

Pacific Northwest Tree Improvement  
Research Cooperative  
**Annual Report**  
**2017-2018**

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

Glenn Howe, Jennifer Kling, Anna Magnuson  
Oguz Urhan, Susan McEvoy, Brad St.Clair



*Photo by Florian Weisenhofer*

# PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

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



2017-2018

Annual Report

## Report editors

Glenn Howe    Jennifer Kling    Anna Magnuson  
Oguz Urhan    Susan McEvoy    Brad St.Clair

*Cover photo: Clumped retention of Douglas-fir and western hemlock on a variable retention harvest unit on WDNR-managed trust land in Clark County, Washington. Photo by Florian Deisenhofer.*

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# CONTENTS

## Introduction

About the PNWTIRC.....	1
PNWTIRC participants.....	2
Highlights of 2017-2018.....	3
Message from the Director.....	4
Annual meeting agenda.....	5
Overview.....	6

## Research Presentations

Developing a Multi-trait Rust Resistance Index for Western White Pine.....	11
Axiom Genotyping Array for Western White Pine.....	23
PNWTIRC/NWTIC Genomic Selection Research.....	31
Introduction to the Species Potential Habitat Tool.....	65
Budget and Other Business.....	87

## Appendices




Appendix I. Collaborations and grants.....	89
Appendix II. Annual meeting minutes.....	90
Appendix III. PNWTIRC research needs.....	93
Appendix VI. Financial statement.....	95

# 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 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 2017-2018

- Research Coordinator, Scott Kolpak, took a job as an area geneticist with the USFS, after nine years with the PNWTIRC.
- Susan McEvoy completed the bioinformatics for the western white pine Axiom genotyping array before leaving for graduate school at the University of Connecticut.
- Graduate student, Oguz Urhan, continued his work on developing a rust index for improving resistance to white pine blister rust in western white pine. This is a collaboration with Marc Rust, Richard Sniezko, and others. After performing principal component analysis (PCA) on a variety of rust traits, Oguz found that PC1 and PC2 are good indices of rust resistance, and seem reflect different rust resistance mechanisms. He showed that rust resistance is highly heritable and that substantial gains in quantitative resistance can be obtained from the eight open-pollinated progeny tests he studied.
- We continued to lay the foundation for an Axiom genotyping array for western white pine. We sequenced RNA samples, producing 66-73 million raw reads. These were combined with existing sequences from the Canadian Forest Service to improve transcriptome assembly. After assembling the transcriptome using *de novo* assembly, we discovered ~1.9M potential SNPs using bioinformatic analyses, and designed an Axiom genotyping array.
- PNWTIRC Director, Glenn Howe, continued to serve on the Conifer SNP Consortium (CSC) Executive Committee. The Conifer SNP Consortium will provide a financially feasible pathway for genotyping SNPs in Douglas-fir for applications such as genotype ID and genomic selection.
- The PNWTIRC continues to work with Keith Jayawickrama and Terrance Ye to develop operational approaches for using genomic selection in Douglas-fir breeding programs.
- The PNWTIRC continues to work with the USFS (Brad St.Clair) and Conservation Biology Institute (Nik Stevenson-Molnar and Brendan Ward) on the development and delivery of the Seedlot Selection Tool (SST; <https://seedlotselectiontool.org/sst/>) and the Species Potential Habitat Tool (SPHT).
- Lauren Magalska (Port Blakely) was elected to continue as the Policy/Technical Committee Chair for the PNWTIRC.

## MESSAGE FROM THE DIRECTOR

Last year was one of transitions. Scott Kolpak, who's been with the PNWTIRC since 2009, took a job as area geneticist with the U.S. Forest Service. He'll be working at the Supervisor's Office on the Umpqua National Forest in Roseburg, Oregon. This is an exciting change for him—he'll be providing technical guidance and training on genetic resource management for the USFS. This includes making recommendations on species and seed sources for reforestation, managing seed orchards, developing conservation plans, using genetics to help forests resist insects and disease, and helping forests adapt to climate change. Scott was involved in many PNWTIRC projects, including the genetics of wood stiffness, Miniaturized Seed Orchard Study, Drought Hardiness Study, development of SNP genetic markers for Douglas-fir, and genomic selection. We will surely miss his talents behind the computer, in the laboratory, and in the field.

Susan McEvoy also moved on to graduate school at the University of Connecticut, to begin a graduate degree (M.S.) in bioinformatics. At OSU, Susan mostly worked on western white pine genomics, which was funded by the USFS Special Technology Development Program. However, she also made important contributions to the PNWTIRC. These include helping on the bioinformatics needed to develop the Axiom genotyping array for Douglas-fir, and using her programming skills to enhance the Tree Genome Simulator, which we're using in our genomic selection research.

So, who's left? Remaining personnel include quantitative geneticist, Jennifer Kling, Program Manager, Anna Magnuson, and graduate student Oguz Urhan. We will also rebuild by welcoming Meridith McClure, a new Master's student, to the Department of Forest Ecosystems and Society. Meridith will be selecting her research topic during the 2018-2019 academic year, and this may involve research with the PNWTIRC. Finally, during 2018-2019, we will fill the hole left by Scott's departure by increasing Anna's PNWTIRC appointment from 10% to full-time employment. We're excited to be able to make additional use of Anna's broad set of skills in genetic research and management. Finally, PNWTIRC research benefits enormously by our collaborations with the Northwest Tree Improvement Cooperative, including Keith Jayawickrama's extensive tree breeding experience and Terrance Ye's deep understanding of quantitative genetics.

Glenn Howe, PNWTIRC Director



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**AGENDA – THURSDAY OCTOBER 18, 2018**  
**– ANNUAL MEETING –**  
**PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH**  
**COOPERATIVE (PNWTIRC)**

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**START TIME** 9:00 AM for coffee; 9:30 AM for presentations  
**LOCATION** North Willamette Research and Extension Center  
15210 NE Miley Rd, Aurora, OR  
**CONTACT TEL** 541-730-3400 (Glenn)  
**LOCATION TEL** 503-678-1264  
**LUNCH** Lunch provided

<b>Time</b>	<b>Topic</b>	<b>Responsibility</b>
9:00-9:30	Coffee	
9:30-9:45	Welcome and introductions	Lauren Magalska
9:45-10:00	Overview <ul style="list-style-type: none"> <li>• <i>PNWTIRC personnel changes</i></li> <li>• <i>PNWTIRC accomplishments for 2017-18</i></li> <li>• <i>PNWTIRC plans for 2018-19</i></li> </ul>	Glenn Howe
10:00-10:30	Breeding for resistance to white pine blister rust in western white pine	Oguz Urhan Glenn Howe
10:30-10:45	Break	
10:45-11:30	PNWTIRC/NWTIC genomic selection research	Glenn Howe
11:30-12:00	Update - Seedlot Selection Tool/Species Potential Habitat Tool	Brad St.Clair
12:00-1:00	Lunch	
1:00-2:00	Research needs – Breakout groups and discussion	Josh Sherrill
2:00-2:15	Break	
2:15-2:30	Budget and other business <ul style="list-style-type: none"> <li>• <i>Budget presentation and vote</i></li> <li>• <i>Elect new Policy/Technical Committee Chair</i></li> </ul>	Glenn Howe Lauren Magalska
2:30-3:00	PNWTIRC engagement with OSU COF and USFS PNWRS	Glenn Howe Brad St.Clair
3:00	Wrap-up and adjourn	Glenn Howe

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## Overview – 2017-2018

By Glenn Howe

Glenn Howe began this year's annual meeting by presenting an overview of PNWTIRC personnel changes, collaborations, and grants for 2017 – 2018. Scott Kolpak, PNWTIRC Research Coordinator, left to take a job as area geneticist with the U.S. Forest Service, and Susan McEvoy, Bioinformatician, left to start a Master's degree at the University of Connecticut. Current PNWTIRC staff include Glenn Howe (Director), Jennifer Kling (Research Scientist), and Anna Magnuson (Program Manager). Oguz Urhan is continuing with the PNWTIRC as a graduate student, Lauren Magalska (Port Blakely) served as the Policy/Technical Committee Chair, and Brian Baltunis (Weyerhaeuser) served as the CAFS representative for OSU.

# **PNWTIRC Annual Meeting 2018**

## **October 18, 2018**

**Glenn Howe**

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

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## **PNWTIRC mission**

### ***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

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## PNWTIRC personnel

### 2017-2018

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

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## Personnel changes in 2017-18

### Scott Kolpak took a job with the USFS

- Scott worked for the PNWTIRC for 9 years!
- Served as PNWTIRC Research Coordinator and OSU Senior Faculty Research Assistant
- Among the many things he did, he played a major role in...
  - *Genetics of wood stiffness*
  - *Miniaturized Seed Orchard Study*
  - *Drought Hardiness Study*
  - *Douglas-fir transcriptome sequencing*
  - *Douglas-fir genomic selection, etc, etc, etc*
- Now an area geneticist with the USFS (Umpqua NF)



## Personnel changes in 2017-18

### Susan McEvoy left for graduate school

- Worked for OSU for a little more than 1 year
- She was hired to work on the western white pine project (USFS STDP project)
- She completed the bioinformatics for the western white pine Axiom genotyping array
- She also worked on the Tree Genome Simulator, which we're using for the PNWTIRC/NWTIC genomic selection project
- She loved the bioinformatics so much that she decided to pursue an M.S. degree with Jill Wegrzyn at the University of Connecticut



## Personnel changes in 2017-18

### Jennifer Kling continues part-time

- Jennifer is a quantitative geneticist that has worked for the PNWTIRC for 2.5 years
- Jennifer reduced her hours substantially during 2017-2018, but will continue working for the PNWTIRC
- She has been focusing on the PNWTIRC genomic selection project



## 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, 2012-2018, \$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.
- **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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## Future of CAFS (Phase II ended in 2018)

### CAFS Phase III will continue via CIPS/VMRC

- Center for Advanced Forestry Systems is part of the NSF Industry/University Research Center Program
- To be led by Jeff Hatten, a soil scientist in the Department of Forest Engineering and Resource Management
- Involved cooperatives are...
  - *Center for Planted-forest Silviculture (CIPS), Doug Maguire, Director*
  - *Vegetation Management Research Cooperative, Carlos Gonzalez-Benecke, Director*
- University of Maine will be the lead institution with a potential focus on lidar



## Developing a Multi-trait Rust Resistance Index for Western White Pine

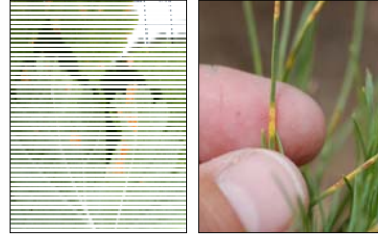
By Oguz Urhan, Marc Rust, Mary Frances Mahalovich, Richard Sniezko, and Glenn Howe

Western white pine (WWP, *Pinus monticola*) is an economically and ecologically important conifer that has been severely impacted by white pine blister rust (WPBR), a disease caused by a non-native fungal pathogen (*Cronartium ribicola*). Resistance to WPBR may be (1) ontogenetic or age-related, (2) qualitative (i.e., controlled by one or a few genes), or (3) quantitative (i.e., exhibiting the characteristics of a quantitatively inherited trait). To evaluate the genetics of quantitative resistance, we measured individual growth and rust traits, and then developed a multi-trait rust resistance index using data from eight open-pollinated progeny tests in Idaho (60 to 700 families each). Data on height (HT), diameter (DBH), rust infection (INF), rust mortality (RMORT), rust location (RLOC), and number of cankers (CANK) were used to estimate heritabilities, inter-trait genetic correlations, age-age genetic correlations, and potential genetic gains. We concluded that multi-trait principal component scores (PC1 and PC2) captured genetic variation associated with different rust resistance mechanisms. Heritabilities for individual rust traits and PC scores (0.00-1.00) were generally higher than heritabilities for growth traits (0.00-0.20). Among the rust traits, the heritabilities were usually largest for INF. Heritabilities were low to moderately high for PC1 (0.00 to 0.63), but consistently low ( $< 0.25$ ) for PC2 and PC3. Genetic correlations were slightly negative to moderately positive (-0.26 to 0.49) between PC1 and PC2 versus growth traits, indicating that rust resistance and growth can be improved simultaneously. The age-age genetic correlations between PC1 and PC2 ranged from 0.11 to 1.00 between ages 10-14 versus age 19. This indicates that early selection for rust resistance is possible.

