, and described the process we used to validate and curate genomic selection data. We isolated DNA and genotyped 1920 trees using the Affymetrix Axiom Array. Three generations of trees were represented, including parents from seed orchards, second-cycle full-sib progeny, and third-cycle seedlings. We calculated the 'A' matrix, which consists of the expected genetic relationships among the 1920 trees based on recorded pedigrees. Data from ~19,822 SNPs were used to create a 'G' matrix of observed genetic relationships, each of which estimates the proportion of DNA shared by the corresponding pair of trees. The observed and expected relationships were compared and large deviations were flagged as potential errors. We then used several approaches to resolve errors. We developed an R program to identify the correct parents of each sample and a SAS program to compare the observed and expected relationships. We corrected systematic errors where a probable cause could be identified; e.g., a shift in data on an Excel spreadsheet, a switch in adjacent samples on an Axiom plate, or a mistake in pedigree records. Data validation began with the parent generation and proceeded sequentially through subsequent generations. We recalculated the A and G matrices after each round of corrections and additional errors were resolved in an iterative manner. We stored the original and corrected genotype identifiers in the database and recorded the type of error. Using this procedure, we corrected most of the discrepancies, resulting in a final correlation between the A and G matrices of 0.96. Unresolved samples were omitted from subsequent genomic selection analyses.

Validation of SNP Data for Genomic Selection in Douglas-fir

**Jennifer Kling, Matt Trappe, Scott Kolpak,
Terrance Ye, Keith Jayawickrama, Glenn Howe**

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

**PNWTIRC Annual Meeting
October 19, 2016**

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Collaborative project

Key funding

PNWTIRC
Conifer Translational Genomics Network (AFRI)
Northwest Advanced Renewables Alliance (AFRI)

Key roles

SNP discovery (PNWTIRC)
SNP chip design (PNWTIRC)
Population design (NARA)
Foliage collection and DNA isolation (NARA)
SNP chip manufacture and genotyping (NARA)
SNP data processing (PNWTIRC)
Genomic selection analyses (PNWTIRC/NARA)

Personnel

PNWTIRC
Glenn Howe
Scott Kolpak
Jennifer Kling
NARA
Keith Jayawickrama
Terrance Ye
Matt Trappe

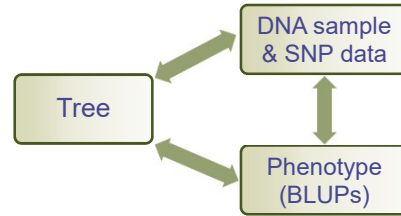
NARA

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Outline of presentation

- Potential sources of error
 - *Experimental material*
 - *Needle collection*
 - *DNA extraction*
 - *Axiom Array → → SNP data*
- Data validation process
 - *Strategy*
 - *Error detection and correction*
 - *Data archiving*
- Benefits of data validation



Future Workshop

Guidelines for data collection and management for genomic selection

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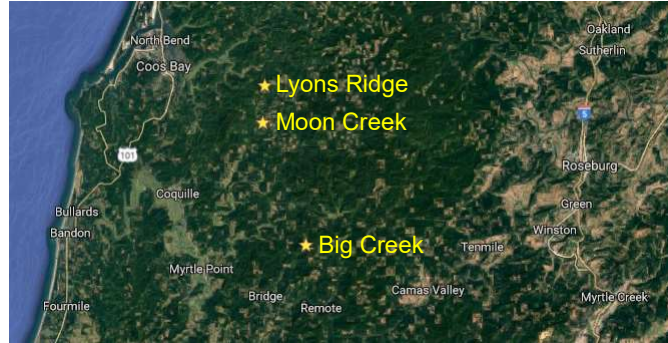
Seed orchards

Orchard	Number of samples
CTC David T. Mason Seed Orchard	102
Roseburg Forest Products Seed Orchard - Lebanon	61
BLM Tyrrell Seed Orchard	6
Plum Creek Seed Orchard	33

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Progeny plantations



Plantation	Samples
Moon Creek	293
Lyons Ridge	208
Big Creek	55

Greenhouse and field test

- Plum Creek greenhouse
- 25 full-sib families
- 1146 trees
- Planted on Roseburg Resources Property near Elkton, Oregon in March, 2015



Photos from Matt Trappe

Needle collection

- Collect 5-10 fresh needles
- Place in vial with desiccant
- 1920 samples were used for genotyping

Potential Errors

- *Crossing errors*
- *Records of identity not accurate*
- *Collect from the wrong tree*



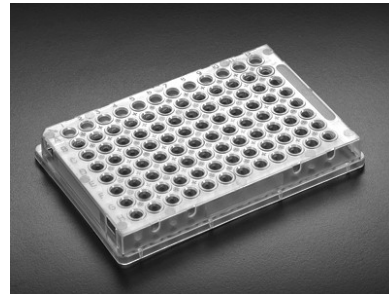
Photo from Matt Trappe

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DNA extraction

- Dry needle samples minced
- Placed in a 96 well plate
- Records of location on the plates maintained in Excel
- Samples sent to the NFGEL Laboratory in Placerville, CA for extraction (Dr. Valerie Hipkins)
- Minimum concentration of 10ng/μl DNA required for genotyping



Potential Errors

- *Samples placed in the wrong well*
- *Contamination of sample or DNA*
- *Need to re-extract some samples leads to errors in record-keeping*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Affymetrix Axiom Array

Large-scale genotyping service from GeneSeek

- High throughput (4x96=384 wells)
- Lower cost per sample

Potential Errors

- DNA samples not properly labelled
- No backup of DNA samples
- Inaccurate records of plate number or well location



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Data validation strategy

- Verify “chain of custody”
field → DNA extraction → genotyping
- Calculate expected relationships based on pedigrees (A matrix)
- Compare to observed relationships (G matrix) based on SNP data
- Start with the parents and work sequentially through subsequent generations
- Make corrections in an iterative manner

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A matrix from pedigrees

Pedigree file

Geno_ID	female	male
7975	0	0
7978	7977	7947
8049	0	0
572309-4777	7978	7975
572309-4778	7978	7975
572330-4813	8049	7975

A matrix

	7975	7978	8049	572309-4777	572309-4778	572330-4813
7975	1	0	0	0.5	0.5	0.5
7978	0	1	0	0.5	0.5	0
8049	0	0	1	0	0	0.5
572309-4777	0.5	0.5	0	1	0.5	0.25
572309-4778	0.5	0.5	0	0.5	1	0.25
572330-4813	0.5	0	0.5	0.25	0.25	1

$$DEV = G_{ij} - A_{ij}$$

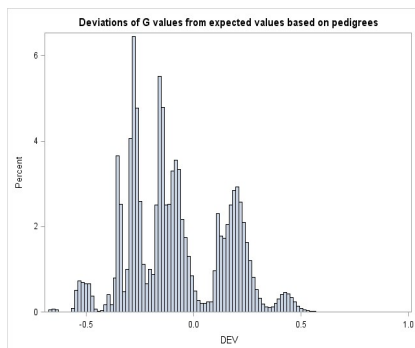
G matrix

	7975	7978	8049	572309-4777	572309-4778	572330-4813
7975	0.890			0.388	0.419	0.411
7978		0.963		0.417	0.442	
8049			0.974			0.467
572309-4777	0.388	0.417		0.913	0.508	0.252
572309-4778	0.419	0.442		0.508	0.960	0.209
572330-4813	0.411		0.467	0.252	0.209	0.936

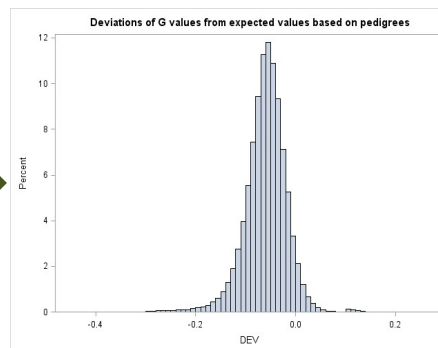
Comparison of A vs G matrices

$$DEV = G_{ij} - A_{ij}$$

BEFORE



AFTER



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Tools for identifying errors

“G Program” (Howe and Kolpak)

- Uses a parent testing approach
 - *Are the parents correct?*
 - *If not, who are the parents?*
- Provides a concise list of probable errors
- Easy to identify pedigree errors for family groups
- Multiple generations and different crossing systems in this study
 - *Not always able to distinguish parents from full-sibs*
- Software can be provided by PNWTIRC for implementation

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Tools for identifying errors

Visual Inspection

Geno_ID	female	male	A value	G value	Geno_ID_col	female_col	male_col
572330-942	13732	13720	1	0.931	572330-942	13732	13720
572330-942	13732	13720	0.5	0.496	572309-940	13732	13720
572330-942	13732	13720	0.5	0.48	41444	13732	13720
572330-942	13732	13720	0.5	0.471	572309-943	13732	13720
572330-942	13732	13720	0.5	0.449	13720		
572330-942	13732	13720	0.5	0.437	572330-933	13732	13720
572330-942	13732	13720	0.5	0.434	13732		
572330-942	13732	13720	0.5	0.424	572309-955	13732	13720
572330-942	13732	13720	0.5	0.417	572309-951	13732	13720
572330-942	13732	13720	0.25	0.253	573370-2044	41444	41466
572330-942	13732	13720	0.25	0.253	573370-2079	41444	41466
572330-942	13732	13720	0.25	0.252	573370-2043	41444	41466
572330-942	13732	13720	0.25	0.249	573370-2053	41444	41466

- Observed and expected relationships are similar

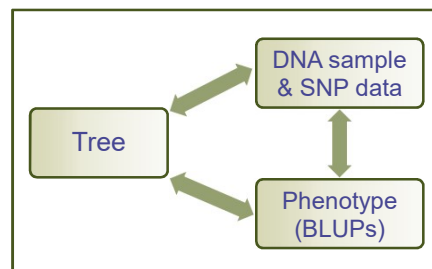
Tools for identifying errors

Geno_ID	female	male	A value	G value	Geno_ID_col	female_c	male_col
573370-1516	33342	33368	1	0.96	573370-1516	33342	33368
573370-1516	33342	33368	0.5	0.015	573370-1505	33342	33368
573370-1516	33342	33368	0.5	-0.015	33368	29934	15532
573370-1516	33342	33368	0.5	-0.016	573370-1496	33342	33368
573370-1516	33342	33368	0.5	-0.031	33342	29942	15382
573370-1516	33342	33368	0.5	-0.031	573370-1507	33342	33368
573370-1516	33342	33368	0.25	0.003	782209-1486	29942	15382
573370-1516	33342	33368	0.25	0	15382		
573370-1516	33342	33368	0.25	-0.006	782209-1483	29942	15382
573370-1516	33342	33368	0	0.492	573370-2190	33371	33379
573370-1516	33342	33368	0	0.485	573370-2153	33371	33379
573370-1516	33342	33368	0	0.456	33379	29924	33010
573370-1516	33342	33368	0	0.451	33371	15491	29872
573370-1516	33342	33368	0	0.449	573370-2167	33371	33379
573370-1516	33342	33368	0	0.264	29924		
573370-1516	33342	33368	0	0.253	782209-2291	29924	33010

- Observed and expected relationships do not match

Data correction and archiving

- Systematic errors can be corrected, such as ...
 - Entire family has the wrong pedigree – crossing error
 - “Shift” in Excel records follows a clear pattern
 - Adjacent samples on the Axiom plate are reversed
 - Plates are mislabeled
- Isolated errors
 - Cause cannot be determined
 - Genotype is lost for the purposes of genomic selection
- Database includes both the original and the corrected data for each sample



Benefits of genotyping and validation

- Correct associations between genotypes and phenotypes are necessary for effective genomic selection
- SNP genotyping helped to improve the accuracy of breeding records
 - *Pedigrees*
 - *Labeling of trees in seed orchards and progeny trials*
 - *Database accuracy*
- Accurate records permit more effective phenotypic selection
 - *Better BLUP estimation*
 - *Better choice of parents for breeding*
 - *Better selection of trees for commercial production*
- A workshop may be offered to highlight best practices for data collection and management for the purposes of genomic selection

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



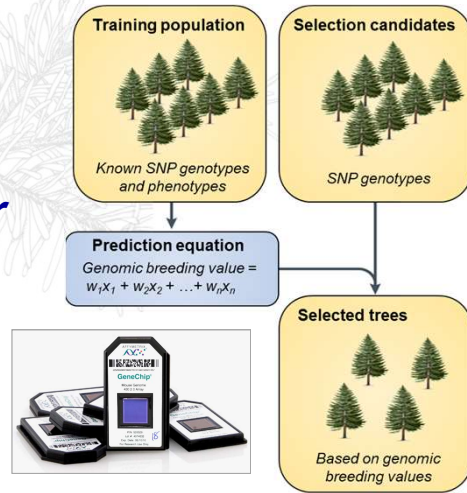
Genomic selection in Douglas-fir

By Glenn Howe

Genomic selection (GS), or whole-genome marker-assisted selection (Meuwissen *et al.*, 2001), could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing, and select for mature traits, such as wood properties at the seedling stage. The objective of GS is to predict breeding values using a genome-wide set of markers, typically tens of thousands of single nucleotide polymorphic markers (SNPs). Genomic selection involves two steps (Hayes and Goddard, 2010). First, a genomic prediction model is developed using phenotypes and marker genotypes measured on a test or 'training' population. Second, superior individuals are selected from a related breeding population based on marker genotypes alone. GS has been highly effective in livestock breeding, and is beginning to be used operationally on *Eucalyptus* in Brazil.