## ***Developing a Multi-trait Rust Resistance Index for Western White Pine***

**Oguz S. Urhan**

*Dept of Forest Ecosystems and Society  
Oregon State University*



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## **Today's talk**

- Western white pine
- White pine blister rust
- Quantitative versus qualitative rust resistance
- Overview of breeding programs
- Why a rust index is needed
- Methods used to develop a rust index
- Results
- Conclusions


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Reaches heights of 40 to 55 meters and diameters of 75 to 100 centimeters


Historically covered 90% of moist forests in the northern Rocky Mountains

Widely used for lumber, especially for interior paneling, windows, and panel doors



*Wood magazine*

Western white pine (WWP) is an economically and ecologically important conifer



Blister rust causes economic and ecological damage

90% of the WWP stands in the inland Northwest have been killed or damaged by rust

Loss of white pines has had serious effects on biodiversity, hydrology, and wildlife






White pine blister rust causes heavy mortality on white pines







Needle spots are the first symptom

Stem or branch infections appear three to nine months after needle infection


Later, stem cankers appear and produce resinosis and necrosis




A



B




C



D

Trees exhibit needle and stem symptoms





Single gene (qualitative) resistance is a successful resistance mechanism

Resistance gene (*Cr1*) in sugar pine

Resistance gene (*Cr2*) in WWP

But the pathogen (*Cronartium ribicola*) evolves over time





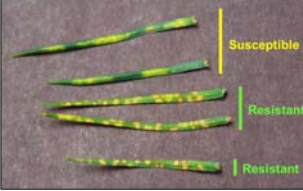



Photo by Richard Sniezko

Single gene resistance




Quantitative (multiple gene) resistance involves a reduction in disease symptoms


Slow canker growth, less stem infection, and higher survival after infection

Also called partial resistance

More durable than single gene resistance



Quantitative resistance



USFS and Inland Empire Tree Improvement Cooperative (IETIC)

USFS Dorena Genetic Resource Center (DGRC)

British Columbia Ministry of Forests

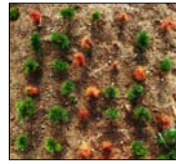


Collaborators  
Three main breeding programs



## 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



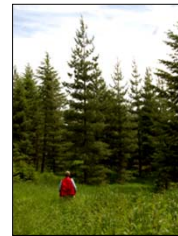
Susceptible family



Resistant family



Seed orchard



Plantation

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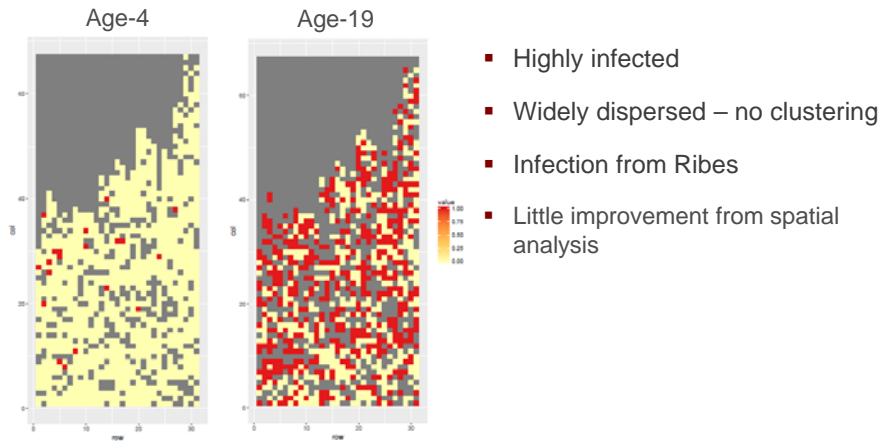
## Developing a multi-trait rust index

### Project

- IETIC tests
  - *Traditional progeny tests*
  - *Performance tests*
  - *Inoculation trials*
- Dorena tests
  - *Dorena RV6 diallel tests*
  - *Inoculation trials*



## Previous work on rust index



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## A good rust index

$$\text{Rust index} = f(\text{???}) + f(\text{???}) + f(\text{???})$$

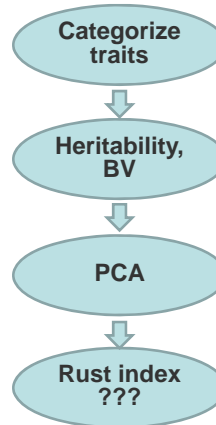
- Simple to measure
- Heritable
- Captures multiple-rust resistance mechanisms
- Can be measured at an early age
- Correlated with long-term performance

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## Methods

- Categorize traits to focus on rust traits that are common to all tests
- Estimate heritability and eliminate traits if they have low heritability
- Conduct principle component analyses across all plantations and ages
- Calculate individual PC scores, heritabilities, and breeding values



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## Analysis methods depend on the trait



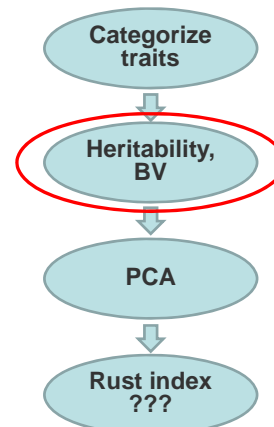
*Journal of Statistical Software*

January 2010, Volume 33, Issue 2. <http://www.jstatsoft.org/>

MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package

Jarrod D. Hadfield  
University of Edinburgh

- Can be used to analyze normally distributed, binary, count, and ordinal data
- Uses a Bayesian approach



## PC1 and PC2 may represent different rust resistance mechanisms

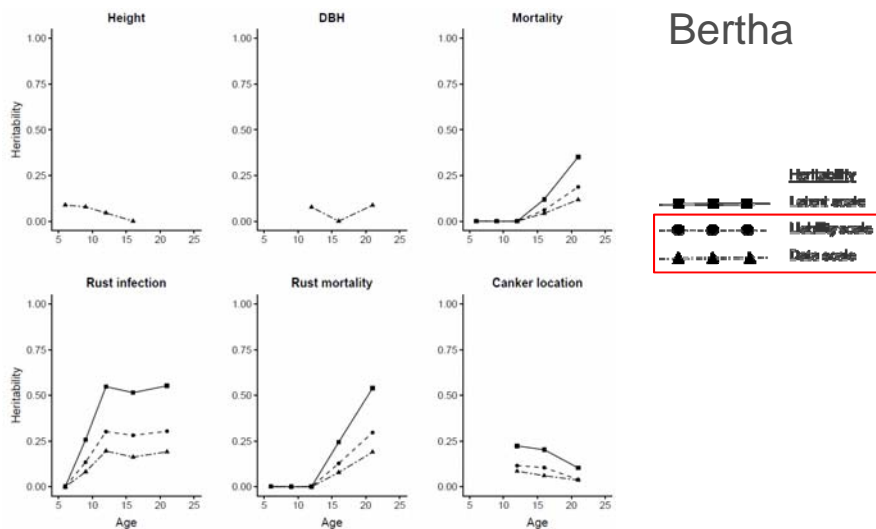
Across site PC scores		
PCs	Proportion %	Cumulative %
PC1	50.88	50.88
PC2	28.13	79.00
PC3	19.75	98.75
PC4	1.25	100.00

PCs	Higher scores indicate genotypes with:
PC1	Less infection, lower mortality, and fewer cankers on the branches and bole
PC2	Higher infection, but lower mortality and fewer cankers on the bole

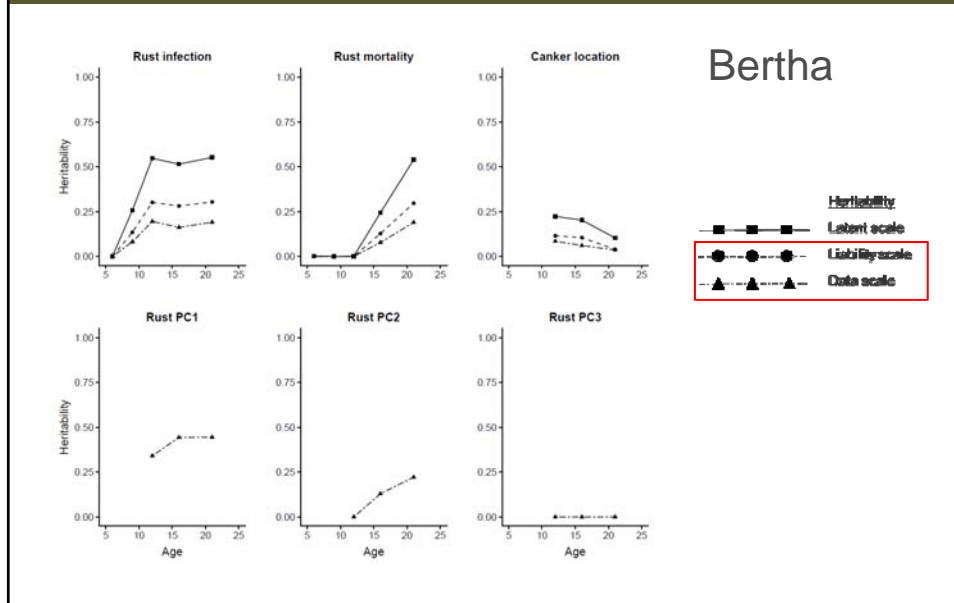
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## Heritabilities for rust are moderately high



## PC1 and PC2 are heritable



## Selection for rust resistance and growth is possible

Bertha genetic correlations			
Trait	Age	Age 19	
		PC1	PC2
HT	10	0.33	0.30
HT	14	0.33	0.31
DBH	10	0.29	0.26
DBH	14	0.36	0.32
DBH	19	0.36	0.24

## Early selection for rust resistance is possible

Bertha genetic correlations			
Trait	Age	Age 19	
		PC1	PC2
INF	10	-0.73	-0.71
INF	14	-0.77	-0.61
INF	19	-0.84	-0.61
MORT	10	-0.32	-0.52
MORT	14	-0.47	-0.71
MORT	19	-0.71	-0.91
LOC	10	-0.64	-0.64
LOC	14	-0.65	-0.37
LOC	19	-0.58	-0.12

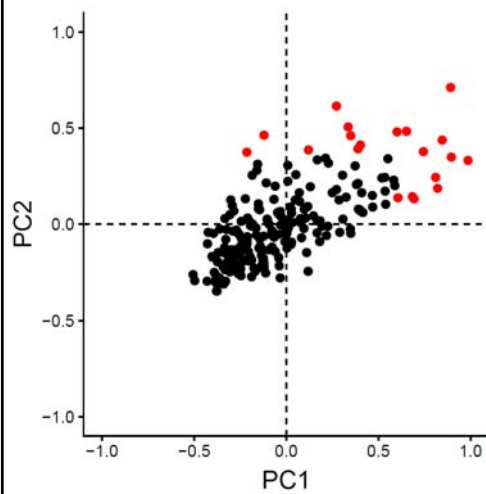
Higher values of PC1 and PC2 indicate greater resistance

## Early selection is possible using PCs (e.g., age 10)

Bertha genetic correlations			
Trait	Age	Age 19	
		PC1	PC2
PC1	10	0.76	0.79
PC2	10	-0.21	0.11
PC1	14	0.87	0.71
PC2	14	0.47	0.74
PC1	19	1.00	0.72



## We can use PCs to select for rust resistance



### Bertha

Ind = mean of all individuals

BV = mean of breeding values for all families

BV24 = mean of breeding values for top 24 families based on PC1 and PC2

Trait	Age	Mean		
		Ind.	BV	BV24
INF	19	0.72	0.00	-0.54
MORT	19	0.58	0.00	-0.52
LOC	19	0.74	-0.00	-0.06

## Conclusions

- PC1 and PC2 are good indices of rust resistance
- PC1 and PC2 may represent different rust resistance mechanisms
- Heritabilities for rust traits are moderately high, especially at older ages
- Early selection is possible (e.g., age 10)
- Resistance and growth traits can be improved simultaneously
- MCMCglmm R package can be used to estimate genetic parameters and breeding values of binary, ordered, and count traits



## **Axiom Genotyping Array for Western White Pine**

By Glenn Howe, Susan McEvoy, and Scott Kolpak

We are developing tools for genomic breeding in western white pine (WWP). Ultimately, we will use these tools to improve resistance to white pine blister rust. Our immediate goal is to develop a high-density (50K SNP) genotyping array for WWP. This tool will allow breeders to use an approach called genomic selection to improve traits such as disease resistance and growth. To accomplish this, we (1) sequenced WWP genes using RNAseq, (2) assembled a transcriptome consisting of 416,923 contigs from 277,011,758 western white pine RNA sequences, (3) evaluated and annotated the transcriptome using a software pipeline called EnTap, (4) discovered ~1.9M potential SNPs using bioinformatic analyses, and (5) designed an Axiom genotyping array. The next steps are to screen a large number of SNPs (e.g., 420K) on a modest number of trees, and then use the resulting data to design the final 50K SNP chip. This work is being planned as part of the Conifer SNP Consortium, but we will need to acquire new funds to complete these next steps.

## Axiom Genotyping Array for Western White Pine

Glenn Howe  
Susan McEvoy  
Scott Kolpak

Single nucleotide polymorphism (SNP)

	SNP		
	1	2	
Tree 1	A	G	Maternal chrom.
	C	A	Paternal chrom.
Tree 2	G	G	Maternal chrom.
	C	G	Paternal chrom.
Tree 3	C	A	Maternal chrom.
	A	A	Paternal chrom.