Genomic Selection in Douglas-fir

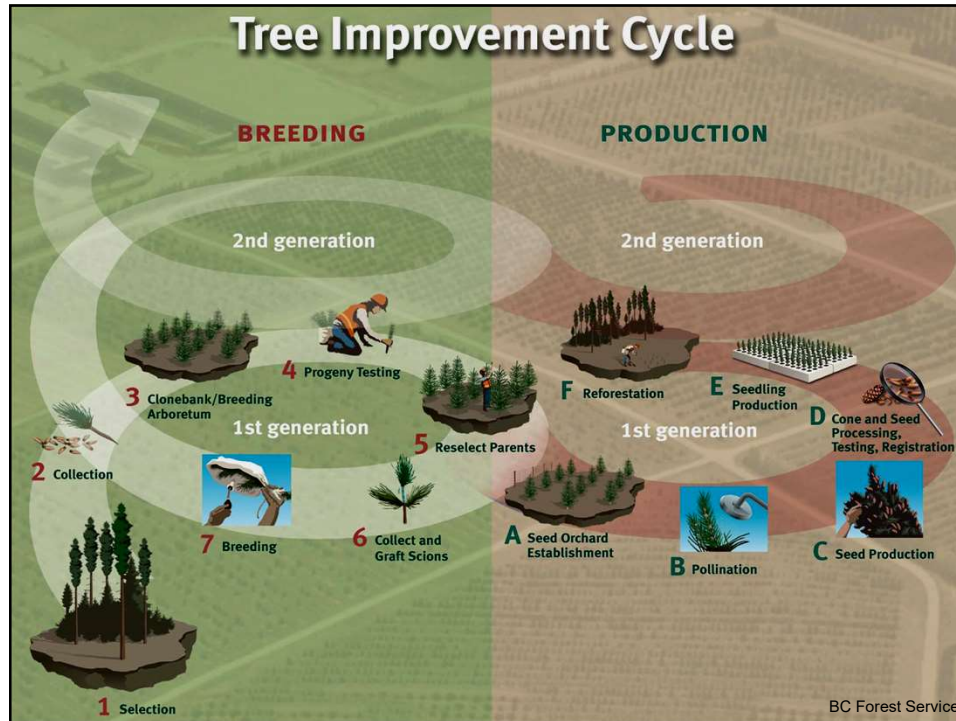
Glenn Howe
 Keith Jayawickrama
 Jennifer Kling
 Scott Kolpak
 Terrance Ye



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Genomic selection

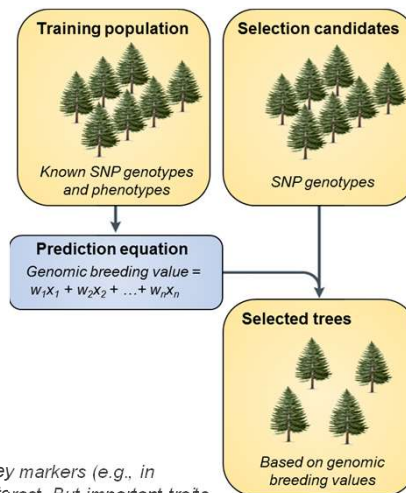


Genomic selection

More promising than association genetics¹

- Objective is to predict breeding values using a genome-wide set of markers (e.g., tens of thousands of SNPs)
- With enough markers, at least one marker will be linked to each important gene
- No need to identify which specific genes or markers are important
- Highly effective in livestock breeding

¹The objective of association genetics is to find a few key markers (e.g., in candidate genes) that are associated with the trait of interest. But important traits probably controlled by tens to hundreds of genes with small effects.



Genomic selection

Unlike candidate gene approaches, genomic selection markers will work for any measured trait

Growth

- Height, diameter, volume growth

Adaptability

- Cold hardiness
- Spring bud flush

Stem form

- Ramicorn branches and forks
- Sinuosity

Wood stiffness

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Livestock breeders have led the way

Technical Note: DNA Analysis

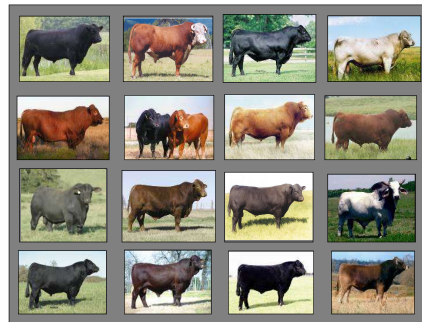


Genomic Selection—A Paradigm Shift in Animal Breeding

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.



By 9/2011, 86,744 young Holstein bulls and heifers had been evaluated through genomic selection without phenotypic testing

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Genotyped Holsteins

*Traditional evaluation
 **No traditional evaluation

Date	Bulls*	Cows*	Young animals**		
			Bulls	Heifers	All animals
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(7)

G.R. Wiggins



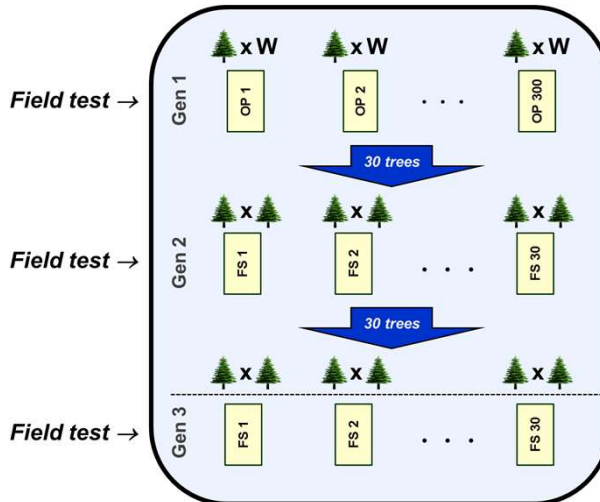
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



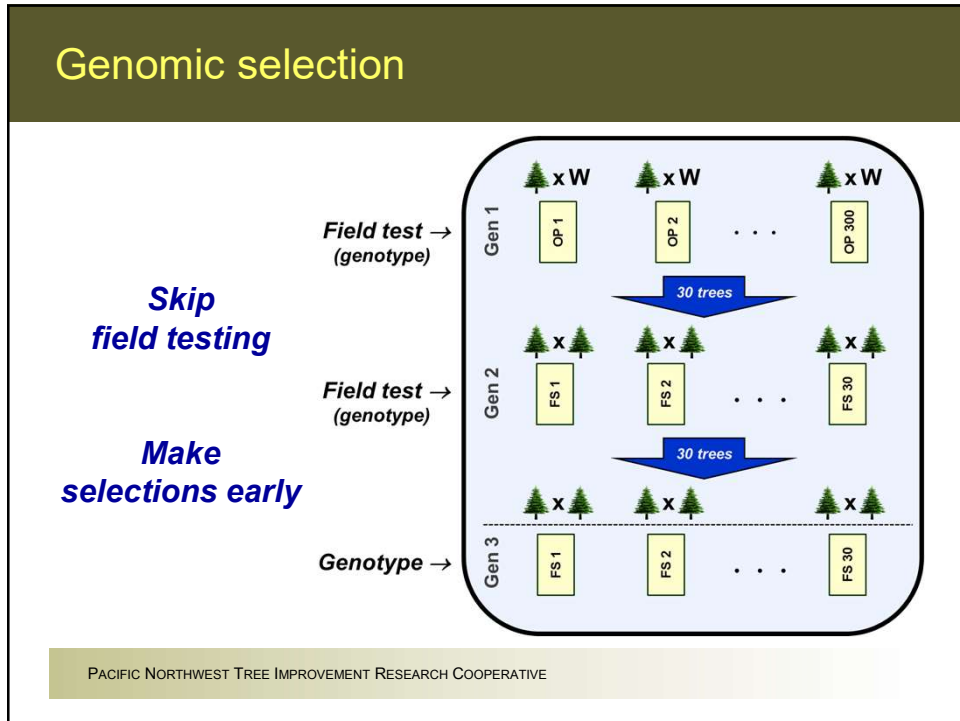
Genomic selection

Field tests are costly

Testing/selection takes a long time



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE




Genomic selection Valuable for within-family 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
- Genomic selection could be used instead

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



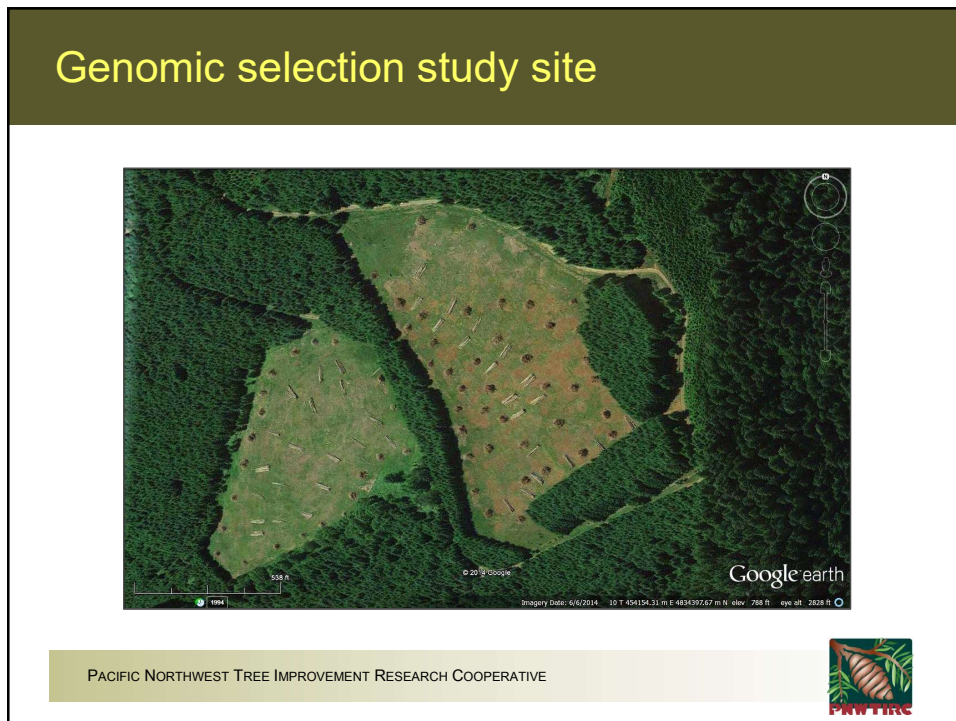
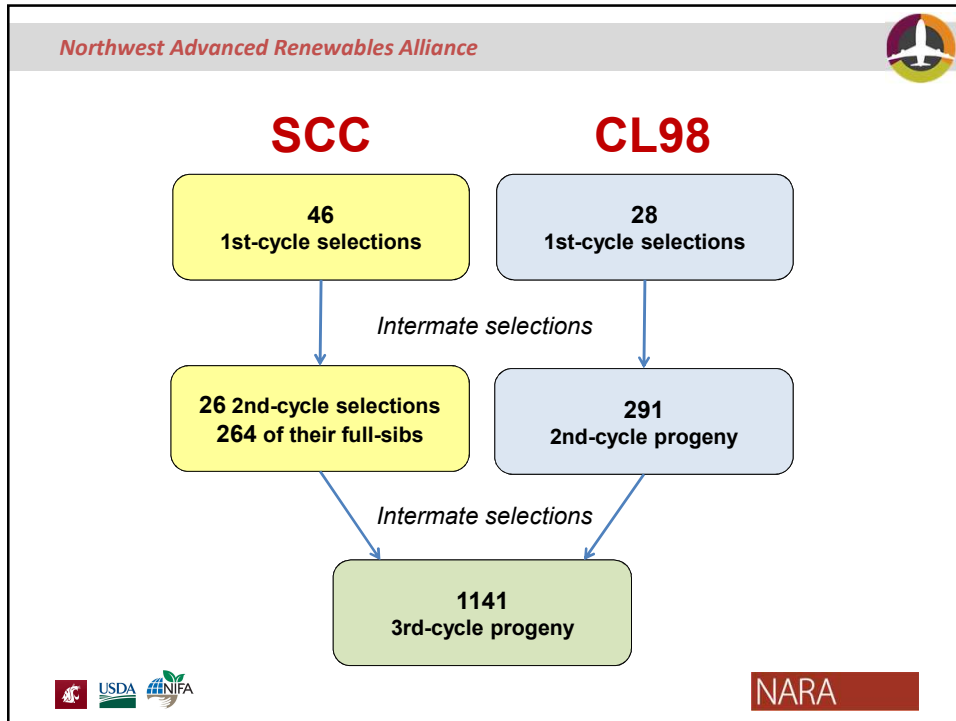
Potential advantages of genomic selection

- Skip entire cycles of field testing
- Reduce the size of field tests by using genomic selection for early culling
- Shorten the generation interval
- Select for mature traits at an early age

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Proof of Concept



Performance of genomic selection

Predictive ability is the correlation between breeding values estimated from phenotypes versus SNPs

Table 4. Performance of genomic selection in Douglas-fir. Predictive ability (PA) was calculated using rrBLUP and 22,458 SNP markers. PA is the correlation between breeding values estimated from phenotypic measurements versus SNP markers using 10-fold cross-validation.

Trait (age 12)	Predictive ability (PA)
Height	0.698
DBH	0.655
Volume	0.612
Ramicorn branching	0.874
Forking	0.887
Sinuosity	0.852
Specific gravity	0.632

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PNWTIRC Five-Year Plan

Next steps for genomic selection

- Eventually integrate the third-cycle trees into the genomic selection analyses
- Develop lower-cost approaches
 - *Optimize the number of SNPs*
 - *Optimize training population size*
 - *Combine low-density and high-density arrays*
- Integrate early testing?
- Integrate Breeding-without-breeding (BWB)?
- Test using simulation approaches plus current and new data

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Genomic selection Valuable for within-family selection

Many more trees per family in third cycle

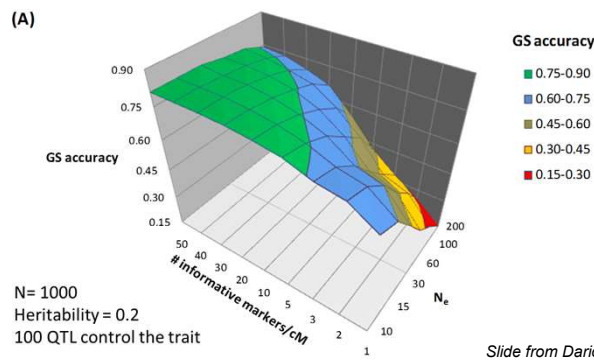


More rigorous test of genomic selection

internal ID	geno ID	plate	well	cell	female	male
41602	578370 1234	4	B01		41608	41624
41602	578370 1235	11	B04		41608	41624
41602	578370 1236	14	B12		41608	41624
41602	578370 1237	12	D05		41608	41624
41603	578370 1241	13	F04		41619	41617
41603	578370 1242	14	B02		41619	41617
41603	578370 1243	14	B11		41619	41617
41603	578370 1244	14	D09		41619	41617
41603	578370 1245	15	D02		41619	41617
41603	578370 1246	13	B07		41619	41617
41603	578370 1247	15	D09		41619	41617
41603	578370 1248	22	F02		41619	41617
41603	578370 1249	13	G04		41619	41617
41603	578370 1250	23	F10		41619	41617
41603	578370 1251	15	D06		41619	41617
41603	578370 1252	14	A08		41619	41617
41603	578370 1253	15	F07		41619	41617
41603	578370 1254	20	A12		41619	41617
41603	578370 1255	13	F11		41619	41617
41603	578370 1256	20	D05		41619	41617
41603	578370 1257	20	A09		41619	41617
41603	578370 1258	15	E11		41619	41617
41603	578370 1259	13	G01		41619	41617
41603	578370 1260	15	H12		41619	41617
41603	578370 1261	14	D07		41619	41617
41603	578370 1262	15	D09		41619	41617
41603	578370 1263	20	D09		41619	41617
41603	578370 1264	20	D05		41619	41617
41603	578370 1265	13	F06		41619	41617
41603	578370 1266	20	C11		41619	41617
41603	578370 1267	22	D1		41619	41617
41603	578370 1268	15	H04		41619	41617
41603	578370 1269	15	D06		41619	41617
41603	578370 1270	13	G03		41619	41617
41603	578370 1271	13	H03		41619	41617
41603	578370 1272	13	G10		41619	41617
41603	578370 1273	13	H04		41619	41617
41603	578370 1274	13	H02		41619	41617
41603	578370 1275	15	F01		41619	41617
41603	578370 1276	15	D01		41619	41617
41603	578370 1277	15	H10		41619	41617
41603	578370 1278	20	A09		41619	41617
41603	578370 1279	13	H08		41619	41617
41603	578370 1280	15	D05		41619	41617
41603	578370 1281	13	G07		41619	41617
41603	578370 1282	13	G06		41619	41617
41603	578370 1283	22	F1		41619	41617
41603	578370 1284	20	F07		41619	41617
41603	578370 1285	13	F03		41619	41617
41603	578370 1286	22	F1		41619	41617
41603	578370 1287	15	F03		41619	41617
41603	578370 1288	15	D04		41619	41617
41603	578370 1289	13	H05		41619	41617
41603	578370 1290	15	D04		41619	41617
41603	578370 1291	15	H03		41619	41617
41603	578370 1293	15	G07		41619	41617
41603	578370 1294	13	H09		41619	41617
41603	578370 1295	14	B07		41619	41617
41603	578370 1296	14	D05		41619	41617
41603	578370 1297	15	H08		41619	41617
41603	578370 1298	14	D06		41619	41617
41603	578370 1299	15	F07		41619	41617
41603	578370 1300	14	A06		41619	41617
41604	578370 1301	13	D06		41661	41666
41604	578370 1302	14	H01		41661	41666
41604	578370 1303	15	G01		41661	41666

Genomic selection - Next steps

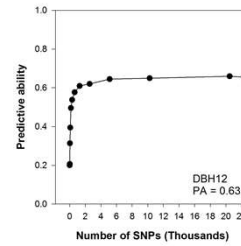
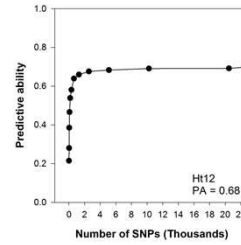
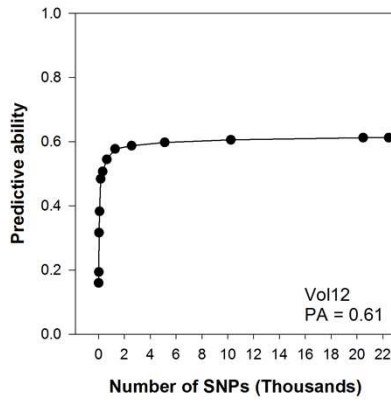
Optimize the number of SNPs
Optimize the training population size



Slide from Dario Grattapaglia



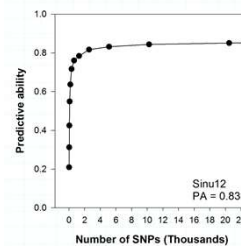
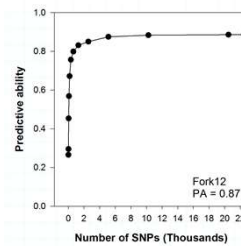
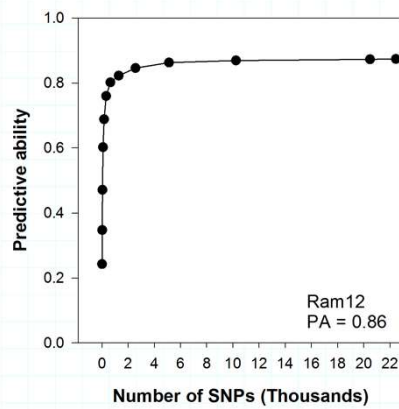
Optimize the number of SNPs



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Optimize the number of SNPs

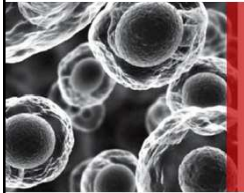


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Genomic selection - Next steps

Use low-density and high-density SNP genotyping arrays



Affymetrix

Wellmann et al. *Genetics Selection Evolution* 2013, **45**:28
<http://www.gsejournal.org/content/45/1/28>



RESEARCH

Open Access

Genomic selection using low density marker panels with application to a sire line in pigs

Robin Wellmann^{1*}, Siegfried Preuß¹, Ernst Tholen², Jörg Heinkel³, Klaus Wimmers⁴ and Jörn Bennewitz²

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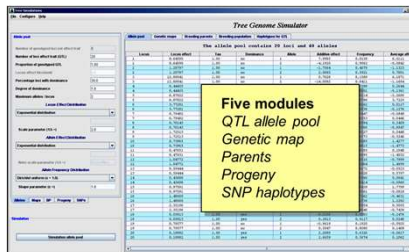


Genomic selection - next steps

Breeding program simulations

Optimize sampling design for SNP genotyping

- Number of SNPs
- Breeding population size
- OP mating designs
- Combine with early testing



Five modules
 QTL allele pool
 Genetic map
 Parents
 Progeny
 SNP haplotypes

Update to account for the structure of NWTIC breeding programs

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Genomic selection - Next steps

Collaborators

Potential collaborators for economies of scale...

PACIFIC NORTHWEST TREE IMPROVEMENT
RESEARCH COOPERATIVE



Genome Canada LSARP Proposal: CoAdapTree: Healthy Trees for New Climates

Project Leaders: Sally Aitken, UBC; Sam Yeaman, University of Calgary;
and Richard Hamelin, Laval University and UBC



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Genomic selection - Next steps

Third-generation SNP chip

Lower the costs of SNP genotyping

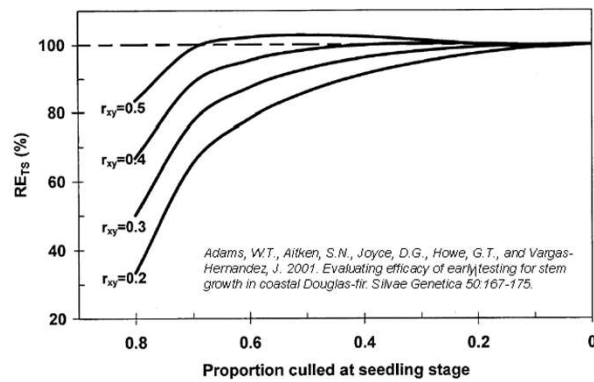
- Only include successful SNPs on the array (20K instead of 50K)
- Use lower-cost, low-density SNP arrays
 - *Sequenom*
 - *Smaller Axiom arrays*
- Combine low-density and high-density arrays for genomic selection

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Next steps - Early selection (early culling)

Early culling may be valuable, even when J-M correlations are low



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Breeding without breeding

Open-pollinated mating designs

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

Approach

- Field test open-pollinated seed from seed orchard parents
- Use SNPs to fingerprint the individuals in the OP families to identify their fathers

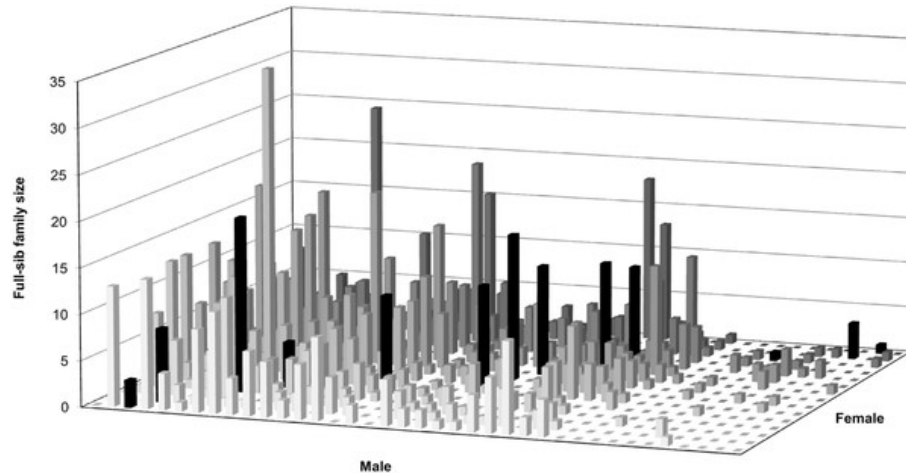
Potential benefits

- Saves time and the cost of controlled crossing, and may increase gain by increasing the number of full-sib families tested
- Downsides are unequal mating in the orchard, increase in N_e , and costs of SNP genotyping

*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.

26

Figure 1. Pedigree reconstruction results showing the formation of full-sib families nested within the maternal and paternal half-sib families (black bars represent selfing).



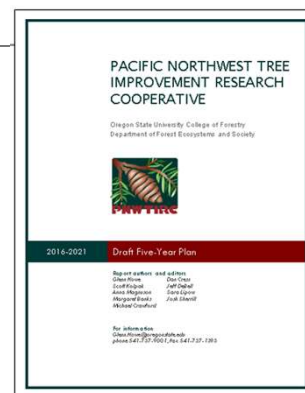
El-Kassaby YA, Cappa EP, Liewlaksaneeyanawin C, Klápště J, et al. (2011) Breeding without Breeding: Is a Complete Pedigree Necessary for Efficient Breeding?. PLoS ONE 6(10): e25737. doi:10.1371/journal.pone.0025737



Five-Year Plan Genomic selection work plan

Objectives

1. Evaluate genomic selection in Douglas-fir breeding programs using recently acquired SNP data and previously measured phenotypes.
2. Reduce the costs of genomic selection by optimizing the number of SNPs and training population size
3. Test whether early testing can be used to increase the efficiency of genomic selection
4. Test whether breeding-without-breeding can be used to increase the efficiency of genomic selection.
5. Develop a new, lower-cost SNP genotyping array for Douglas-fir
6. Conduct genomic selection workshops and other training for PNWTIRC members.



Draft Five Year Plan

By Glenn Howe

Glenn Howe led a discussion of the PNWTIRC Five-year plan to guide future cooperative activities. A Five-Year Plan subcommittee was formed last year and met occasionally to help shape the Draft Five-year plan. Topics included an overview of PNWTIRC organization and current membership, PNWTIRC research projects (core, other, future), technology transfer (e.g., workshops), and a discussion of past and current trends in PNWTIRC budgets.