Tree 1 is heterozygous    Trees 2 and 3 are homozygous



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## Background

- Objectives
  - Design an Axiom genotyping array for western white pine
  - Use the array as a foundation to attract funds to manufacture the array
  - Ultimately, use genomic selection to breed for resistance to white pine blister rust
- Funding
  - USFS Special Technology Development Program (STDP)
  - Center for Advanced Forestry Systems (CAFS)

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## Steps

- Tissue samples
- RNA sequences
- Assembly of the RNA sequences
- Gene annotation
- SNP discovery
- Array design
- Genomic selection

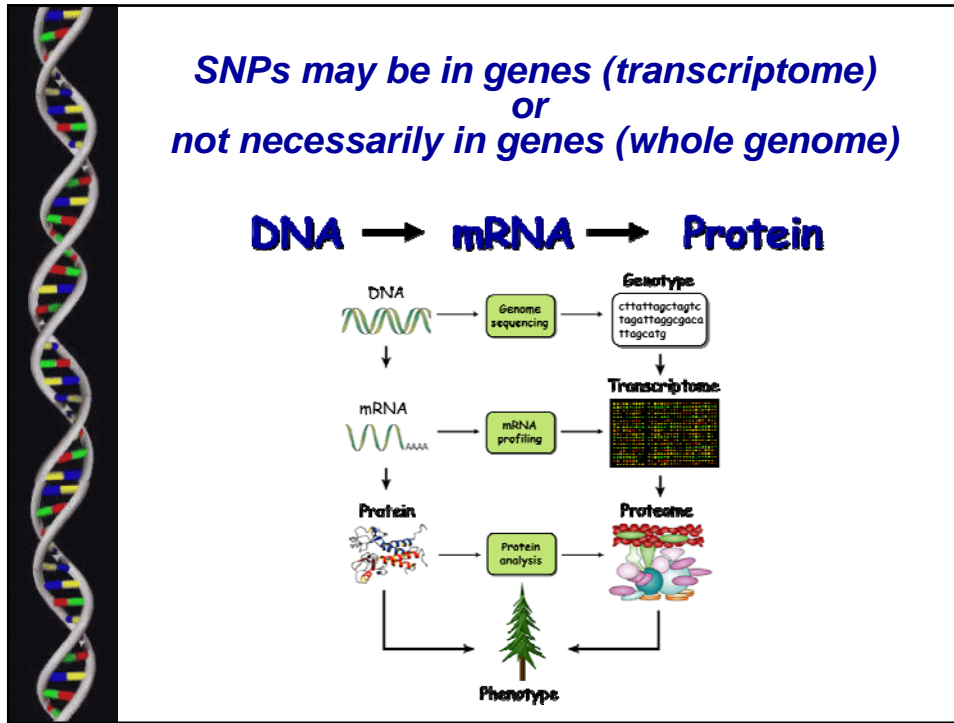
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## Single nucleotide polymorphism (SNP)

							<b>SNP</b>									
							↓									
<b>Tree 1</b>	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.	
<b>Tree 2</b>	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.	
<b>Tree 3</b>	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*



## Tissue samples

- Tissues included in the transcriptome
  - Needles, branches, stems, roots, buds
- Three WWP breeding programs<sup>1</sup>
  - USFS DGRC
  - USFS / IETIC
  - BC Ministry of Forests
- Adjusted the final RNA pool to maximize the variety of genes

**Table 1.** Western white pine tissues were collected from the greenhouse or field, and then used for extracting RNA for high-throughput sequencing. The numbers in parentheses represent the approximate numbers of unrelated trees, full-sib families, or half-sib families in the tissue collection.

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)	—	—

<sup>1</sup> DGRC = Dorena Genetic Resource Center, IETIC = Inland Empire Tree Improvement Cooperative

## OSU RNA Sequences

### RNA sequencing

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



**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
<b>66 to 73 million reads produced</b>		<b>277,011,758</b>

## Canadian Forest Service sequences

### Collaborator is Jun-Jun Liu

- Take advantage of existing sequences to improve assembly
- Retrieved from the European Nucleotide Archive (ENA)
- Tissues – *Pinus monticola* primary needles and shoot-tip cDNA libraries



Run accession	Tax ID	Scientific name	Instrument model	Library layout	FASTQ files (FTP)	FASTQ files (Galaxy)
SRR3273237	3345	<a href="#">Pinus monticola</a>	Illumina HiSeq 2000	PAIRED	<a href="#">File 1</a> <a href="#">File 2</a>	<a href="#">File 1</a> <a href="#">File 2</a>



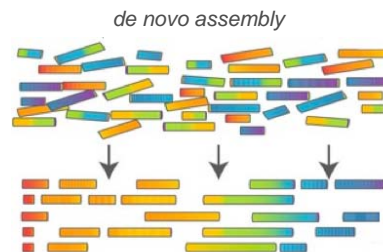
## Assembling the transcriptome

Sequences 'reads' are joined into longer sequences (contigs) using overlaps



Illumina HiSeq 2500

Sequence Reads  
TATCACGATCTCTCTGATTCCG



## EnTAP pipeline used for gene annotation

Goal is to clean the assembly and infer what the genes do

### EnTAP: Bringing Faster and Smarter Functional Annotation to Non-Model Eukaryotic Transcriptomes

Alexander J. Hart<sup>1</sup>, Samuel Ginzburg<sup>1</sup>, Muyang (Sam) Xu, Cera R. Fisher,<sup>1</sup> Nasim Rahmatpour<sup>1</sup>, Jeffrey B. Mitton<sup>2</sup>, Robin Paul<sup>1</sup>, Jill L. Wegrzyn<sup>1\*</sup>

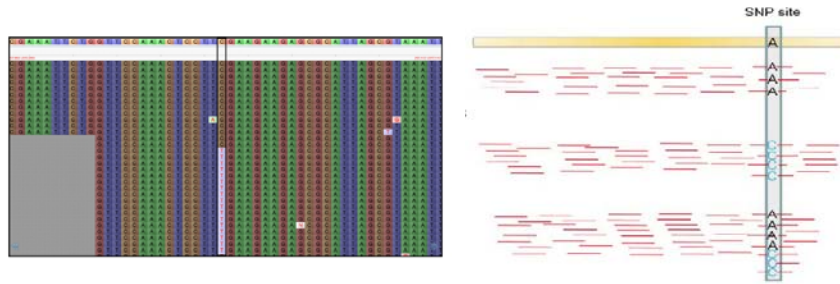
<sup>1</sup>Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

<sup>2</sup>Department of Ecology and Evolutionary Biology, University of Colorado Boulder, Boulder, CO, USA 80309

Corresponding Author: Jill L. Wegrzyn: [jill.wegrzyn@uconn.edu](mailto:jill.wegrzyn@uconn.edu)

# SNP discovery

- Mapping original reads to the assembly gives us a pileup
- Scripts look for SNPs in pileup
- Differences can be SNPs or sequencing errors – the more reads the better



# Final SNP file to send to ThermoFisher

Axiom design and manufacture will occur when funds become available for the WWP array

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	
Organism	SNP ID	REF	ALT	SEQ	CHR	POS	IMP_NEIGHBOR	IMP_ORI	IMP_VAL	IMP_TSS																								
Pinus	Pinus SNP_PNF01C_00005_2054	variant	G	T	AT	2054	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2055	variant	T	G	T	2055	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2056	variant	G	A	T	2056	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2057	variant	G	A	T	2057	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2058	variant	A	T	2058	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2059	variant	T	T	2059	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2060	variant	T	T	2060	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2061	variant	G	A	T	2061	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2062	variant	G	A	T	2062	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2063	variant	T	T	2063	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2064	variant	T	T	2064	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2065	variant	G	A	T	2065	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2066	variant	T	T	2066	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2067	variant	T	T	2067	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2068	variant	G	A	T	2068	0	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2069	variant	T	T	2069	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2070	variant	T	T	2070	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2071	variant	T	T	2071	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2072	variant	T	T	2072	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2073	variant	T	T	2073	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2074	variant	T	T	2074	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2075	variant	T	T	2075	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2076	variant	T	T	2076	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2077	variant	T	T	2077	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2078	variant	T	T	2078	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2079	variant	T	T	2079	0	+	+	+	+																								
Pinus	Pinus SNP_PNF01C_00005_2080	variant	T	T	2080	0	+	+	+	+																								



## Axiom genotyping array for western white pine

### *Large-scale genotyping and genomic selection*



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## Acknowledgements

### Thanks to....

- Mike Crawford, BLM, Tyrell Seed Orchard
- Alvaro Hernandez, University of Illinois, Roy J. Carver Biotechnology Center
- Angelia Kegley, USFS, Dorena Genetic Resource Center
- John King, British Columbia Ministry of Forests and Range
- Marc L. Rust, University of Idaho, Inland Empire Tree Improvement Cooperative
- Mary Frances Mahalovich, USFS
- 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 STDP Program
- CAFS, Center for Advanced Forestry Systems
- Center for Genome Research and Biocomputing, Oregon State University

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## **PNWTIRC/NWTIC Genomic Selection Research**

By Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak

Genomic selection uses a genome-wide set of markers designed to predict breeding values for tree improvement. It has been widely used in the livestock breeding industry, and should be valuable to tree breeders as well. Genomic selection can be directly incorporated into current breeding programs by using early marker-assisted selection to reduce breeding intervals and minimize the amount of progeny testing needed to identify seed orchard candidates. This approach also offers the ability to select for difficult-to-measure traits and increase heritabilities. We identified ~28k reliable SNPs that can be assayed using an Affymetrix Axiom genotyping array for Douglas-fir, and successfully demonstrated the potential of genomic selection. Although genomic selection can reduce field testing, genotyping costs remain high. Thus, further research is needed to overcome this hurdle and make implementation of genomic selection economically favorable. Moving forward, our research will focus on further validation tests for genomic selection and finding ways to reduce the cost of implementation to tree breeders. Specifically, our objectives are to (1) develop the tools (e.g., protocols and software) needed to practice genomic selection in a cost-effective way, (2) compare baseline phenotypic selection and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use multi-stage selection to substantially reduce genotyping costs, (4) obtain new breeding values from the NARA genomic selection field test, (5) test whether we can use a combination of high-density and low-density arrays (HD/LD arrays) to substantially reduce genotyping costs, (6) develop a high-density SNP linkage map for Douglas-fir, and (7) hold workshops on how to practice genomic selection in Douglas-fir.

## PNWTIRC/NWTIC Genomic Selection Research

**Glenn Howe**  
PNWTIRC  
Oregon State University  
October 18, 2018

The flowchart illustrates the genomic selection process. It starts with a 'Training population' (trees) with 'Known SNP genotypes and phenotypes'. This leads to a 'Prediction equation' box containing the formula: 
$$\text{Genomic breeding value} = w_1x_1 + w_2x_2 + \dots + w_px_p$$
. Below this equation is an image of an Affymetrix GeneChip microarray. The 'Training population' also leads to 'Selection candidates' (trees) with 'SNP genotypes'. The 'Prediction equation' and 'Selection candidates' both lead to 'Selected trees' (trees), which are 'Based on genomic breeding values'.

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## Collaborative project

### Key funding

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

### 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/NWTIC)

**PNWTIRC**  
Glenn Howe  
Jennifer Kling  
Scott Kolpak  
Susan McEvoy

**NARA**  
Keith Jayawickrama  
Terrance Ye  
Hao Truong  
Matt Trappe

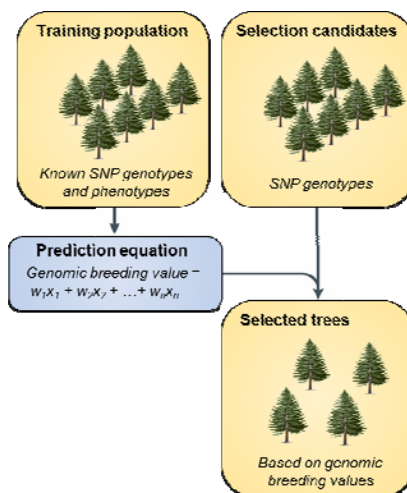
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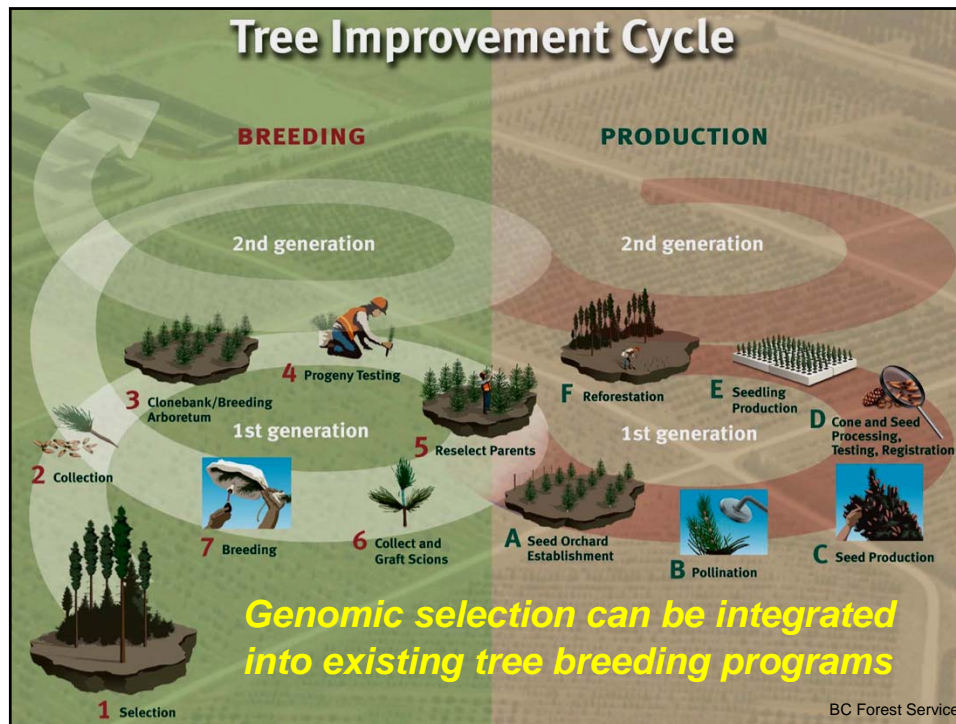
# Background

## Genomic selection

### *How does it work?*

- 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





## Potential advantages of genomic selection

*The selection of genetically superior trees based on genomic information rather than on directly measured phenotypes*

- Skip an entire cycle of field testing
- Shorten the generation interval
- Reduce the size of field tests by using genomic selection for early culling
- Increase heritabilities
- Select for difficult to measure traits (e.g., mature traits at an early age)



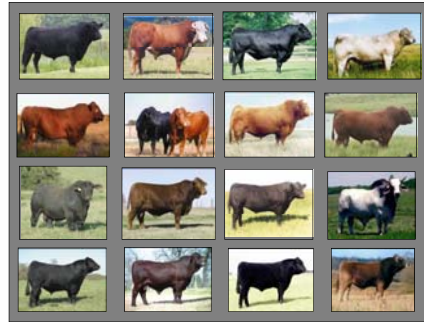
## Livestock breeders have led the way

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.