PNWTIRC 'core' research projects include those that are largely or partially funded by PNWTIRC dues. Currently, these include research on (1) development of SNP markers for Douglas-fir, (2) genomic selection in Douglas-fir, (3) Douglas-fir site characterization, and (4) the genetics of Douglas-fir drought hardiness. Research projects that are solely funded by external grants and agreements are considered 'other' research projects. Current projects in this category include (1) genomic selection for Douglas-fir tree improvement; (2) development of genetic markers for western white pine and Douglas-fir; (3) meta-analysis of Douglas-fir provenance tests; (4) assisted migration; (5) Seedlot Selection Tool; and (6) genetic markers for western white pine. Potential future topics include: breeding-without-breeding, seed zones and breeding zones for future climates, economic weights for genetic selection, and a new facilitated research model.

In addition to discussing research activities, potential up-coming workshops topics were discussed including, (1) how to implement genomic selection in Douglas-fir breeding programs, and (2) consideration of climate change in tree breeding programs. Finally, trends in past and current cooperative funding were presented and a discussion was held on increasing dues to meet PNWTIRC budget short-falls that have been previously augmented with external funds.

Five-Year Plan

Glenn Howe

*PNWTIRC
Dept Forest Ecosystems and Society
Oregon State University*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Plans for 2016-2017

PNWTIRC Five-Year Plan activities (after PNWTIRC annual meeting)

Activity	Deliverable	Target date
Five-Year Plan survey	PNWTIRC report on survey results	Nov 18, 2016
Dues increase	Vote on dues increase	Dec 31, 2016
Affymetrix Axiom array	PNWTIRC report	Dec 31, 2016
Douglas-fir site characterization	PNWTIRC report	Dec 31, 2016
Genomic selection work plan	Approved work plan	Dec 31, 2016
Five-Year Plan	Approved plan	Dec 31, 2016
Drought hardiness study	Master's thesis	Mar 15, 2017
Genomic selection (array design)	PNWTIRC report	June 30, 2017
Facilitated research plan	Work plan or no-go decision	June 30, 2017
Workshop plans for FY2017-2018	Workshop proposal	June 30, 2017

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Draft Five-Year Plan

PNWTIRC Overview

PNWTIRC OVERVIEW

Purpose and scope

The purpose of the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) is to conduct genetics and breeding research on Pacific Northwest tree species with the goal of providing priority information that will enhance the efficiency of tree improvement efforts. Emphasis is on region wide problems dealing with major coniferous species. The PNWTIRC is concerned with both tree breeding and mass production of genetically improved materials. The intent is to complement and supplement research by other organizations in the region and to avoid duplication. Another important objective of the Cooperative is to foster communication among tree improvement workers throughout the Pacific Northwest.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

PNWTIRC Personnel

Table 1. PNWTIRC personnel 2016-2017.

Position	Time	Name
Director	45%	Glenn Howe
Research Coordinator	80%	Scott Kolpak
Research Scientist	50%	Jennifer Kling
Research Assistant	10%	Lauren Magalska
Research Manager	15%	Anna Magnuson
Policy/Technical Committee Chair	–	Sara Lipow

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

PNWTIRC Members 2016-2017

Table 2. PNWTIRC members and annual dues (in parentheses). Categories of membership are described in Appendix I.

Regular Members (\$8000)	Associate Members (\$4000)
Bureau of Land Management	Starker Forests
Cascade Timber Consulting	Contractual Participants (\$2000)
Green Diamond Resource Company	Lone Rock Timber Company
Hancock Forest Management	Liaison Members
Olympic Resource Management	Inland Empire Tree Improvement Coop.
Oregon Department of Forestry	Northwest Tree Improvement Coop.
Port Blakely Tree Farms	USFS, PNW Research Station
Rayonier Forest Products	University Members
Roseburg Forest Products	Oregon State University
Stimson Lumber Company	
Washington DNR	
Weyerhaeuser Company	

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

Planning

- Discussion at last annual meeting
- Five-Year Plan Committee
- Occasional meetings
- Presentation of Draft Plan (today)
- Survey by November 11
- Five-Year Plan updates
- Five-Year Plan vote by Dec 31

Table 3. Five-Year Plan Committee members.

Sara Lipow	Roseburg Forest Products
Margaret Banks	Stimson Lumber Company
Michael Crawford	Bureau of Land Management
Dan Cress	Olympic Resource Management
Jeff DeBell	Washington DNR
Josh Sherrill	Rayonier Forest Products

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

Core PNWTIRC Research

Overview

Because of the wide variety of tree improvement programs in the Pacific Northwest, it is difficult to find research problems that are of equal interest to all PNWTIRC members. Therefore, the PNWTIRC research program consists of a suite of individual research projects that reflect a broad range of interests and needs. The administrative costs of all major research projects are borne by all contributing members. In contrast, active participation and in-kind contributions will vary by project. The PNWTIRC may also undertake subprojects that are financed by additional project-specific contributions from a subset of the members. More details on these approaches are given in Appendix IV.

- Genomic selection for Douglas-fir tree improvement
- Douglas-fir site characterization and effects of climate change
- Genetics of drought hardiness

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Core Research

Development of SNP markers for Douglas-fir

Objectives of SNP development research

1. Discover Douglas-fir SNPs using transcriptome sequencing (completed as part of PNWTIRC/CTGN research) (Howe et al. 2013).
2. Develop a high-density Illumina Infinium genotyping array (completed as part of PNWTIRC/CTGN research) (Howe et al. 2013).
3. Design and test a low-density SNP genotyping array (e.g., Sequenom) using a subset of SNPs (completed as part of PNWTIRC/CAFS research).
4. Develop a high-density Affymetrix Axiom genotyping array for Douglas-fir (completed as part of PNWTIRC/NARA research; collaborator Keith Jayawickrama).
5. Characterize the SNPs on the Axiom array (i.e., minor allele frequency, observed heterozygosity, call rate, Hardy-Weinberg Equilibrium). This is nearing completion.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Core Research

Genomic selection for Douglas-fir tree improvement

Objectives of genomic selection research

1. Evaluate genomic selection in Douglas-fir breeding programs using recently acquired SNP data and previously measured phenotypes.
2. Reduce the costs of genomic selection by optimizing the number of SNPs and training population size.
3. Test whether early testing can be used to increase the efficiency of genomic selection.
4. Test whether breeding-without-breeding can be used to increase the efficiency of genomic selection.
5. Develop a new, lower-cost SNP genotyping array for Douglas-fir.
6. Conduct genomic selection workshops and other training for PNWTIRC members.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Core Research

DF site characterization / effects of climate change

- Identify environmental (site) characteristics that explain variation in the Douglas-fir growth and stem defects
- Project the effects of near-term climate change on Douglas-fir growth

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Core Research

Genetics of drought hardiness

- Identify the impacts of drought on seedling growth and survival
- Develop recommendations for practicing assisted migration
- Characterize genetic variation in drought hardiness
- Study the adaptability of families to climate conditions in southern Oregon using ClimateNA models
- Obtain baseline measurements to help in the analysis and interpretation of future measurements in the drought hardiness study

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

Other Research Projects

Overview

The PNWTIRC is associated with other research projects to varying degrees, including research funded by the NSF Center for Advanced Forestry Systems, USFS Pacific Northwest Research Station, and the USFS National Forest System (see Table 6 under Funding). These projects are described below.

- Development of genetic markers for WWP and DF (CAFS)
- Meta-analysis of Douglas-fir provenance tests (USFS PNWRS)
- Assisted migration (USFS PNWRS)
- Seedlot Selection Tool
- Genetic markers for western white pine (WWP) (STDP)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Future Research

Overview

Based on recent guidance from the Policy/Technical Committee, we will be pursuing research on the development of SNP markers for Douglas-fir and genomic selection for Douglas-fir tree improvement during FY2016-2017, and probably beyond. We will consider the other topics described below (or additional projects) as well.

- Development of SNP markers for Douglas-fir
- Genomic selection for Douglas-fir tree improvement
- Genetics of Douglas-fir drought hardiness
- Breeding for future climates
- Facilitated research

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Future Research

Facilitated Research

- Facilitated research projects will be led by one of the PNWTIRC members
- The project lead will have primary responsibility for the project
- PNWTIRC members will be responsible for all field work
- Other PNWTIRC members may participate, but are not required to do so
- PNWTIRC funds will be allocated to the project using the normal budgeting process
- PNWTIRC staff will help the project lead develop a work plan
- PNWTIRC staff will help coordinate project activities by helping organize meetings, managing budgets, supervising field work, and facilitating communication with other PNWTIRC members
- PNWTIRC staff will archive data, help with statistical analyses, and help report research results

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Facilitated Research

Examples of potential research topics

- Genetics and economic impact of stem defects
- Techniques for early flowering in Douglas-fir
- Economic weights for genetic selection

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

Technology Transfer

Potential new workshops

“How to implement genomic selection in Douglas-fir breeding programs.” This workshop would cover the practical aspects of practicing genomic selection for tree improvement managers, including design of breeding populations, collection and storage of foliage samples, contracting of DNA isolation and SNP genotyping, and data analysis.

“Consideration of climate change in tree breeding programs.” This workshop would cover how materials from breeding programs can be climatically matched to planting sites based on current and future climates, how breeders can use the Seedlot Selection Tool for making these decisions, and how forest managers can ensure sufficient improved seed for the lands in the future.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Funding

PNWTIRC Funds

FUNDING

PNWTIRC funds

Summary

PNWTIRC dues have not increased in the past 18 years, although costs have. Dues increases have been averted because of external grant and contract funds that have been used to support core PNWTIRC projects and other projects of interest to PNWTIRC members. Using the historic PNWTIRC 'target budget' as a guide, total membership dues should now be closer to \$160K to \$170K, instead of the current \$102K. We will help balance the internal PNWTIRC budget by increasing dues by \$2000 to \$4000 per Regular Member over the next few years. Four options for the amount and timing of dues increases are described.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan – Funding

PNWTIRC Funds

Table 5. Current and 1998 target budgets. Budgets are shown for FY2015-2016 and FY1998-1999, the year of the last dues increase to \$8,000 per Regular Member. The 1998 budgets are shown in 1998 USD and 2016 USD based on a cumulative inflation rate of 47.8%.

Item	Current target budget		Target budget in 1998 (last dues increase)	
	2016 USD	1998 USD	2016 USD	
Income				
Regular member dues	8,000	8,000	11,824	
Total income from dues	102,000	116,000	171,448	
Expenses				
Permanent employee (full-time salary and OPE)	89,877	61,030	90,202	
Graduate student (0.49 FTE, excluding tuition)	30,637	12,863	19,012	
Supplies, equipment, and travel	22,170	15,000	22,170	
Indirect costs (13%)	18,549	0	0	
Total expenses	161,233	88,893	131,384	
Balance	-59,233	27,107	40,064	

*Historically, the target budget has consisted of (1) income from membership dues and (2) expenses associated with a permanent employee; graduate student; and supplies, equipment, and travel.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft
Five-Year
Plan – Funding

PNWTIRC Funds

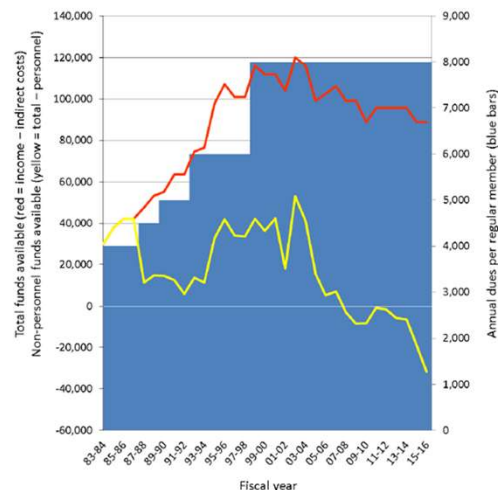


Figure 2. Target PNWTIRC budget (1983-2016). The blue bars show the annual dues per regular member, the red line shows the total operational funds available (total dues – indirect costs), and the yellow line shows funds available for supplies, equipment, and travel (red line – fixed personnel costs for one permanent employee and one graduate student). The yellow line dropped substantially below zero in recent years, indicating that current PNWTIRC membership dues are less than what are needed to support the historic target budget.

Draft Five-Year Plan – Funding

Income from non-PNWTIRC sources

Table 6. Funds available from non-PNWTIRC sources. External funds have been used to (1) support core PNWTIRC research projects and other research of interest to PNWTIRC members and (2) maintain full employment for personnel that are on part-time appointments with the PNWTIRC.

Project	Source	Available balance (\$)	End date
Genomic selection for Douglas-fir tree improvement	NSF-CAFS*	14,548	9/30/17
Development of genetic markers for western white pine and Douglas-fir	NSF-CAFS	14,548	9/30/17
Meta-analysis of Douglas-fir provenance tests	USFS PNWRS†	88,997	9/30/17
Assisted migration	USFS PNWRS	39,665	8/31/19
Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust	USFS NFS‡	83,500	7/31/18
Total		241,258	

*NSF-CAFS is the National Science Foundation Center for Advanced Forestry Systems.

†USFS PNWRS is the USDA Forest Service Pacific Northwest Research Station.

‡USFS NFS is the USDA Forest Service National Forest System.



Draft Five-Year Plan – Funding

Options for future PNWTIRC dues

Table 7. Options for future PNWTIRC dues. Projections of PNWTIRC income and expenses suggests that total PNWTIRC membership dues should rise to at least \$150,000 in the next 5-year period (Table 5). PNWTIRC members will consider the following four options, with discussion, possible modifications, and a final vote to be completed by the end of the 2016 calendar year.

Option	Dues increase (Regular Members)*	Total dues	Comments
1	\$8000 to \$10,000 in FY2017-2018 Budget assessment in FY2018-2019	\$127,500 \$127,500?	Pros: Small dues increase Cons: PNWTIRC research will be curtailed
2	\$8000 to \$11,000 in FY2017-2018 Budget assessment in FY2018-2019	\$140,250 \$140,250?	Pros: Moderate dues increase Cons: PNWTIRC research will likely be curtailed
3	\$8000 to \$10,000 in FY2017-2018 \$10,000 to \$12,000 in FY2018-2019	\$127,500 \$153,000	Pros: Incremental increase, long-term continuity Cons: Larger dues increase
4	\$8000 to \$12,000 in FY2017-2018	\$153,000	Pros: Short-term and long-term continuity Cons: Larger dues increase

*Percentage increases would be comparable for Associate and Contractual Members.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

APPENDIX I

PNWTIRC membership categories and fee structure.

Regular Members (\$8,000)

Annual membership fees and in-kind support – Regular members contribute an annual membership fee and in-kind support (e.g., study sites, labor, equipment, and materials).

Acreage requirements and voting privileges – Regular members include organizations that own, lease, or manage 100,000 acres or more of forestland in the Pacific Northwest. Organizations with more than 500,000 acres may purchase one additional membership for each additional 500,000 acres. This additional membership is subject to approval by two-thirds of the Regular members of the Policy/Technical Committee. Each Regular membership entitles an organization to one vote on the Policy/Technical Committee.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

APPENDIX II

Responsibilities of PNWTIRC staff and members.

The Cooperative Director:

1. Provides overall leadership and coordination for PNWTIRC activities.
2. Helps the Policy/Technical Committee set research priorities.
3. Plans and designs studies that meet the practical needs of the Cooperators, yet conform to the standards of scientific experimentation.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

APPENDIX III

PNWTIRC Five-Year Plan activities.

Activity	Deliverable	Target date
FY2016-2017		
Five-Year Plan	Draft plan	Oct 19, 2016
FY2016-2017 budget	Approved budget	Oct 19, 2016
Five-Year Plan survey	PNWTIRC report on survey results	Nov 18, 2016
Dues increase	Vote on dues increase	Dec 31, 2016
Affymetrix Axiom array	PNWTIRC report	Dec 31, 2016
Douglas-fir site characterization	PNWTIRC report	Dec 31, 2016
Genomic selection work plan	Approved work plan	Dec 31, 2016
Five-Year Plan	Approved plan	Dec 31, 2016
Drought hardiness study	Master's thesis	Mar 15, 2017
Genomic selection (array design)	PNWTIRC report	June 30, 2017
Facilitated research plan	Work plan or no-go decision	June 30, 2017
Workshop plans for FY2017-2018	Workshop proposal	June 30, 2017

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Draft Five-Year Plan

APPENDIX IV

PNWTIRC research approach.

1. Research is defined on a project basis.
 - a. Major projects are projects that (1) require a substantial amount of financial and/or in-kind resources and (2) are mostly financed by annual membership fees or grants.
 - b. Subprojects are projects that (1) require a substantial amount of financial and/or in-kind resources and (2) are mostly financed by contracts or additional project-specific contributions from a subset of the members. These subprojects may be specific, short-term projects conducted by individual scientists or graduate students, or projects carried out by one or more of the Cooperative members.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Budget

By Glenn Howe and Sara Lipow


Glenn Howe presented last year's budget (FY2015-2016) and the proposed budget for next year (FY2016-2017). During this portion of the annual meeting, we also elected a new Policy/Technical Committee Chair and an OSU representative for the NSF Center for Advanced Forestry Systems (CAFS).

Budget and Other Business

Glenn Howe

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Budget 2015-16

Main points

- 2015-16 income = \$102K
- 2016-17 income = \$102K
- Indirect = 13%

Attachment #1
Financial Support Received in 2015-16

Organization	Financial Support
Regular Members	
Cascade Timber Consulting	8,000
Bureau of Land Management	8,000
Green Diamond Resource Company	8,000
Hancock Forest Management	8,000
Olympic Resource Management	8,000
Oregon Department of Forestry	8,000
Plum Creek Timber Company	8,000
Port Blakely Tree Farms	8,000
Rayonier	8,000
Roseburg Forest Products	8,000
Stimson Lumber Company	8,000
Washington State Dept. of Natural Resources	8,000
Associate Members	
Starker Forests	4,000
Contractual Members	
Lone Rock Timber Company	2,000
Total	102,000

Budget 2015-16

Main points

- Summarizes personnel costs
- Personnel costs were covered by PNWTIRC members and OSU (Director)
- Carryover increased
- CAFS and STDP funds were used to pay some salaries

Attachment #2
PNWTIRC Income and Expenditures by Source
FY 2015-2016

Income and Expenditures	OSU	Members	Total
Income			
OSU Forest Research Laboratory	124,341	0	124,341
Membership fees and contracts	0	102,000	102,000
Carryover from previous year	0	96,368	96,368
Total income	124,341	198,368	322,709
Expenditures			
Salaries and OPE*			
Director (0.45 FTE; OSU funded)	71,771	0	71,771
Program Manager	0	10,181	10,181
Research Coordinator	0	18,048	18,048
Research Scientist	0	15,500	15,500
Faculty Research Assistant	0	7,367	7,367
Graduate students	0	0	0
Student employees	0	613	613
OPE reimbursement	0	-111	-111
Supplies and Services	0	4,805	4,805
Travel	0	809	809
Total direct costs (TDC)	71,771	57,302	129,073
Indirect costs**	52,570	7,449	60,019
Direct + Indirect Costs	124,341	64,751	189,092
Carryover to next year	0	133,617	133,617

Budget 2015-16

Main points

- Summarizes costs by project
- Expenditures on 'New research' (e.g., genomic selection were delayed)
- We now have all SNP data needed for genomic selection research

Attachment #3
Proposed and Actual PNWTIRC Budgets for 2015-2016*

Income	Proposed (10/15)	Actual (7/16)
Members fees and contracts	102,000	102,000
Carryover from previous year	96,365	96,368
Total income	198,365	198,368
Expenses		
SNP marker assisted selection	21,681	27,518
New research (e.g., Drought)	75,797	4,389
Site characterization (CAFS)	4,110	3,684
WWP genetic markers (UI/CAFS)	4,110	3,610
Technology transfer	0	0
Administration	23,869	18,102
Total direct costs (TDC)	129,567	57,302
Indirect costs**	16,844	7,449
Direct + Indirect costs	146,411	64,751
Carryover to next year	51,954	133,617

Budget details for 2015-16

Attachment #4
Expenditures of Cooperator Funds for Fiscal Year 2015-2016 by Project and Activity

Expense*	SNP MAS	Drought Hardiness	Site Char. (w/CAFS)	WWP	Tech Transfer	Admin.	Total
Director (funded by OSU) (approx. FTE)	0 0.10	0 0.10	0 0.05	0 0.10	0 0.00	0 0.10	0 0.45
Program Manager (approx. FTE)	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	10,181 0.14	10,181 0.14
Research Coordinator (approx. FTE)	9,024 0.10	3,610 0.04	0 0.00	3,610 0.04	0 0.00	1,805 0.02	18,048 0.20
Research Scientist (approx. FTE)	14,811 0.13	780 0.01	0 0.00	0 0.00	0 0.00	0 0.00	15,590 0.14
Faculty Research Assistant (approx. FTE)	3,684 0.05	0 0.00	3,684 0.05	0 0.00	0 0.00	0 0.00	7,367 0.10
Graduate students (approx. FTE)**	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00
Undergraduate students	0	0	0	0	0	613	613
OPE reimbursement	0	0	0	0	0	-111	-111
Personnel sub-total	27,518	4,389	3,684	3,610	0	12,488	51,688
Supplies & Services	0	0	0	0	0	4,805	4,805
Travel	0	0	0	0	0	809	809
Non-personnel sub-total	0	0	0	0	0	5,614	5,614
Total direct costs (TDC)	27,518	4,389	3,684	3,610	0	18,102	57,302
Indirect (13% of TDC)	3,577	571	479	469	0	2,353	7,449
Total costs	31,095	4,960	4,162	4,079	0	20,455	64,751

Budget 2016-17

Main points

- Summarizes proposed costs of personnel for 2016-2017
- Oguz Urhan and Erda Celer are associated with the PNWTIRC, but are supported by the Turkish government
- Focus on SNP marker assisted selection
- Five-Year Plan addresses long-term dues structure

Attachment #5
Proposed Expenditures of Cooperator Funds for Fiscal Year 2016-2017

Income and Expenditures	FY 2015-16	FY 2016-17
Income from Cooperators		
Membership fees and contracts	102,000	102,000
Carryover from previous year	96,368	133,617
Total income	198,368	235,617
Expenditures		
Salaries and OPE*		
Director (0.45 FTE; OSU funded)	0	0
Program Manager	10,181	10,709
Research Coordinator	18,048	71,851
Research Scientist	15,560	57,150
Faculty Research Assistant	7,367	7,398
Graduate students	0	0
Student employees	613	1,000
OPE reimbursement	-111	0
Supplies and Services	4,805	6,000
Travel	809	2,000
Total direct costs (TDC)	57,302	156,108
Indirect costs**	7,449	20,234
Direct + Indirect Costs	64,751	176,402
Carryover to next year	133,617	59,214

Budget 2016-17

Main points

- Summarizes proposed costs by project for 2016-2017
- Focus on SNP marker assisted selection

Attachment #6

Proposed Expenditures of Cooperator Funds for Fiscal Year 2016-2017

Income	FY 2015-16	FY 2016-17
Members fees and contracts	102,000	102,000
Carryover from previous year	96,368	133,617
Total income	198,368	235,617
Expenses	FY 2015-16	FY 2016-17
SNP marker assisted selection	27,518	126,455
Drought hardness	4,389	2,694
Site characterization (CAFS)	3,684	2,959
WWP genetic markers (UI/CAFS)	3,610	2,694
Technology transfer	0	0
Administration	18,102	21,305
Total direct costs (TDC)	57,302	156,108
Indirect costs*	7,449	20,294
Direct + Indirect costs	64,751	176,402
Carryover to next year	133,617	59,214

Budget details for 2016-17

Attachment #7

Proposed Expenditures of Cooperator Funds for Fiscal Year 2016-2017

Expense*	SNP MAS	Drought Hardiness	Site Char. (w/CAFS)	WWP	Tech Transfer	Admin.	Total
Director (funded by OSU) (approx. FTE)	0 0.15	0 0.05	0 0.05	0 0.05	0 0.00	0 0.15	0 0.45
Program Manager (approx. FTE)	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	10,709 0.15	10,709 0.15
Research Coordinator (approx. FTE)	64,666 0.72	2,694 0.03	0 0.00	2,694 0.03	0 0.00	1,796 0.02	71,851 0.80
Research scientist (approx. FTE)	57,150 0.50	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	57,150 0.50
Faculty Research Assistant (approx. FTE)	4,439 0.06	0 0.00	2,959 0.04	0 0.00	0 0.00	0 0.00	7,398 0.10
Graduate students (approx. FTE)**	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00
Student employees (proportion of expense)	200 0.20	0 0.00	0 0.00	0 0.00	0 0.00	800 0.80	1,000 1.00
Personnel sub-total	126,455	2,694	2,959	2,694	0	13,305	148,108
Supplies & Services	0	0	0	0	0	6,000	6,000
Travel	0	0	0	0	0	2,000	2,000
Non-personnel sub-total	0	0	0	0	0	8,000	8,000
Total direct costs (TDC)	126,455	2,694	2,959	2,694	0	21,305	156,108
Indirect (13% of TDC)	16,439	350	385	350	0	2,770	20,294
Total costs	142,894	3,045	3,344	3,045	0	24,075	176,402

Budget and other business

Vote on budget

Elect new Policy/Technical Committee Chair

Elect new CAFS OSU Site Representative

Other business?

Seedlot Selection Tool

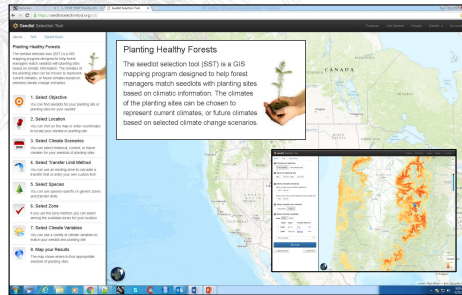
By Glenn Howe, Brad St. Clair, Dominique Bachelet, Brendan Ward, and Nik Stevenson-Molnar

The Seedlot Selection Tool (SST) has been redesigned and launched with the collaboration of Dominique Bachelet and staff at the Conservation Biology Institute. The original version of the SST was developed through a collaboration of Glenn Howe (OSU, PNWTIRC) and Brad St.Clair (US Forest Service, Pacific Northwest Research Station). Partnering with CBI will promote better long-term maintenance and integration as updated climate information or seed zones become available. The new SST is available online at <https://seedlotselectiontool.org/sst/>.