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## However...

We're engaged in genomic selection research because...

***In theory, there's no difference between theory and practice....***

***In practice, there is!***

— attributed to Jan L. A. van de Snepscheut

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# How does genomic selection work?

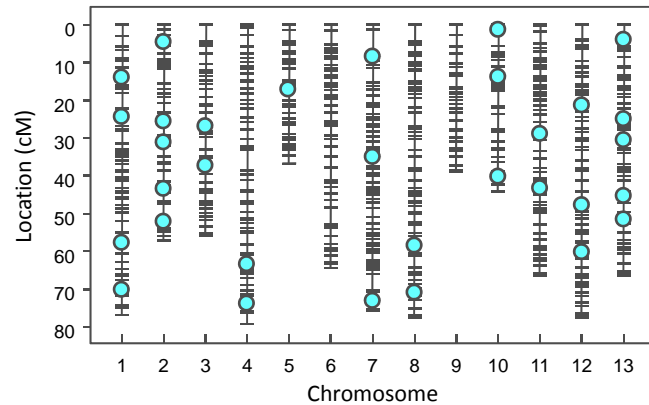
## Genomic selection

Relies on markers linked to quantitative trait loci

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## Genomic selection

Relies on markers linked to quantitative trait loci



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## Genomic selection

**Genomic selection markers 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**

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## Genomic selection

Particularly 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

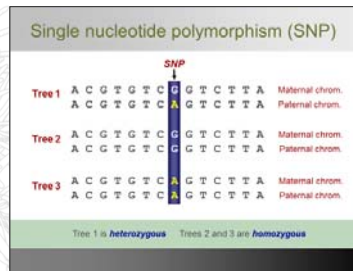
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## Axiom Genotyping Array

## Affymetrix Axiom Genotyping Array for Douglas-fir

Glenn Howe  
Keith Jayawickrama  
Scott Kolpak  
Jennifer Kling  
Matt Trappe  
Valerie Hipkins  
Terrance Ye  
Stephanie Guida  
Rich Cronn  
Sam Cushman  
Susan McEvoy



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## Axiom SNP characteristics (CR = 60%)

*Unrelated Coastal Douglas-fir only*

**55,766 SNPs attempted**

**27,699 SNPs polymorphic and 'called'**

**24,574 SNPs = polymorphic, 'called', HWE**

Statistic	Mean	Median	Min	Max
Call rate (%)	95.7	99.2	52.7	1.000
Polymorphic information content	0.261	0.284	0.004	0.375
Heterozygosity	0.319	0.338	0.004	0.635
Minor allele frequency	0.236	0.220	0.002	0.500

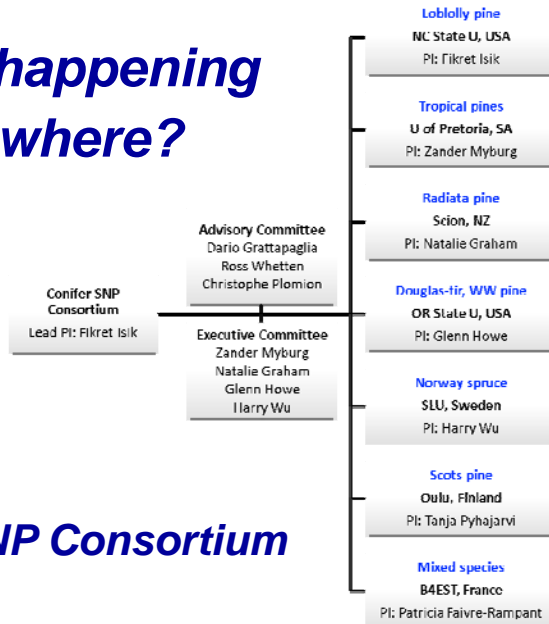
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# What's happening elsewhere?

## What's happening elsewhere?

### Conifer SNP Consortium



# Specialty Wood Products

Research Partnership



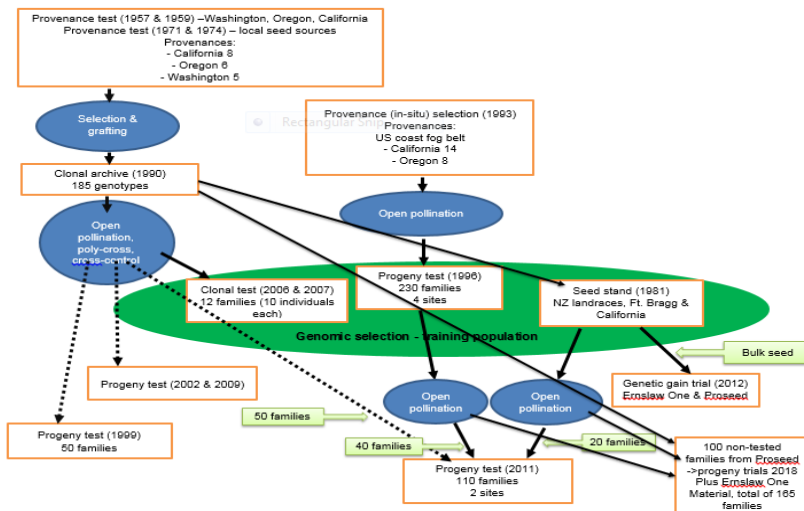
## Update on Genomic Selection in Douglas-fir

Jaroslav Klapšte, Mari Suontama, Toby Stovold, Mark Miller, Kane Fleet, Heidi Dungey, Charlie Low

Meeting Date: 6 April 2017

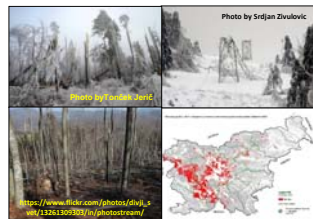


## Scion Training Population



## Identification of planted Douglas-fir stands in Slovenia, central Europe

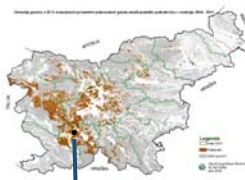
Marjana Westergren, Slovenian Forestry Institute, Slovenia  
Santiago González-Martínez, INRA, France



Ice sleet in February 2014

**Renewed interest:** Douglas-fir is currently considered as a species that will be widely planted in central Europe in the near future

No knowledge on the origin of Douglas-fir planted around 100 years ago in Slovenia (and other countries)



Bark beetle attacks 2014-2017



Douglas-fir was neither damaged by ice sleet nor by bark beetles, photo from March 2018

Funding: Slovenian Ministry of Agriculture, Forestry and Food; Research carried out by: Slovenian Forestry Institute, with the collaboration of INRA-Bordeaux (France)

## Objectives

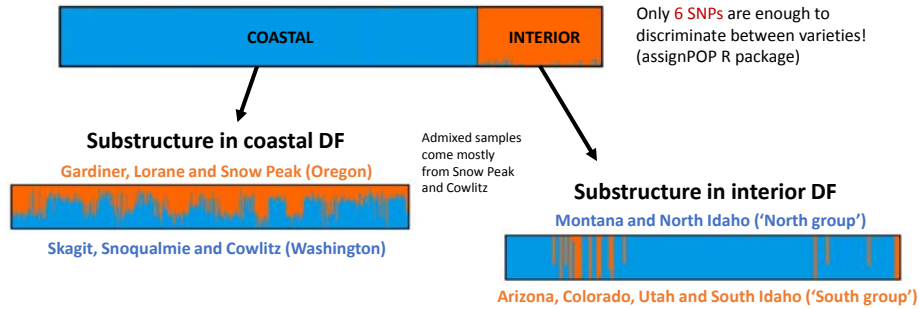
- Develop a small assay (40 SNPs) with discriminating power to identify the origin of European plantations of Douglas-fir
- Test the assay in common gardens (known origin) and plantations (unknown origin) in Slovenia (380 samples)



*Pilot study to be sourced locally using the MassArray (Sequenom) System, suitable to genotype small SNP sets for operative forestry*

## Assay based on SNPs from the Infinium dataset

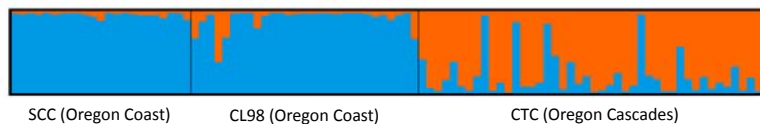
703 trees & 7,084 SNPs (after strict filtering)



Selection of best SNPs from the Infinium dataset for distinguishing gene pools within coastal and interior DF varieties on-going (available end of October)

## Assay based on SNPs from the Axiom dataset

114 coastal DF trees from Oregon & 16,599 SNPs (after strict filtering)



AX-118159886	AX-124407960	AX-119027471	AX-124418631
AX-118167575	AX-124408206	AX-119030530	AX-124421025
AX-119004563	AX-124408764	AX-119032994	AX-124421074
AX-119005864	AX-124408781	AX-119035916	AX-124423977
AX-119012663	AX-124409785	AX-123136618	AX-124426518
AX-119017017	AX-124412268	AX-124398848	AX-124427672
AX-119018022	AX-124414290	AX-124399236	AX-124428516
AX-119019479	AX-124418049	AX-124399294	AX-124430121
AX-119023665	AX-124418580	AX-124401087	AX-124430262

Best 36 SNPs discriminating between OR Coast and OR Cascade origins

# Genomic Selection Workplan

## Genomic selection workplan

Genomic selection workplan | Page 1

### Genomic Selection Workplan

*A Joint project between the PNWTIRC and NWTIC*

*Glenn Howe, Jennifer Kling, Keith Jayavickrama, Terrance Ye, and Scott Kolpak*


*October 18, 2017*

#### Summary

Genomic selection, or whole-genome marker-assisted selection, could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. The potential of genomic selection has been demonstrated in key forest tree species, and by our preliminary results in Douglas-fir. However, genotyping costs are high, probably much higher than testing trees in standard progeny tests. The purpose of this research is to directly address this cost issue. We will conduct research specifically designed to reduce genotyping costs and make genomic selection financially attractive. Our specific objectives are to (1) develop a high-density SNP linkage map for Douglas-fir, (2) compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use a combination of high-density and low-density arrays to substantially reduce genotyping costs, (4) test whether we can use early phenotypic culling to substantially reduce genotyping costs, (5) develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way, (6) hold workshops on how to practice genomic selection in Douglas-fir, and (7) obtain new breeding values from the Roseburg genomic selection field test.