The SST is a GIS mapping tool designed to help forest managers match seedlots with planting sites based on climatic information. The climates of the planting sites can be chosen to represent current climates or future climates based on selected climate change scenarios. In contrast to traditional seed zones and breeding zones, the SST uses zones that are centered on a chosen focal point (a planting site or a seed collection site), and utilizes the climate at that point to determine other areas of similar climate now and into the future (e.g., based on selected climate change scenarios).

Seedlot Selection Tool

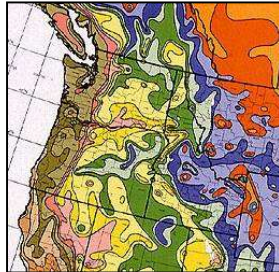
Glenn Howe
 Brad St. Clair
 Dominique Bachelet
 Brendan Ward
 Nik Stevenson-Molnar



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



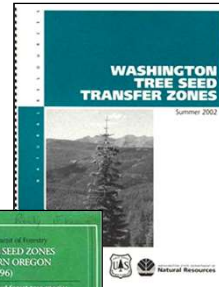
What to plant? Seed zones and breeding zones



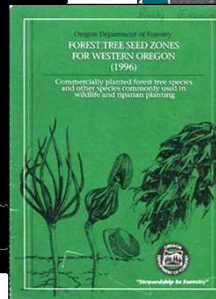
Seed zones and breeding zones are largely delineated based on climate



Randall (1996) OR Dept of Forestry



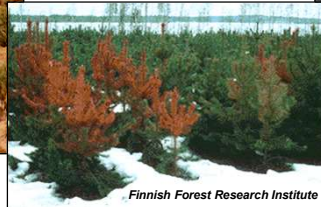
Randall and Berrang (2002) WA Dept Nat Resources



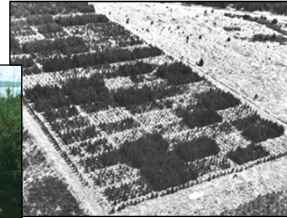
Transfer limits from provenance tests



Superior adaptability of a Douglas-fir seed source from California growing in Spain (Hernandez et al 1993)



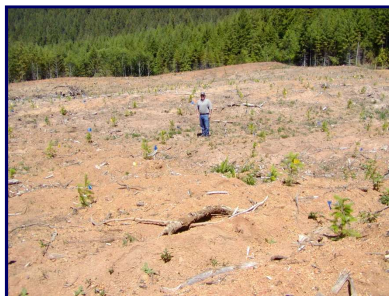
Lodgepole pine provenances from maritime areas are not adapted to the winters of eastern Finland



Lodgepole pine provenance test in New Zealand (Wright 1976)

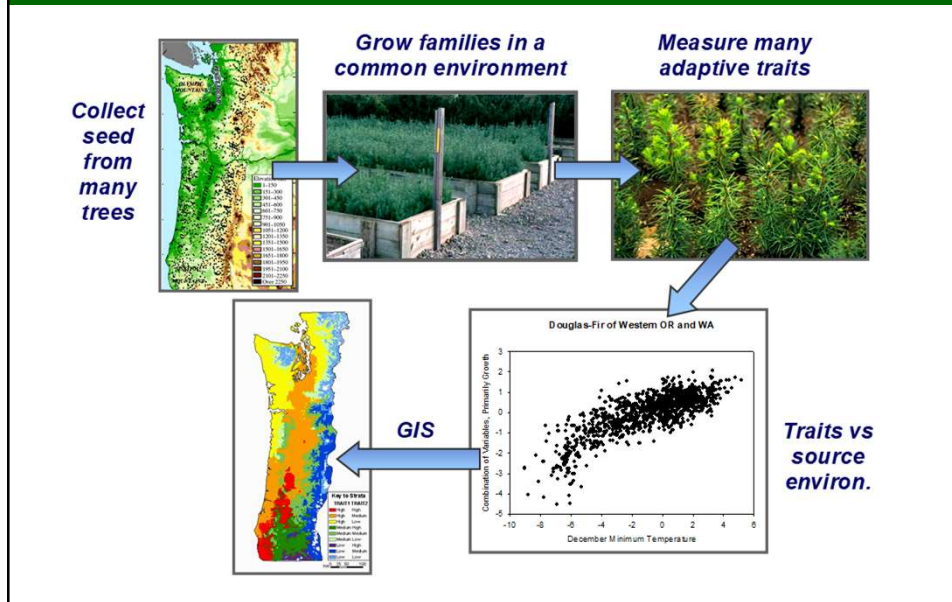
- Transfer limits are derived from direct observations of seed transfer
- Large climatic distances are often tested
- Sufficiently large provenance tests are rare
- Results are most relevant to plantations rather than naturally regenerated forests

Transfer limits from progeny tests



- Transfer limits are derived from direct observations of seed transfer
- Small climatic distances are often tested
- Progeny testing available for relative few species
- Results are most relevant to plantations rather than naturally regenerated forests

Transfer limits from seedling tests



Transfer limits based on experience



- Simply put...have seed zones, breeding zones, deployment zones worked?
- If so, they provide a safe lower limit for seedlot transfer

Types of zones

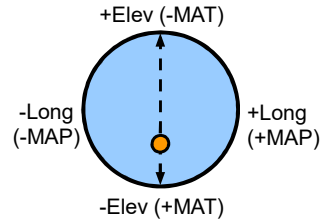
Traditional zones

- Defined 'circles on a map'
- Transfers in different geographic directions may be limited at different climatic distances

Geography

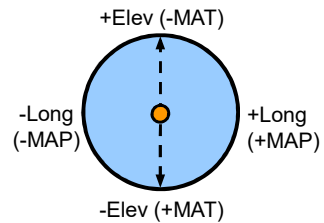
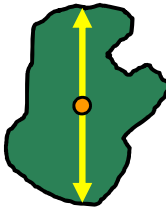


Climate space

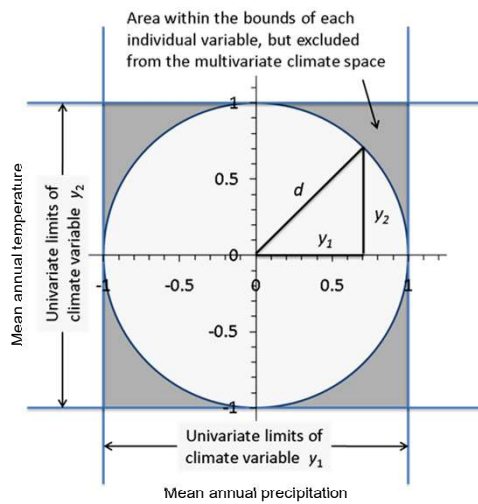


Focal point zones

- Zones 'float'
- Centered on your focal point
- Transfers are always limited at the same climatic distance



Climate space and transfer limit



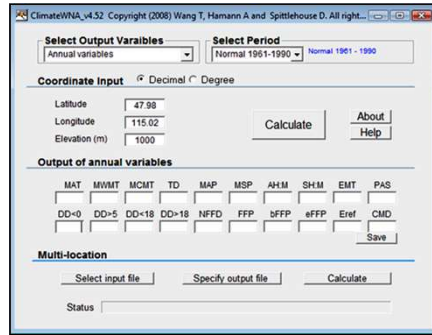
Transfer limit = radius

On the standardized scale, the transfer limit = 1.0

Transfer distance = d

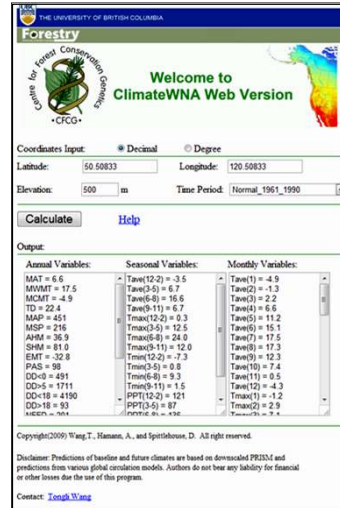
$$d = \sqrt{y_1^2 + y_2^2}$$

ClimateWNA

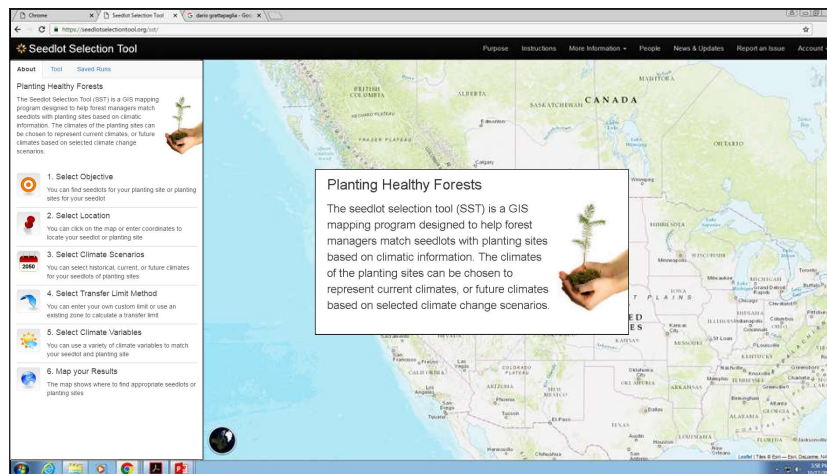


ClimateWNA provides easy access to over 20,000 climate surfaces

Slide from Tongli Wang



Seedlot Selection Tool (SST)




How the tool works







- Select objective
- Select location
- Select climate scenarios
- Select transfer limit method
- Select climate variables
- Map your results

About | Tool | Saved Runs

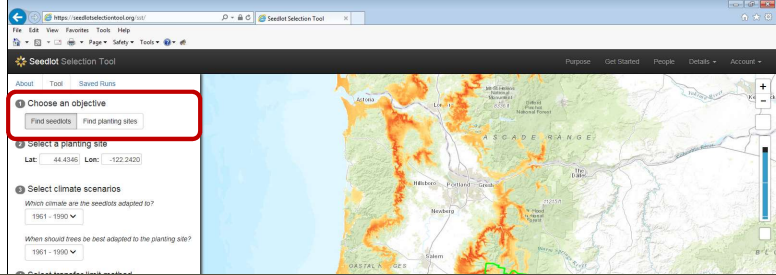
Planting Healthy Forests

The Seedlot Selection Tool (SST) is a GIS mapping program designed to help forest managers match seedlots with planting sites based on climatic information. The climates of the planting sites can be chosen to represent current climates, or future climates based on selected climate change scenarios.



- 
1. Select Objective
 You can find seedlots for your planting site or planting sites for your seedlot
- 
2. Select Location
 You can click on the map or enter coordinates to locate your seedlot or planting site
- 
3. Select Climate Scenarios
 You can select historical, current, or future climates for your seedlots of planting sites
- 
4. Select Transfer Limit Method
 You can enter your own custom limit or use an existing zone to calculate a transfer limit
- 
5. Select Climate Variables
 You can use a variety of climate variables to match your seedlot and planting site
- 
6. Map your Results
 The map shows where to find appropriate seedlots or planting sites

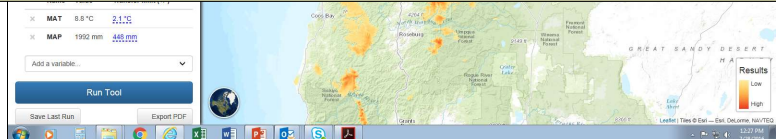
Select your objective



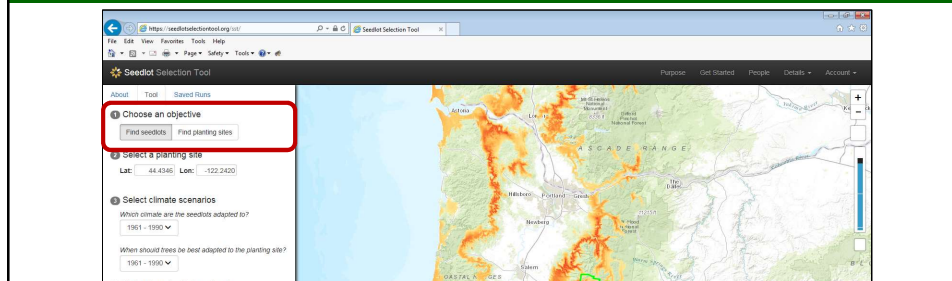
Given a specific planting site ...

Which seedlot is well adapted today?...

And in the future given a climate change scenario?