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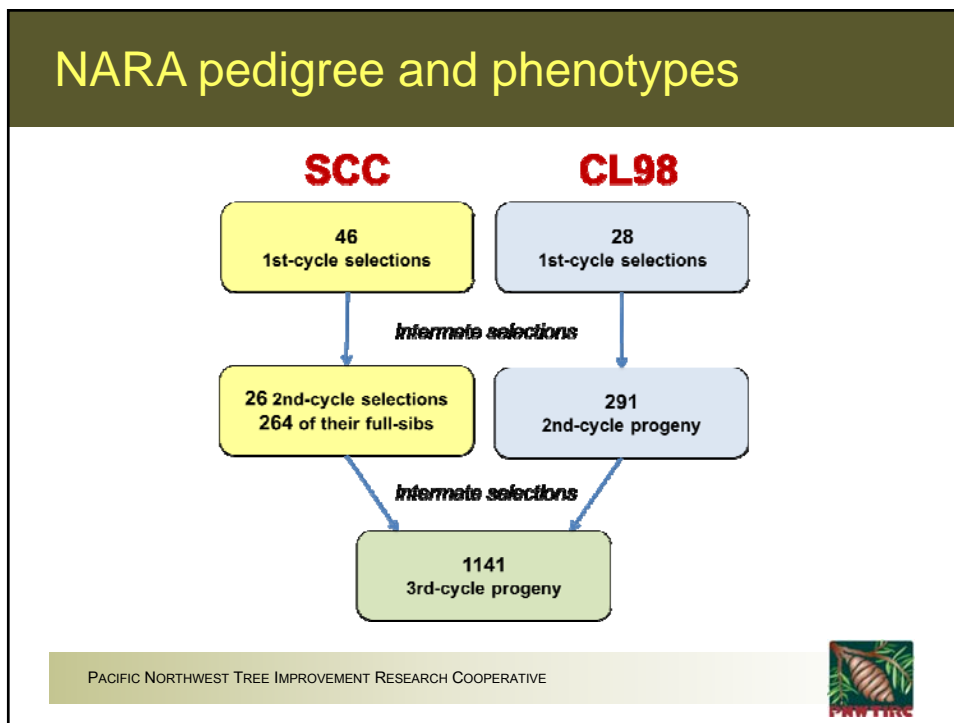




## Northwest Advanced Renewables Alliance

Keith Jayawickrama







## New crosses were outplanted

Plum Creek nursery

25 full-sib families

1146 trees

Planted on Roseburg  
property near Elkton,  
Oregon in March, 2015



*Photos from Matt Trappe*

## Genomic selection validation – NARA field test



## Genomic selection workplan

***Long-term goals are to test the effectiveness and reduce the costs of genomic selection in Douglas-fir***

Objective 1 – Tools for GS – manuals, software

Objective 2 – Baseline protocols for PS, GS

Objective 3 – Multi-stage testing

Objective 4 – Additional phenotypes for validation

Objective 5 – Combine LD/HD arrays

Objective 6 – Linkage map

Objective 7 – GS workshops

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**Objective 1:** Develop the tools (e.g., protocols and software) needed to practice genomic selection in a cost-effective way

**Objective 2:** Compare baseline phenotypic selection and genomic selection scenarios based on genetic gain per unit time and cost

**Objective 3:** Test whether we can use multi-stage selection to substantially reduce genotyping costs


# What have we learned so far?

## Across family genomic selection works

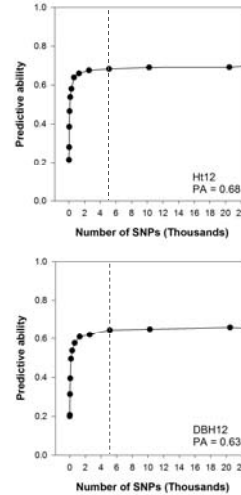
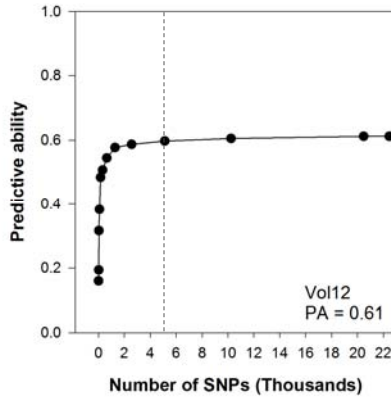
***Predictive ability is the correlation between breeding values estimated from phenotypes versus SNPs***

**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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## How many SNPs are needed?



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## Genomic selection Particularly valuable for within-family selection

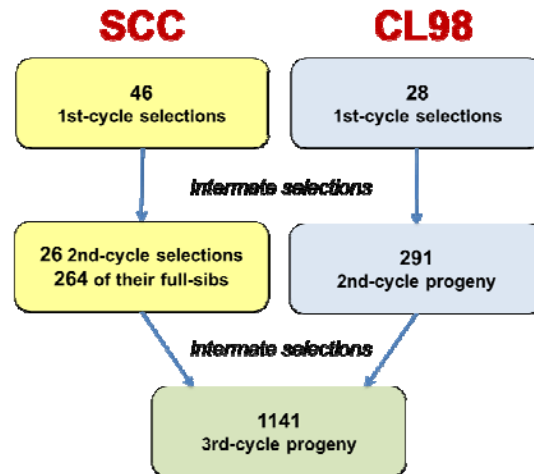
**Parent 1**    **x**    **Parent 2**  
*offspring 1*  
*offspring 2*  
*offspring 3*  
*etc*

- 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

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## NARA pedigree and phenotypes



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## 'A' matrix versus 'G' matrix

### 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

### 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

**A matrix** = Additive relationship matrix

**G matrix** = Genomic relationship matrix



## No difference between A and G regression

**BV method** = different methods for estimating breeding values in genomic regression analysis (Garrick et al 2009)

BV method	Height		DBH		Vol		Ram		Sin	
	A	G	A	G	A	G	A	G	A	G
Phenotype	0.38	0.40	0.53	0.54	0.54	0.55	0.17	0.16	0.35	0.30
EBV	0.82	0.79	0.80	0.77	0.75	0.72	0.98	0.97	0.96	0.95
dEBV	0.17	0.18	-0.06	-0.06	0.05	0.05	0.37	0.37	0.13	0.13
dEBV + PA	0.40	0.39	0.19	0.17	0.21	0.23	0.40	0.41	0.35	0.34

**Conclusion** = No apparent advantage of genomic selection

# WHY?



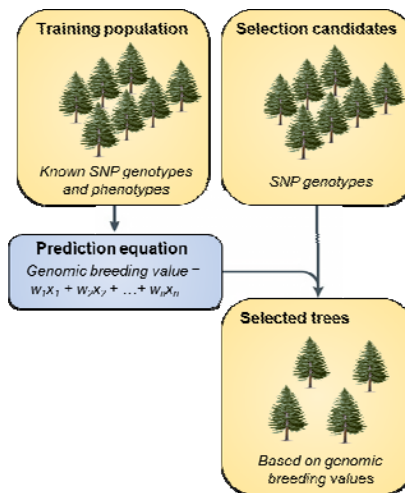
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



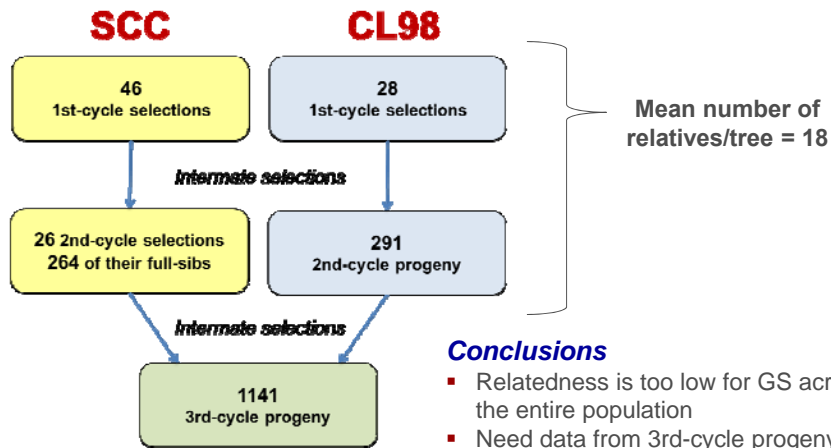
## Genomic selection

### How does it work?

- 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



## NARA pedigree and phenotypes



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



## Objective 4

Obtain new breeding values from the NARA genomic selection field test

## Genomic selection Valuable for within-family selection

Many more related trees in third cycle



Internal ID	Gene ID	plate	well	cell	female	male
41602	573070-1234	4	B01	41408	41404	
41602	573070-1235	11	F04	41408	41404	
41602	573070-1236	14	B12	41408	41404	
41602	573070-1237	12	G05	41408	41404	
41603	573070-1241	13	F04	41419	41417	
41603	573070-1242	14	H02	41419	41417	
41603	573070-1243	14	B11	41419	41417	
41603	573070-1244	14	D09	41419	41417	
41603	573070-1245	13	G02	41419	41417	
41603	573070-1246	13	H07	41419	41417	
41603	573070-1247	15	G09	41419	41417	
41603	573070-1248	22	H2	41419	41417	
41603	573070-1249	13	G04	41419	41417	
41603	573070-1250	23	F10	41419	41417	
41603	573070-1251	15	G06	41419	41417	
41603	573070-1252	14	A08	41419	41417	
41603	573070-1253	15	F07	41419	41417	
41603	573070-1254	20	A12	41419	41417	
41603	573070-1255	13	F11	41419	41417	
41603	573070-1256	20	B05	41419	41417	
41603	573070-1257	20	A09	41419	41417	
41603	573070-1258	15	F14	41419	41417	
41603	573070-1259	13	G01	41419	41417	
41603	573070-1260	15	H12	41419	41417	
41603	573070-1261	14	D07	41419	41417	
41603	573070-1262	15	B09	41419	41417	
41603	573070-1263	20	D09	41419	41417	
41603	573070-1264	20	G05	41419	41417	
41603	573070-1265	13	F06	41419	41417	
41603	573070-1266	20	C11	41419	41417	
41603	573070-1267	22	D1	41419	41417	
41603	573070-1268	15	H04	41419	41417	
41603	573070-1269	15	B06	41419	41417	
41603	573070-1270	13	G03	41419	41417	
41603	573070-1271	13	H03	41419	41417	
41603	573070-1272	13	G10	41419	41417	
41603	573070-1273	13	H04	41419	41417	
41603	573070-1274	13	H02	41419	41417	
41603	573070-1275	15	F05	41419	41417	
41603	573070-1276	15	G03	41419	41417	
41603	573070-1277	15	H10	41419	41417	
41603	573070-1278	20	H09	41419	41417	
41603	573070-1279	13	H08	41419	41417	
41603	573070-1280	15	B05	41419	41417	
41603	573070-1281	13	G07	41419	41417	
41603	573070-1282	13	G06	41419	41417	
41603	573070-1283	22	F1	41419	41417	
41603	573070-1284	20	F07	41419	41417	
41603	573070-1285	13	F03	41419	41417	
41603	573070-1286	22	F1	41419	41417	
41603	573070-1287	15	F03	41419	41417	
41603	573070-1288	15	F04	41419	41417	
41603	573070-1289	13	H05	41419	41417	
41603	573070-1290	15	B04	41419	41417	
41603	573070-1292	15	H02	41419	41417	
41603	573070-1293	15	G07	41419	41417	
41603	573070-1294	13	H09	41419	41417	
41603	573070-1295	14	H07	41419	41417	
41603	573070-1296	14	G05	41419	41417	
41603	573070-1297	15	H08	41419	41417	
41603	573070-1298	14	G06	41419	41417	
41603	573070-1299	13	F07	41419	41417	
41603	573070-1300	14	A06	41419	41417	
41604	573070-1301	13	D08	41461	41456	
41604	573070-1302	14	H01	41461	41456	
41604	573070-1303	15	G01	41461	41456	

Empirical test of genomic selection

## Tree Genome Simulator (TGS)

- Can simulate realistic tree genomes
- Assumptions/parameters can be modified
- Can simulate genomes using an input pedigree

The screenshot shows the 'Tree Genome Simulator' interface. On the left, there are configuration options for the QTL allele pool, including the number of loci, number of alleles per locus, and dominance parameters. The main window displays a table of simulated loci with columns for Locus ID, Locus effect, Additive effect, and Frequency. A central image shows a forest with the text 'Tree Simulations' overlaid.

Locus	Locus ID	Locus effect	Additive effect	Frequency	Average effect
1	1	0.97739	1.00	0.00	0.97739
2	2	0.97739	1.00	0.00	0.97739
3	3	0.97739	1.00	0.00	0.97739
4	4	0.97739	1.00	0.00	0.97739
5	5	0.97739	1.00	0.00	0.97739
6	6	0.97739	1.00	0.00	0.97739
7	7	0.97739	1.00	0.00	0.97739
8	8	0.97739	1.00	0.00	0.97739
9	9	0.97739	1.00	0.00	0.97739
10	10	0.97739	1.00	0.00	0.97739
11	11	0.97739	1.00	0.00	0.97739
12	12	0.97739	1.00	0.00	0.97739
13	13	0.97739	1.00	0.00	0.97739
14	14	0.97739	1.00	0.00	0.97739
15	15	0.97739	1.00	0.00	0.97739



## Simulate QTL alleles

**Tree Genome Simulator**

**QTL allele pool**

Number of loci genotyped (GL)

Number of loci affecting the trait (QTL)

Percent of QTL / non-QTL with dominance

Degree of dominance for QTL and non-QTL

Maximum alleles / locus

**Locus Effect Distribution**

Exponential distribution

Scale parameter (1/λ =)

**Allele Effect Distribution**

Exponential distribution

Scale parameter (1/λ =)

**Allele Frequency Distribution**

Dirichlet uniform (c = 1.0)

Shape parameter (c =)

Stop by step simulation  Multiple simulation

**Simulate QTL allele pool**

**The QTL allele pool contains 20 loci**

Locus	Locus ID	Locus effect	tau	QTL dominance	Genotyped	Allele	Additive effect	Frequency	Average effect
1	1	1.90789	1.00	no	yes	1	10.3962	0.0191	6.5459
1	1	1.90789	1.00	no	yes	2	3.0946	0.2186	2.9011
1	1	1.90789	1.00	no	yes	3	-5.4744	0.7164	-1.3644
2	2	4.45505	1.00	yes	yes	1	-14.2340	0.4205	-5.0960
2	2	4.45505	1.00	yes	yes	2	0.7089	0.4815	2.6151
2	2	4.45505	1.00	yes	yes	3	5.7166	0.0980	6.1039
3	3	1.99275	3						
3	3	1.99275	3						
4	4	1.19959	4						
4	4	1.19959	4						
5	5	3.78993	5						
5	5	3.78993	5						
6	6	4.42833	6						
6	6	4.42833	6						
7	7	0.76655	7						
8	8	6.31769	8						
8	8	6.31769	8						
9	9	1.47155	9						
9	9	1.47155	9						
10	10	4.39859	10						
10	10	4.39859	10						
11	11	7.84452	11						
11	11	7.84452	11						
12	12	1.64024	12						
12	12	1.64024	12						
13	13	6.16384	13						
13	13	6.16384	13						
14	14	4.79706	14						
14	14	4.79706	14						

**Can modify...**

- Number of loci genotyped
- Number of QTL
- Percent of QTL with dominance
- Degree of dominance
- Locus effect distribution
- Allele effect distribution
- Allele frequency distribution

## Simulate the genetic map

**Tree Genome Simulator**

**Genetic map**

Default genome simulation  User defined linkage group(s)

Number of linkage groups

**Genetic map function**

Haldane

**Distribution for Loci on Chromosomes**

Uniform distribution

Stop by step simulation  Multiple simulation

**Simulate genetic map**

**1000 loci (including 980 neutral loci) on 13 of the 13 chromosomes**

Chrom	Num of loci	Locus ID	QTL	Map position	Locus effect	Num of allel.	G_var	A_var	D_var	Std_G_var
1	76	988	no	0.52	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	478	no	2.95	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	477	no	37.01	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	391	no	27.00	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	210	no	24.70	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	734	no	32.89	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	623	no	37.93	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	626	no	38.18	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	290	no	46.40	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	181	no	46.63	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	725	no	48.04	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	828	no	49.22	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	938	no	49.41	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	172	no	49.64	0.0000	2	0.0000	0.0000	0.0000	0.0000
1	76	715	no	49.79	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	210	no	52.02	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	417	no							
1	76	874	no							
1	76	329	no							
1	76	921	no							
1	76	014	no							
1	76	863	no							
1	76	009	no							
1	76	196	no							
1	76	876	no							
1	76	508	no							
1	76	544	no							
1	76	186	no							
1	76	489	no							
1	76	352	no							
1	76	320	no							
1	76	250	no	66.40	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	729	no	74.22	0.0000	3	0.0000	0.0000	0.0000	0.0000
1	76	616	no	77.36	0.0000	2	0.0000	0.0000	0.0000	0.0000

**Can modify...**

- Number of linkage groups
- Genetic map function
- Distribution of loci on chromosomes

## Simulate first-generation parents

**Tree Genome Simulator**

File Configure Help

**Parents**

Mating system  
 Mixed mating  Random mating

Number of breeding parents: 20  
 Heritability of the trait: 0.2  
 Number of OP progeny / breeding parent: 20

Mixed mating parameters  
 Offspring from non-local males (P<sub>nl</sub>): 0.600  
 Offspring from local males (P<sub>l</sub>): 0.400

Softing (P<sub>s</sub>): 0.050  
 Parent (P<sub>p</sub>): 0.010  
 Offspring (P<sub>o</sub>): 0.010  
 Full-sib (P<sub>fs</sub>): 0.090  
 Half-sib (P<sub>hs</sub>): 0.140  
 Unrelated and local (P<sub>ul</sub>): 0.100

Inbreeding  
 Expected  User input  
 Equilibrium inbreeding (F<sub>e</sub>): 0.0909

Step by step simulation  Multiple simulation

Simulate parents

**Tree Genome Simulator**

OTL alleles Genetic map Parents Progeny SNP haplotypes

**20 breeding parents**

ID	GEN	FEM	MALE	OTL	Phen	
1	0	0	0	-1.1083	-0.3952	3, 1, 2, 2
2	0	0	0	0.1049	2.4103	3, 2, 1, 1
3	0	0	0	-0.4364	2.0520	2, 2, 1, 1
4	0	0	0	-0.3173	-4.0557	3, 1, 2, 2
5	0	0	0	-1.5240	1.2725	2, 2, 2, 2
21	1	1	0	-0.6385	-3.7504	1, 3, 2, 1
22	1	1	0	-0.6947	-0.6559	3, 2, 2, 2
23	1	1	0	-1.0675	-2.6624	1, 3, 2, 2
24	1	1	0	-1.1882	-3.4193	3, 2, 2, 2
41	1	2	0	0.1049	1.0862	3, 2, 1, 1
42	1	2	0	0.1103	-0.2042	3, 2, 1, 1
43	1	2	0	0.1049	-4.0979	3, 2, 1, 1
61	1	3	0	-0.6947	-0.6559	1, 1, 1, 1
62	1	3	0	-0.6947	-0.6559	3, 1, 1, 1
63	1	3	0	-0.6947	-0.6559	2, 1, 1, 1
64	1	3	0	-0.6947	-0.6559	1, 2, 2, 1
81	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
82	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
83	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
84	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
101	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
102	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
103	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
104	1	5	0	-0.6947	-0.6559	1, 2, 1, 1

**Can modify...**  
 Number of parents  
 Number of progeny  
 Heritability  
 Mixed-mating parameters (OP)

## Simulate advanced generations

**Tree Genome Simulator**

File Configure Help

**Parents**

Progeny/advanced-generation cross: 10  
 Number of advanced generations: 2

Mating design  
 Double-pair mating (recurrent)

Number of parents used: 10

Genetic relatedness  
 Proportion of unrelated matings (P<sub>u</sub>):  
 Proportion of related matings:  
 Number of progeny / local male:  
 Unequal progeny  Equal progeny  
 Average progeny/local male:

Estimate relatedness  
 Do not estimate  Estimate

Simulation model

Step by step simulation  Multiple simulation

Simulate progeny

**Tree Genome Simulator**

OTL alleles Genetic map Parents Progeny SNP haplotypes

**20 breeding parents**

ID	GEN	FEM	MALE	OTL	Phen	
1	0	0	0	-1.1083	-0.3952	3, 1, 2, 2
2	0	0	0	0.1049	2.4103	3, 2, 1, 1
3	0	0	0	-0.4364	2.0520	2, 2, 1, 1
4	0	0	0	-0.3173	-4.0557	3, 1, 2, 2
5	0	0	0	-1.5240	1.2725	2, 2, 2, 2
21	1	1	0	-0.6385	-3.7504	1, 3, 2, 1
22	1	1	0	-0.6947	-0.6559	3, 2, 2, 2
23	1	1	0	-1.0675	-2.6624	1, 3, 2, 2
24	1	1	0	-1.1882	-3.4193	3, 2, 2, 2
41	1	2	0	0.1049	1.0862	3, 2, 1, 1
42	1	2	0	0.1103	-0.2042	3, 2, 1, 1
43	1	2	0	0.1049	-4.0979	3, 2, 1, 1
44	1	2	0	0.1049	2.9973	3, 2, 1, 1
61	1	3	0	-0.6947	-0.6559	2, 2, 1, 1
62	1	3	0	-2.3581	-5.1241	2, 2, 1, 1
63	1	3	0	-0.6947	-0.6559	2, 2, 1, 1
64	1	3	0	-0.6947	-0.6559	2, 2, 1, 1
81	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
82	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
83	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
84	1	4	0	-0.6947	-0.6559	1, 2, 2, 1
101	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
102	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
103	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
104	1	5	0	-0.6947	-0.6559	1, 2, 1, 1
421	2	58	23	0.9922	1.0498	3, 1, 2, 2
422	2	59	23	0.1296	-4.5242	3, 1, 2, 2
423	2	58	23	0.5103	1.5278	3, 1, 2, 2
431	2	230	17	0.1049	1.0862	3, 1, 2, 2
432	2	230	17	0.1049	1.0862	3, 1, 2, 2
433	2	230	17	0.1049	1.0862	3, 1, 2, 2
441	2	179	184	0.9922	1.0498	3, 1, 2, 2
442	2	179	184	0.1296	-4.5242	3, 1, 2, 2
443	2	179	184	0.5103	1.5278	3, 1, 2, 2

**Can modify...**  
 Mating design  
 Number of parents  
 Number of progeny per cross

## Simulate locus haplotypes

The screenshot shows the 'Tree Genome Simulator' interface. On the left, there are several configuration panels:

- SNP haplotypes**: Includes 'Coalescent parameters' with fields for Effective population size (Ne) set to 1000, Population-scaled recombination rate (4Nr) set to 0.0015, Population-scaled mutation rate (4Nμ) set to 0.0053, Selection for QTL (enter non QTL value in GlobalJava) set to 0.15, Max DNA sequence for candidate locus (bp) set to 3001, and Min DNA sequence for candidate locus (bp) set to 1001.
- Genotyping SNPs**: Includes 'SNP discovery panel size' set to 30, 'SNP genotype notations' with radio buttons for homo. major (0), hetero. (1), homo. minor (2), and a set of radio buttons for -1, 0, 1.
- Genotyping all SNPs/locus?**: Radio buttons for No and Yes (Yes is selected).
- Simulation**: Radio buttons for Step by step simulation (selected) and Multiple simulation.

On the right, the 'SNP haplotypes' tab is active, displaying a table titled 'SNPs for QTL loci'. The table has columns: Locus, Locus ID, Allele, QTN, QTN location, and Genotyped SNP locations. The data is as follows:

Locus	Locus ID	Allele	QTN	QTN location	Genotyped SNP locations
1	1	1	0	1543	900 1543
1	1	2	1	1543	900 1543
2	2	1	1	176	176 1370
2	2	2	1	176	176 1370
2	2	3	1	176	176 1370
3	3	1	0	1139	202 1139
3	3	2	1	1139	202 1139
4	4	1	1	925	290 925
4	4	2	1	925	290 925
4	4	3	1	925	290 925
5	5	1	1	127	127 669
5	5	2	1	127	127 669
5	5	3	1	127	127 669

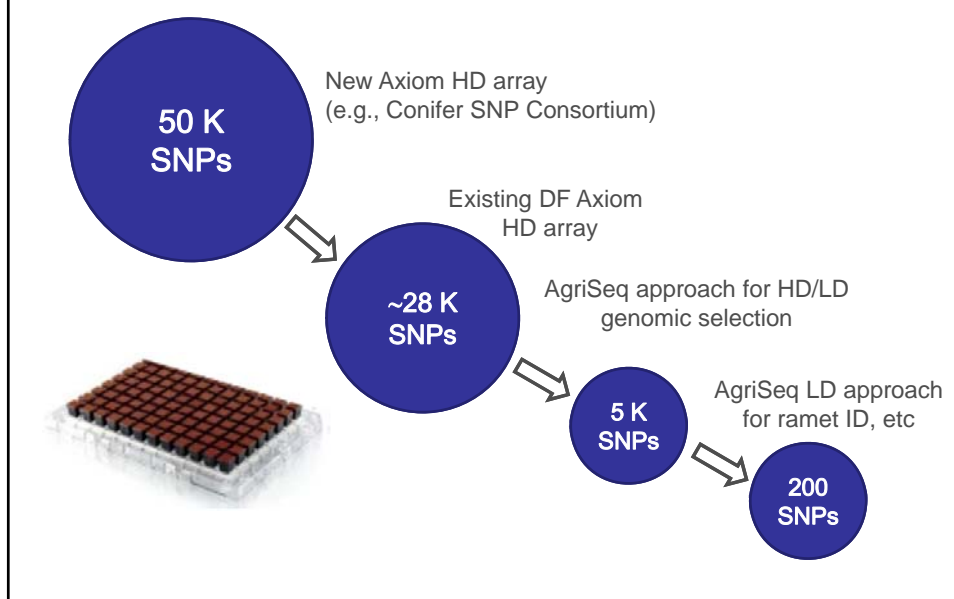
A yellow callout box with the text 'Can modify...' lists the following items:

- Coalescent parameters
- Genotyped SNPs
- SNP genotype code

## Objective 5

Test whether we can use a combination of high-density and low-density arrays (HD/LD arrays) to substantially reduce genotyping costs

## High density vs low density genotyping



## Genotyping costs have been an obstacle

### ***NARA genotyping costs***

\$142,500 for 1,920 trees and 50K SNPs (\$75 / tree)

### ***Conifer SNP Consortium costs***

<b><u>No. of samples</u></b>	<b><u>Cost/sample</u></b>
< 5K	\$32.50
5K-10K	\$25.00
> 10K	\$20.00



## Low density arrays are cheaper

### Coastal Douglas-fir SNPs

Statistic	Number of SNPs	Percent
SNPs assayed	40	100.00
Called SNPs (frequency > 0.85)	36	90.00
Called SNPs that are polymorphic	36	100.00

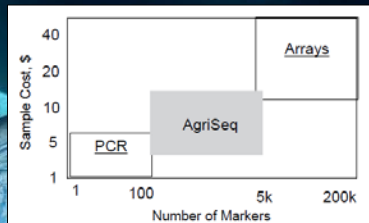
Genotyping cost per sample using Sequenom			
No. of trees	No. of SNPs		
	300	400	500
<b>University of Arizona Genome Center</b>			
1000	\$102.62	\$136.83	\$171.04
1500	90.69	120.92	151.15
2000	101.42	135.23	169.04
<b>GeneSeek</b>			
1000	27.00	34.00	42.50
1500	24.00	29.50	37.00
2000	22.00	27.50	34.00