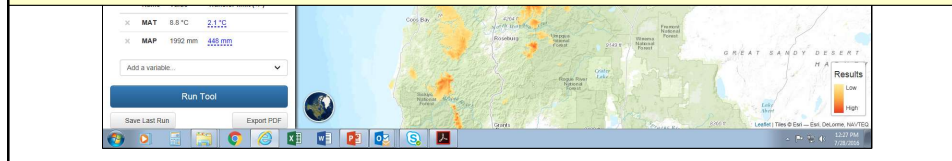
Select your objective



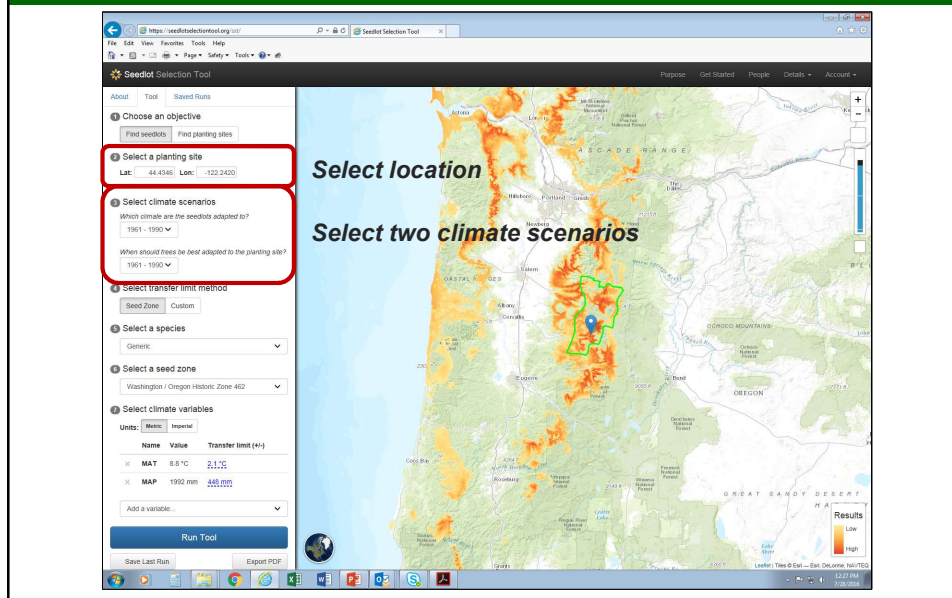
Given a specific seedlot ...

Where is it expected to be well adapted today?...

And in the future given a climate change scenario?



Select location and climate scenarios



Transfer limit – Two options

Use a zone or enter custom transfer limits

The screenshot shows the 'Seedlot Selection Tool' interface. On the left, the 'Select transfer limit method' step is highlighted with a red box, showing two options: 'Seed Zone' and 'Custom'. The 'Seed Zone' option is selected. The 'Select a seed zone' step below it shows 'Washington / Oregon Historic Zone 462' selected. The map on the right shows a green outline around a specific area in the Pacific Northwest, with text 'Use a zone or enter custom transfer limits' overlaid on the map.

Select a species – Shows available zones

Select species Shows available zones

The screenshot shows the 'Seedlot Selection Tool' interface. On the left, the 'Select a species' step is highlighted with a red box, showing a dropdown menu for 'Generic'. The 'Select a seed zone' step below it shows 'Washington / Oregon Historic Zone 462' selected. The map on the right shows a green outline around a specific area in the Pacific Northwest, with text 'Select species Shows available zones' overlaid on the map.

Select climate variables

The screenshot shows the 'Seedlot Selection Tool' interface. On the left sidebar, step 7 'Select climate variables' is highlighted with a red box. The main map area shows a geographical region with a color-coded overlay. A pop-up window titled 'Select climate variables' is open, displaying a table of selected variables:

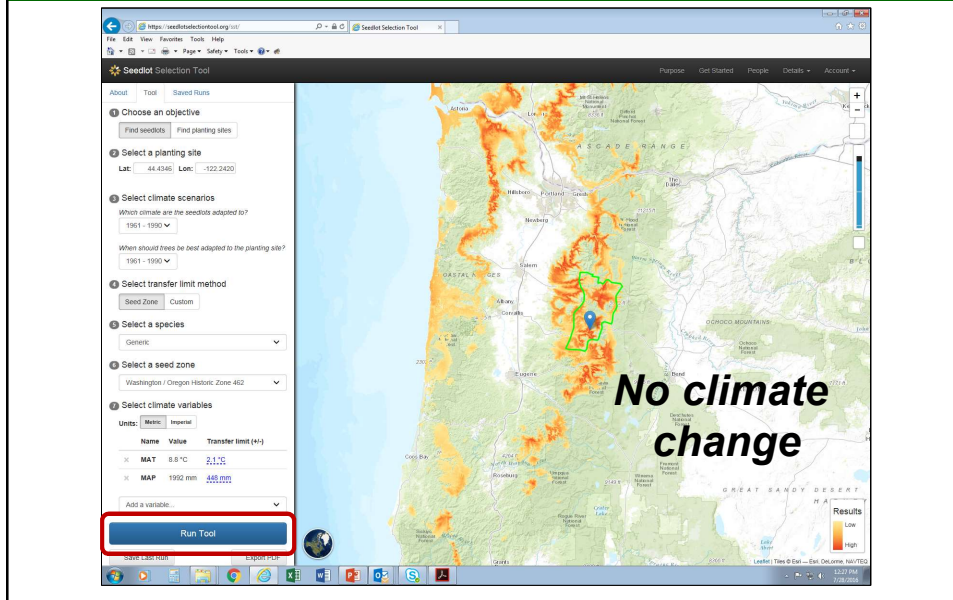
Name	Value	Transfer limit (+/-)
MAT	8.8 °C	2.1 °C
MAP	1992 mm	448 mm

Below the table is an 'Add a variable...' dropdown menu. At the bottom of the sidebar, the 'Run Tool' button is highlighted with a red box.

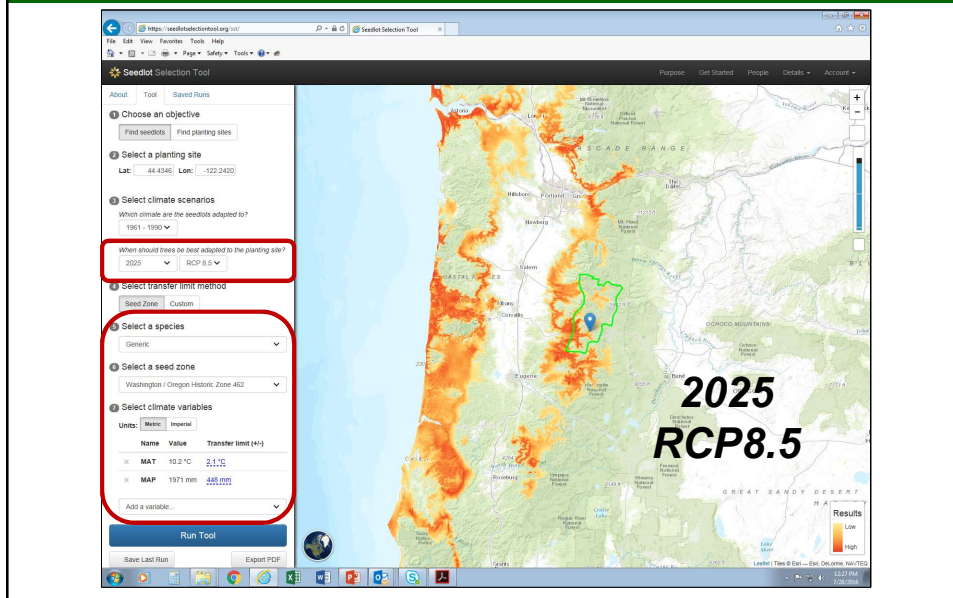
Map your results

This screenshot is identical to the one above, showing the 'Seedlot Selection Tool' interface. The 'Run Tool' button at the bottom of the sidebar is highlighted with a red box, indicating the next step in the process.

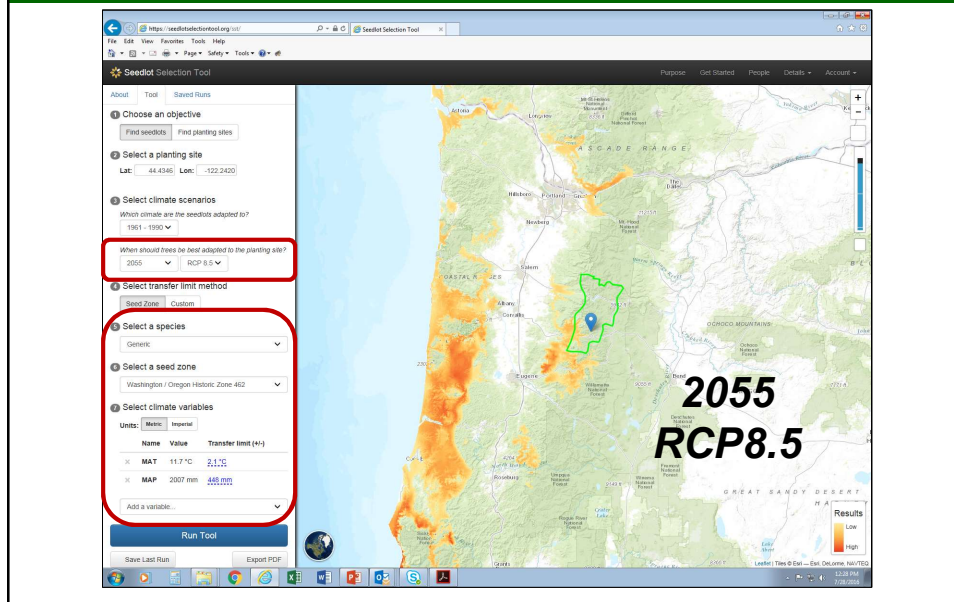
Seedlots for planting site



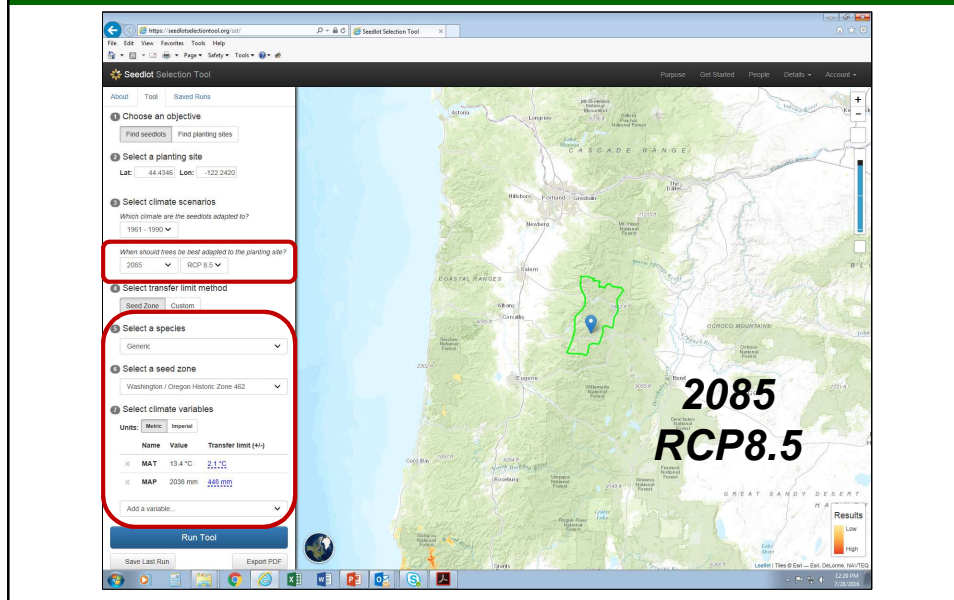
Seedlots for planting site adapted to 2025



Seedlots for planting site adapted to 2055



Seedlots for planting site adapted to 2085



Zoomed out for California sources

The screenshot displays the Seedlot Selection Tool interface. The left sidebar contains several configuration steps:

- 1 Choose an objective**: Find seedlots, Find planting sites
- 2 Select a planting site**: Lat: 44.4345, Lon: -122.2420
- 3 Select climate scenarios**: Which climate are the seedlots adapted to? 1961 - 1990
 - When should trees be best adapted to the planting site? 2085 RCP 8.5
- 4 Select transfer limit method**: Seed Zone, Custom
- 5 Select a species**: Generic
- 6 Select a seed zone**: Washington / Oregon Historic Zone 462
- 7 Select climate variables**:

Name	Value	Transfer limit (4+)
MAT	13.4 °C	2.3 °C
MAP	2038 mm	400 mm

The main map shows a zoomed-out view of California with a blue dot indicating a selected location. A large text overlay on the map reads "2085 RCP8.5". The bottom of the interface includes a "Run Tool" button and a "Results" panel.