Statistic	Mean	Median	Range
Call frequency	0.99	1.00	0.93 - 1.00
Minor allele frequency (MAF)	0.40	0.41	0.22 - 0.50
Heterozygosity (observed)	0.47	0.44	0.27 - 0.96
Heterozygosity (expected)	0.47	0.49	0.35 - 0.50

SEQUENOM



## AgriSeq



**ThermoFisher**  
SCIENTIFIC

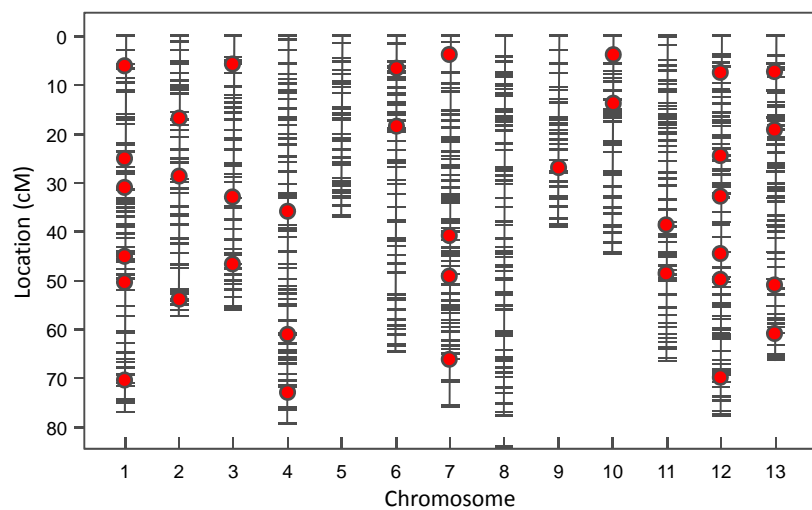
High Throughput Genotyping by Targeted Next-Generation Sequencing for Agricultural Applications

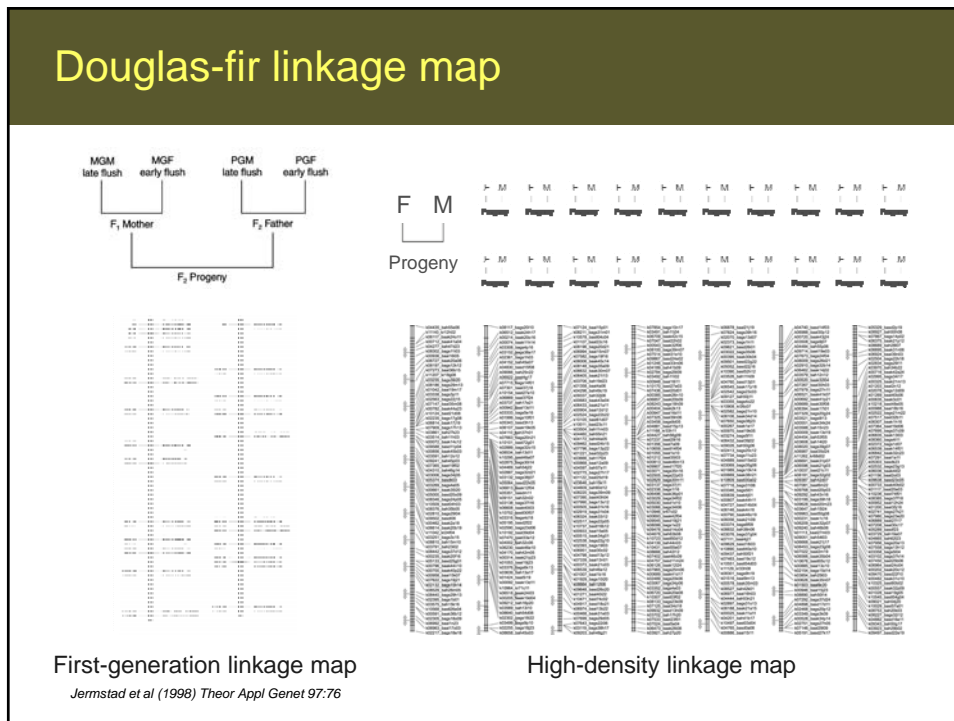
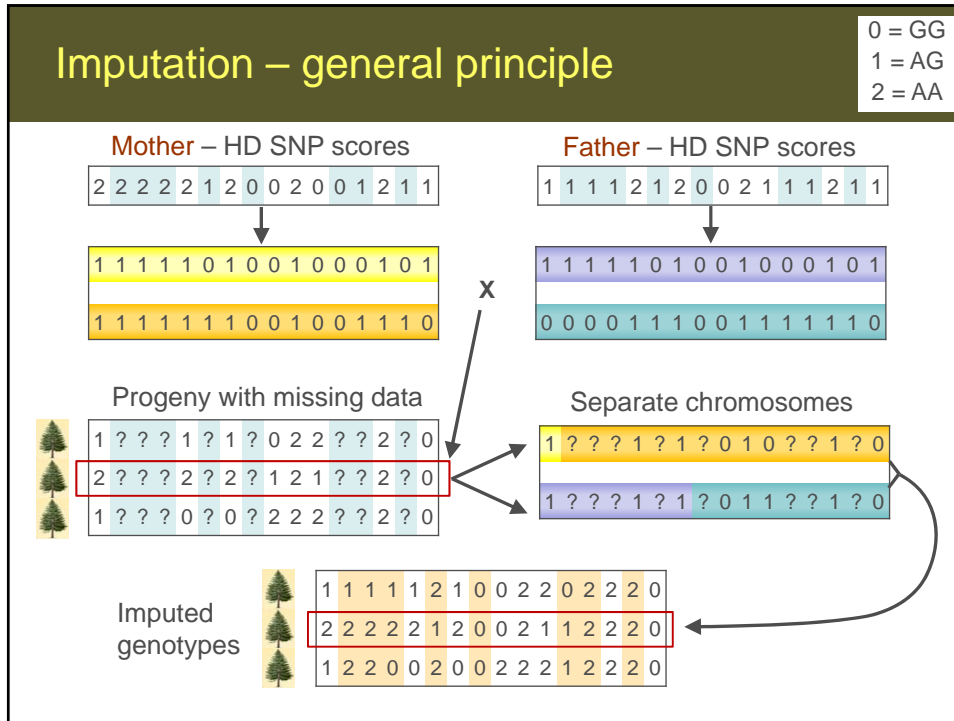
The world leader in serving science

## Objective 6

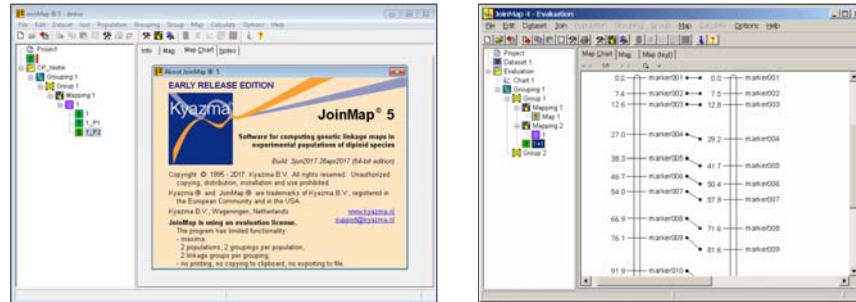
Develop a high-density SNP linkage map for Douglas-fir

### Linkage map – Why?





## We have been using JoinMap



### Other approaches are needed too...

- Van Ooijen, J.W. 2006. JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations, Kyazma B.V., Wageningen, Netherlands, 57pp.
- Strnadov-Neeley, V., Buluc, A., Chapman, J., Gilbert, J.R., Gonzalez, J., and Oliker, L. 2015. Efficient data reduction for large-scale genetic mapping. In Proceedings of the 6th ACM Conference on Bioinformatics, Computational Biology and Health Informatics. ACM, Atlanta, Georgia. pp. 126-135.
- Preedy, K.F., and Hackett, C.A. 2016. A rapid marker ordering approach for high-density genetic linkage maps in experimental autotetraploid populations using multidimensional scaling. Theor Appl Genet 129:2117-2132.

## We have been using JoinMap



***We should conduct additional framework mapping using the AgriSeq platform to increase the reliability of the genetic map***

### Other approaches are needed too...

- Van Ooijen, J.W. 2006. JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations, Kyazma B.V., Wageningen, Netherlands, 57pp.
- Strnadov-Neeley, V., Buluc, A., Chapman, J., Gilbert, J.R., Gonzalez, J., and Oliker, L. 2015. Efficient data reduction for large-scale genetic mapping. In Proceedings of the 6th ACM Conference on Bioinformatics, Computational Biology and Health Informatics. ACM, Atlanta, Georgia. pp. 126-135.
- Preedy, K.F., and Hackett, C.A. 2016. A rapid marker ordering approach for high-density genetic linkage maps in experimental autotetraploid populations using multidimensional scaling. Theor Appl Genet 129:2117-2132.



# Proposed PNWTIRC AgriSeq Project

## Proposed PNWTIRC activity for 2018-2019

### Low-density genotyping via AgriSeq (ThermoFisher)

#### **Objectives**

- Test cost-effective genotyping approach(e.g., 100-5000 SNPs) for...
  - *Ramet ID, pollen contamination, mating systems, HD/LD genomic selection*
- Obtain data for framework mapping of Axiom SNPs
  - *A few large full-sib families*
- Potential collaborators
  - *CIPS, Scion, Slovenia Forestry Institute/INRA*
- Cost = maximum of \$15K for genotyping (minus contributions from collaborators?)



## Potential collaboration with CIPS

Use genetic markers to identify source of seed and families planted in New Zealand by Cascade Timber Consulting



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

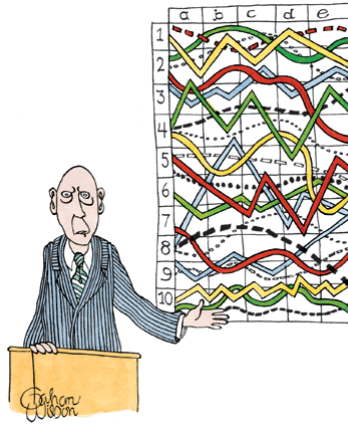


## Objective 7

Hold workshops on how to practice genomic selection in Douglas-fir

Once we know what we're doing?

Thank you!



"I'll stop here so you can let this information sink in"

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



## Introduction to the Species Potential Habitat Tool and Updates for the Seedlot Selection Tool


By Brad St.Clair, Glenn Howe, Nikolas Stevenson-Molnar, and Brendan Ward

The Seedlot Selection Tool (SST) continues to be developed and expanded as a collaboration between Glenn Howe (OSU, PNWTIRC), Brad St.Clair (US Forest Service, Pacific Northwest Research Station), Dominique Bachelet (OSU), and staff at the Conservation Biology Institute (Brendan Ward and Nik Stevenson-Molnar). The SST is available online at <https://seedlotselectiontool.org/sst>. A second tool, the Species Potential Habitat Tool (SPHT), is being developed to allow users to identify suitable species for sites under current or future climates (<https://specieshabitattool.org/spht/>). Together, the SST and SPHT will allow users to examine assisted migration at both the within-species and species levels.

In 2017-2018, the SPHT underwent a lot of development, including linking the SPHT to the SST. New features were added, such as the ability to zoom into areas of interest, look at different time periods and RCPs, and export the results as a GIS file. Currently, only five species are available in the SPHT (lodgepole pine, Douglas-fir, Sitka spruce, ponderosa pine, and Engelmann spruce), but more will be incorporated next year.

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. Key updates to the SST for 2017-2018 included adding more regions (i.e., Central US, Eastern US, and Mexico), adding more seed zones, and incorporating more functions that can be used to customize the mapped results.


We are also developing new tools with funding from the USDA Forest Service. A Climate Smart Restoration Tool (CSRT) is being developed that uses the same methods as the SST, but this tool targets non-tree restoration species, particularly species of concern to managers in the Great Basin (<https://consbio.org/products/projects/climate-smart-restoration-tool>).