Summary

- New Seedlot Selection Tool has been launched
- What other functions are needed?
- What other zones or information is needed?
- Thanks to Ron Beloin and Lauren Magalska

APPENDIX I

Literature Cited

- Adams, W.T., Aitken, S.N., Joyce, D.G., Howe, G.T., Vargas-Hernandez, J. 2001. Evaluating efficacy of early testing for stem growth in coastal Douglas-fir. *Silvae Genetica* 50: 167-175.
- Anekonda, T.S., Lomas, M.C., Adams, W.T., Kavanagh, K.L., Aitken, S.N. 2002. Genetic variation in drought hardiness of coastal Douglas-fir seedling from British Columbia. *Canadian Journal of Forest Research* 32: 1701-1716.
- Bower, A.D., Adams, W.T., Birkes, D., Nalle, D. 2005. Response of annual growth ring components of soil moisture deficit in young, plantation-grown Douglas-fir in coastal British Columbia. *Canadian Journal of Forest Research* 35(10): 2491-2499.
- Dalton, R. 2009. No bull: genes for better milk. *Nature* 457: 369.
- El-Kassaby, Y.A., Lstibůrek, M., Liewlaksaneeyanawin, C., Slavov, G.T., Howe, G.T. 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.*
- El-Kassaby Y.A., Cappa E.P., Liewlaksaneeyanawin C, Klápště J, Lstibůrek, M. 2011. Breeding without breeding: Is a complete pedigree necessary for efficient breeding?. *PLoS ONE* 6(10): e25737. doi:10.1371/journal.pone.0025737
- Hayes, B., Goddard, M. 2010. Genome-wide association and genomic selection in animal breeding. *Genome* 53(11): 876-883.
- Howe, G.T., Yu, J., Knaus, B., Cronn, R., Kolpak, S., Dolan, P., Lorenz, W.W., Dean, J.F. 2013. A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation. *BMC Genomics* 14(1): 137.
- Illumina. 2011. Genomic selection – A paradigm shift in animal breeding: Illumina next-generation sequencing and genotyping technologies are revolutionizing animal breeding. *Technical Note: Agrogenomics, Illumina Inc.: 2.*
- Goddard, M.E., Hayes, B.J., Meuwissen, T.H.E.. 2010. Genomic selection in livestock populations. *Genet. Res.* 92: 413-421
- Magalska, L., Howe, G.T. 2014. Genetic and environmental control of Douglas-fir stem defects. *Forest Ecology and Management* 318: 228-238

- Meuwissen, T.H., Hayes, B.J., Goddard, M.E. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(4): 1819-1829.
- Müller, T., Ensminger, I., and Schmid, K.J. 2012. A catalogue of putative unique transcripts from Douglas-fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings. *BMC Genomics* 13(1): 673.
- Randall, W.K. 1996. Forest Tree seed zones for western Oregon. Salem, OR, Oregon Dept. of Forestry: 82.
- Randall, W.K., Berrang, P. 2002. Washington tree seed transfer zones. Olympia, WA, Washington Dept. Nat. Resources: 63.
- Sabatia, C.O., Harold E.B. 2014. Predicting site index of plantation Loblolly pine from biophysical variables. *Forest Ecology and Management* 326: 142-56.
doi:10.1016/j.foreco.2014.04.019.
- Slavov, G.T., Howe, G.T., Adams, W.T. 2005. Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers. *Canadian Journal of Forest Research* 35(7): 1592-1603.
- Slavov, G.T., Howe, G.T., Yakovlev, I., Edwards, K.J., Krutovskii, K.V., Tuskan, G.A., Carlson, J.E., Strauss, S.H., Adams, W.T. 2004. Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. *Theoretical and Applied Genetics* 108(5): 873-880.
- 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.
- Visser, E.A., Wegrzyn, J.L., Steenkmap, E.T., Myburg, A.A., Naidoo, S. 2015. Combined de novo and genome guided assembly and annotation of the *Pinus patula* juvenile shoot transcriptome. *BMC Genomics* 16: 1057.
- Wang, T., Hamann, A., Spittlehouse, D., Carroll, C. 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS ONE* 11(6): e0156720.
- Wellmann, R., Preuß, S., Tholen, E., Heinkel, J., Wimmers, K., Bennewitz, J. 2013. Genomic selection using low density marker panels with application to a sire line in pigs. *Genetic Selection Evolution* 45(1): 28.

APPENDIX II

Workshops, Presentations, and Abstracts by PNWTIRC personnel 2015-2016

- Lu, H., Howe, G.T., Horvath, D.P., Dharmawardhana, P., Priest, H.D., Mockler, T.C., and Strauss, S.H. 2016. Extensive transcriptome changes during natural onset and release of vegetative bud dormancy in *Populus*. Abstract in: Plant Dormancy Workshop, Plant & Animal Genome XXIV, January 9-13, 2016, San Diego, CA.
- Howe, G.T. and Jayawickrama, K.J. 2016. Genomic selection for Douglas-fir tree improvement. Presentation in: Center for Advanced Forestry Systems Annual Meeting, April 26-28, 2016, Pensacola Beach, Florida.
- Urhan, O., Rust, M.L., Davis, A., Howe, G.T., Hipkins, V. 2016. Development of genetic markers for western white pine and Douglas-fir. Presentation in: Center for Advanced Forestry Systems Annual Meeting, April 26-28, 2016, Pensacola Beach, Florida.
- Howe, G.T. 2016. Douglas-fir breeding and the Pacific Northwest Tree Improvement Research Cooperative. Scion, June 7, 2016, Rotorua, New Zealand.
- Pluess, A.R., Frank, A., Rellstab, C., Vendramin, G.G., Howe, G.T., Sperisen, C., Heiri, C., and Oddou-Muratorio, S. 2016. Evidence for local adaptation and potential maladaptation to climate change in *Fagus sylvatica*: Genome-environment and phenotype-environment associations at regional scale. Abstract in: Genomics and Forest Tree Genetics: A conference jointly organized by the four working in parties of IUFRO Subdivision 2.4 (Genetics), May 30-June 3, 2016, Arcachon, France.
- Howe, G.T. 2016. Possibilities for genomics in Douglas-fir breeding. Presentation in: Douglas-fir Breeding Workshop, organized by Scion and the Specialty Wood Products (SWP) Research Partnership, June 9, 2016, University of Canterbury, Christchurch, New Zealand.
- Howe, G.T. 2016. Douglas-fir breeding and geneecology, University of Forestry, June 23, 2016, Sofia, Bulgaria.
- Howe, G.T. 2016. Forest genetics from science to management, Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL), June 30, 2016, Zurich, Switzerland.

APPENDIX III

Collaborations and Grants 2015-2016

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 Forest Health Protection, Special Technology Development Program. Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust. Howe, G.T., Davis, A., Hipkins, V., Liu, J.-J., Mahalovich, M.F., Rust, M., and Sniezko, R., 2014-2018, \$99,500.

University of Idaho and the Inland Empire Tree Improvement Cooperative. Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust. Howe, G.T., 2013-2016, \$60,000.

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, 2011-2015, \$28,755.

U.S. Endowment for Forestry and Communities. Forest health biotechnologies: What are the drivers of public acceptance? Needham, M.D. and Howe, G.T. 2013-2015, \$100,000.

USFS Pacific Northwest Research Station. Meta-analysis of Douglas-fir provenance tests to estimate responses to seed transfer and climate change. Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2018, \$100,000.

USFS Pacific Northwest Research Station. Evaluating assisted migration options for adapting to climate change. Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2019, \$40,000.

APPENDIX IV

Annual Meeting Minutes

October 19, 2017, Mt. Scott Fire Station 5, Happy Valley, OR

I. Attendees

Michael Crawford – Bureau of Land Management	Anna Magnuson – PNWTIRC, OSU
Darian Domes – Cascade Timber Consulting	Scott Kolpak – PNWTIRC, OSU
John Jayne – Cascade Timber Consulting	Erda Celer – PNWTIRC, OSU
Florian Deisenhofer – Hancock Forest Management	Oguz Urhan – PNWTIRC, OSU
Keith Jayawickrama – NWTIC, OSU	Josh Sherrill – Rayonier Forest Resources
Terrance Ye – NWTIC, OSU	Sara Lipow – Roseburg Forest Products
Dan Cress – Olympic Resource Management	Fred Pfund – Starker Forests
Andrew Wodnik – Olympic Resource Management	Margaret Banks – Stimson Lumber Co.
Don Kaczmarek – Oregon Dept. Forestry	Jeff DeBell – Washington State DNR
Brad St. Clair – PNW Research Station, USFS	Brian Baltunis – Weyerhaeuser
Glenn Howe – PNWTIRC, OSU	Graham Ford – Weyerhaeuser
Jennifer Kling – PNWTIRC, OSU	

II. Welcome

Sara Lipow, PNWTIRC Policy/Technical Chair, called the meeting to order at 9:30 am.

III. PNWTIRC highlights for 2015-2016

Glenn Howe presented an overview of major accomplishments for 2015-16.

1. Administration and members
 - Director – Glenn Howe
 - Research Coordinator – Scott Kolpak
 - Research Scientist – Jennifer Kling
 - Program Manager – Anna Magnuson
 - Graduate student – Erda Çeler, Oguz Urhan
 - Faculty Research Assistant – Lauren Magalska
 - Policy/Technical Committee Chair – Sara Lipow
2. Research
3. Publications
4. Presentations
5. Collaborations and grants

IV. PNWTIRC plans for 2016-17

Glenn Howe presented plans for 2016-2017. Discussions were based around the Genomic Selection Workplan. Specific objectives include:

- Complete a Five-Year Plan survey of co-op research and outreach activities and report survey results
- Vote on a dues increase
- Complete the Affymetrix Axiom array analysis and write a PNWTIRC report on the research
- Complete the analyses and write a PNWTIRC report on the Douglas-fir site characterization research
- Develop a genomic selection work plan that will lead to implementation of genomic selection in Douglas-fir and approve the work plan
- Complete the Five-Year Plan and vote to approve the plan
- Complete the Drought Hardiness Study research and write a Master's thesis
- Complete the genomic selection (array design) analysis and distribute a PNWTIRC report
- Develop a 'facilitated research' plan and approve the work plan or no-go decision
- Develop workshop plans for FY2017-2018 and write a workshop proposal

V. PNWTIRC research presentations

1. *Genetics of western white pine.* Oguz Urhan, Glenn Howe, Marc Rust, Richard Sniezko, Scott Kolpak
2. *SNP chip for western white pine.* Scott Kolpak, Glenn Howe, Brent Kronmiller.
3. *Douglas-fir drought hardiness.* Erda Çeler, Glenn Howe.
4. *Effects of climate change on Douglas-fir.* Lauren Magalska, Glenn Howe, Doug Maguire, Scott Kolpak.
5. *Next-generation SNP chip.* Glenn Howe, Keith Jayawickrama, Scott Kolpak, Stephanie Guida, Sanjuro Jogdeo, Rich Cronn, Callum Bell.
6. *Validation of SNP data.* Jennifer Kling, Matt Trappe, Scott Kolpak, Terrance Ye, Keith Jayawickrama, Glenn Howe.
7. *Genomic selection in Douglas-fir.* Glenn Howe, Keith Jayawickrama, Jennifer Kling, Scott Kolpak, Terrance Ye.
8. *Draft Five-Year Plan.* Glenn Howe.
9. *Seedlot Selection Tool.* Glenn Howe, Brad St.Clair, Dominique Bachelet, Brendan Ward, Nik Stevenson-Molnar.

VI. Budget

Glenn Howe presented the budget for FY 2015-2016. The proposed budget for FY 2016-2017 was also presented. A motion to approve the budgets was offered by Josh Sherrill, seconded by Brian Baltunis, and approved by unanimous voice vote.

VII. Draft Five-Year Plan. Glenn distributed and presented the Draft Five-Year Plan. The full plan was previously sent to PNWTIRC members on October 17, 2016.

1. **Workshops.** Glenn proposed to hold two workshops over the next two years: (1) genomic breeding and (2) climate change. Members suggested that a focus on genomic data analysis would be helpful. This proposal was well received, but topic areas should be described in more detail before we make a go/no-go decision.

2. **Facilitated research.** Members of the PNWTIRC have an interest in applied and basic research. However, because of limited resources, it has been challenging to address all of the important applied and basic research problems of interest to PNWTIRC members. We propose to add a new research model to enhance our portfolio of applied research. Glenn led a discussion on the concept of ‘facilitated research,’ which was described in the Draft Five-Year Plan. As described in the Five-Year Plan, we will develop a ‘facilitated research’ plan to be completed by June 30, 2017.
3. **PNWTIRC dues increase.** Glenn presented the rationale and four options for a dues increase (see below).
4. **Approval.** The Draft-Five Year Plan was approved by unanimous voice vote. Glenn will make some editorial changes that were noted in his presentation, and then distribute the final version.

VII. PNWTIRC dues increase. PNWTIRC dues have not increased in the past 18 years, although costs have. Dues increases have been averted because of external grant and contract funds that have been used to support core PNWTIRC projects and other projects of interest to PNWTIRC members. Using the historic PNWTIRC ‘target budget’ as a guide, total membership dues should now be closer to \$160K to \$170K, instead of the current \$102K. We propose to help balance the PNWTIRC budget by increasing dues by about \$2000 to \$4000 per Regular Member over the next few years.

Glenn presented four options for a dues increase. The consensus among members is that a dues increase is warranted; but most members are not interested in Option #4 (see Draft Five-Year Plan). Therefore, members decided to vote on the remaining three options (plus a no dues increase alternative) by December 31, 2016. If a dues increase is approved, it would show up in the invoices members will receive in July 2017.

VII. Policy/Technical Committee Chair

Sara Lipow was nominated as new Policy/Technical Committee Chair by Dan Cress. The nomination was seconded and approved by unanimous voice vote.

VIII. CAFS representative

Brian Baltunis was nominated as the new CAFS Site Representative. The nomination was seconded and approved by unanimous voice vote. Soon, CAFS members will need to decide whether to develop a Phase 3 CAFS proposal.

IX. PNWTIRC annual meeting

Next year’s meeting will be held Thursday, October 19, 2017

IX. Meeting adjourned

The meeting adjourned about 3:00 pm.

APPENDIX V

Financial Statement 2015-2016

PNWTIRC Financial Support for Fiscal Year 2015-2016

Regular members ¹	96,000
Associate members ¹	4,000
Contracts	2,000
Forest Research Laboratory, Oregon State University ²	124,341
Total	226,341

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.