***Introduction to the Species Potential Habitat Tool  
and Update for the Seedlot Selection Tool***

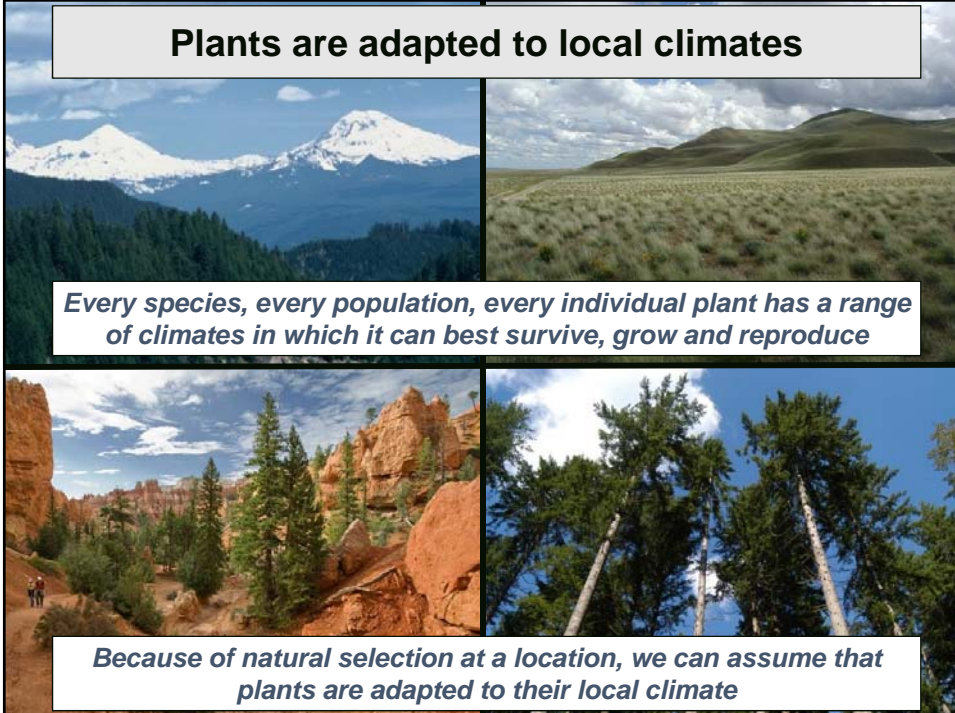
***Brad St.Clair***

*USDA Forest Service, Pacific Northwest Research Station,  
Corvallis, Oregon*



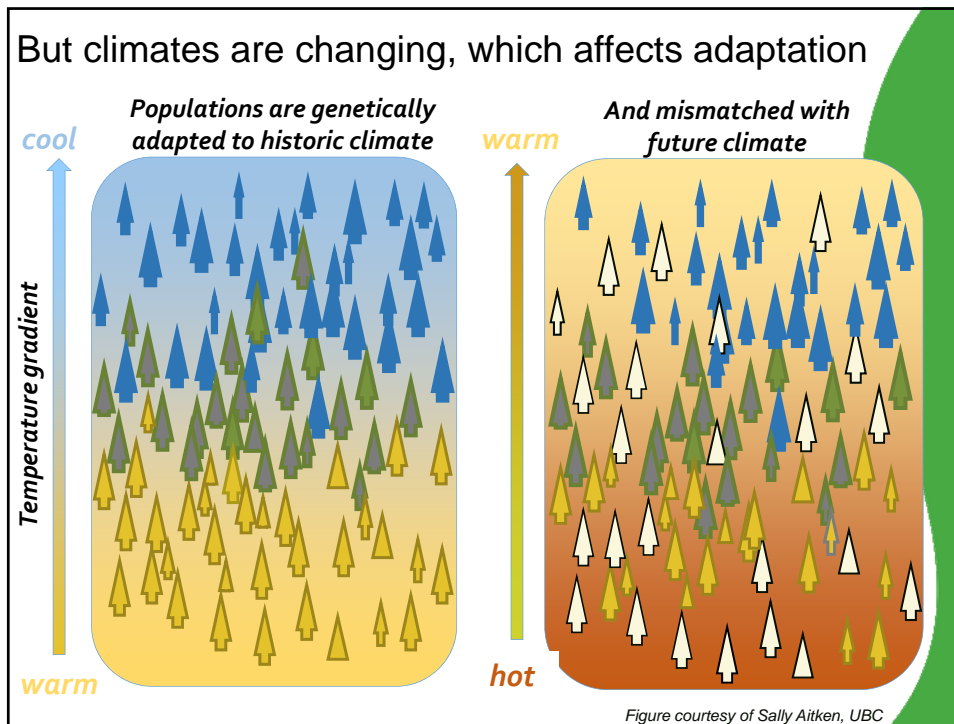
***PNWTIRC Annual Meeting, October 18, 2018***

**Plants are adapted to local climates**



*Every species, every population, every individual plant has a range of climates in which it can best survive, grow and reproduce*

*Because of natural selection at a location, we can assume that plants are adapted to their local climate*



## Reforestation decisions

1. Natural regeneration or planting?
  - Can I get sufficient stocking of the desired species in a reasonable time frame?
  - Can I improve productivity using select planting stock?
  - Will trees be adapted?
    - Local species and seed sources have been the default choice
    - But perhaps should consider other seed sources and species
2. Choice of species?
3. Choice of seed source?
  - Will trees be adapted?
  - What species and seed sources are available?
  - Is select planting stock available?





## Environmental Niche Modelling

Modelling to predict the distribution of species in geographic space based on their known distribution in environmental space (their realized ecological niche)

- Also called climatic niche modelling, species distribution modelling, predictive habitat distribution modelling, and climate envelope modelling.
- Criticism that it does not always reflect actual species distribution.
- Actual distribution may depend on a number of other factors including dispersal ability, evolutionary history, biotic interactions.

### Error rates:

**Predict present, but absent 5.4%**

**Predict absent, but present 0.5%**

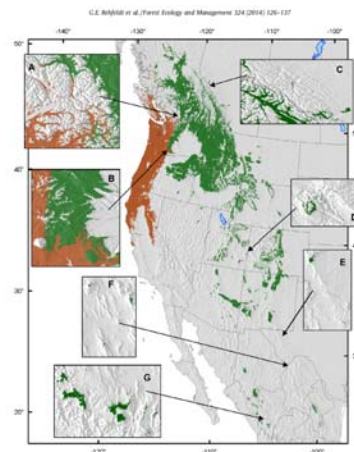
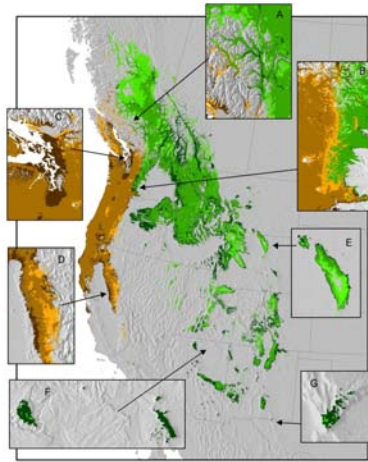


Fig. 3. Mapped prediction for climate niche for *Pseudotsuga menziesii* var. *menziesii* (brown) and var. *glauca* (green)

Rehfeldt et al. 2014. Comparative genetic responses to climate for varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Realized climate niches. *Forest Ecology and Management* 324: 126-137

### Predicted climatic niches by 2060 for *Pseudotsuga menziesii* varieties

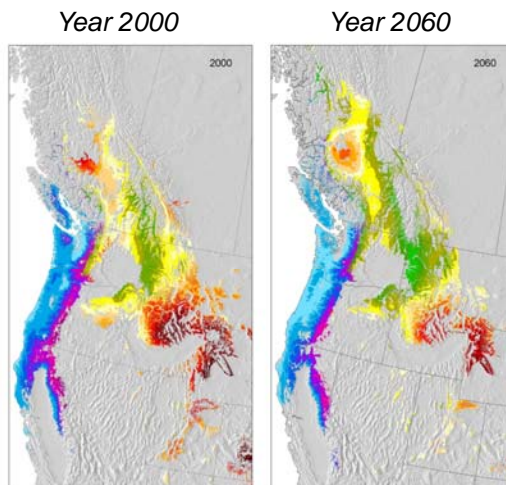


	Habitat lost (dark color)	Remains suitable (middle color)	Habitat gained (light color)
var. menziesii (browns)	18%	82%	18%
var. glauca (greens)	35%	65%	32%

- Habitat is lost at the trailing edge (lower elevations and further south)
- Gained at the leading edge (higher elevations and further north)

Rehfeldt et al. 2014. Comparative genetic responses to climate for varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Realized climate niches. *Forest Ecology and Management* 324: 126-137

### Populations variation: Clines in growth potential within current and future (2060) climatic niches

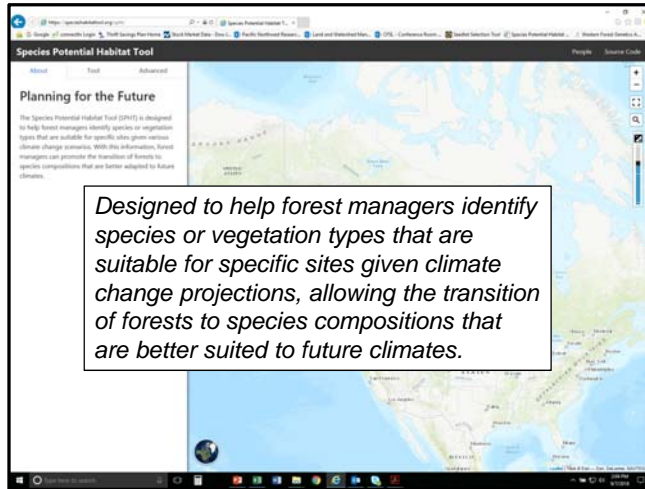


	Remaining suitable from today	Current climatype suitable through 2060
var. menziesii (light blue = high growth magenta = low)	82%	58%
var. glauca (Dark green = high Dark red = low)	68%	1%

Rehfeldt et al. 2014. Comparative genetic responses to climate for varieties of *Pinus ponderosa* and *Pseudotsuga menziesii*: Clines in growth potential. *Forest Ecology and Management* 324: 138-146.



## Species Potential Habitat Tool

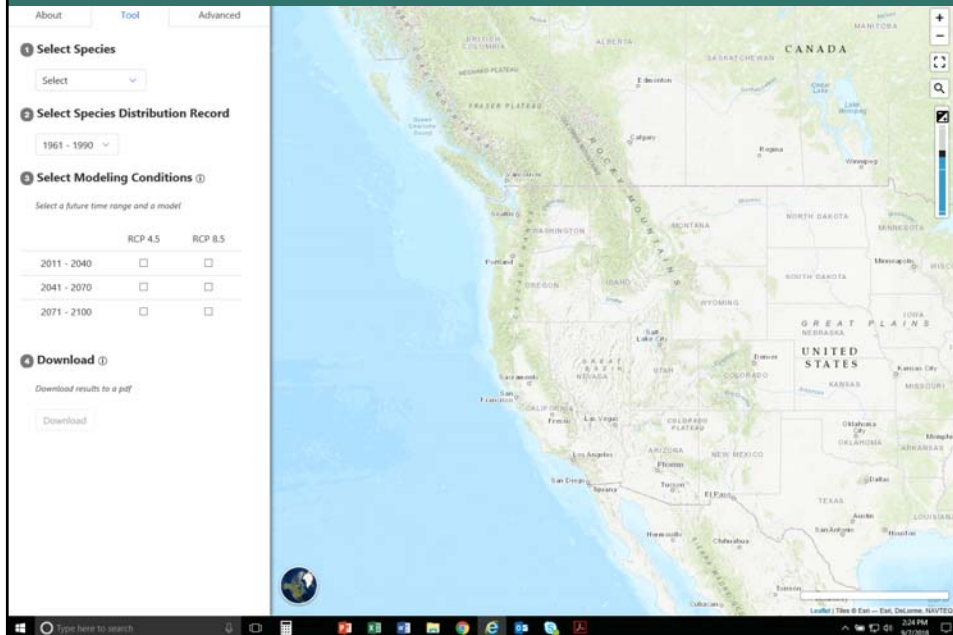


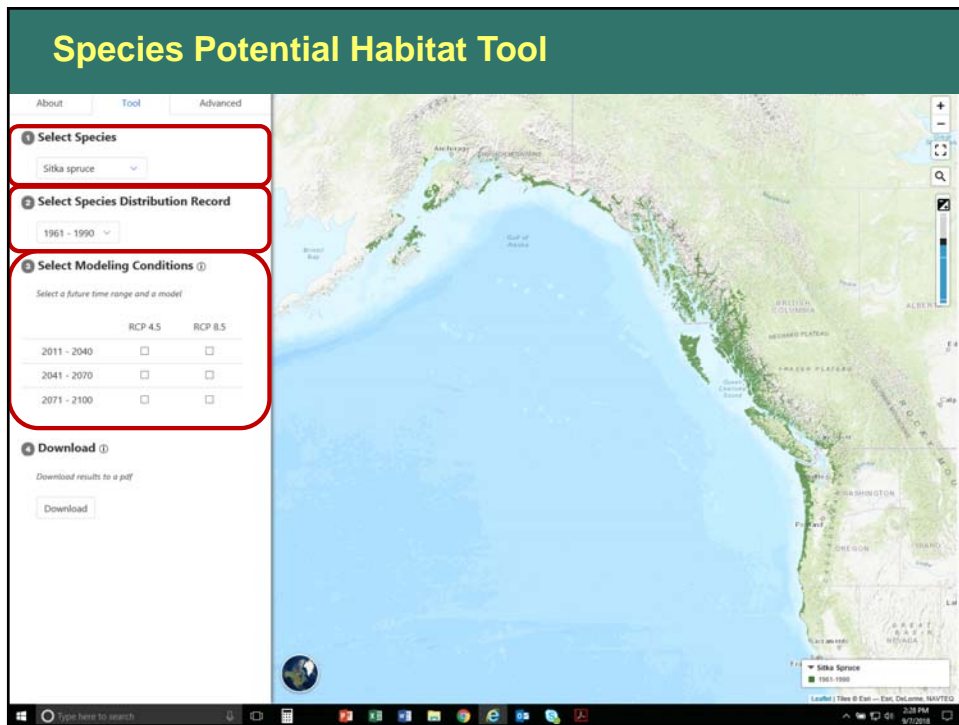
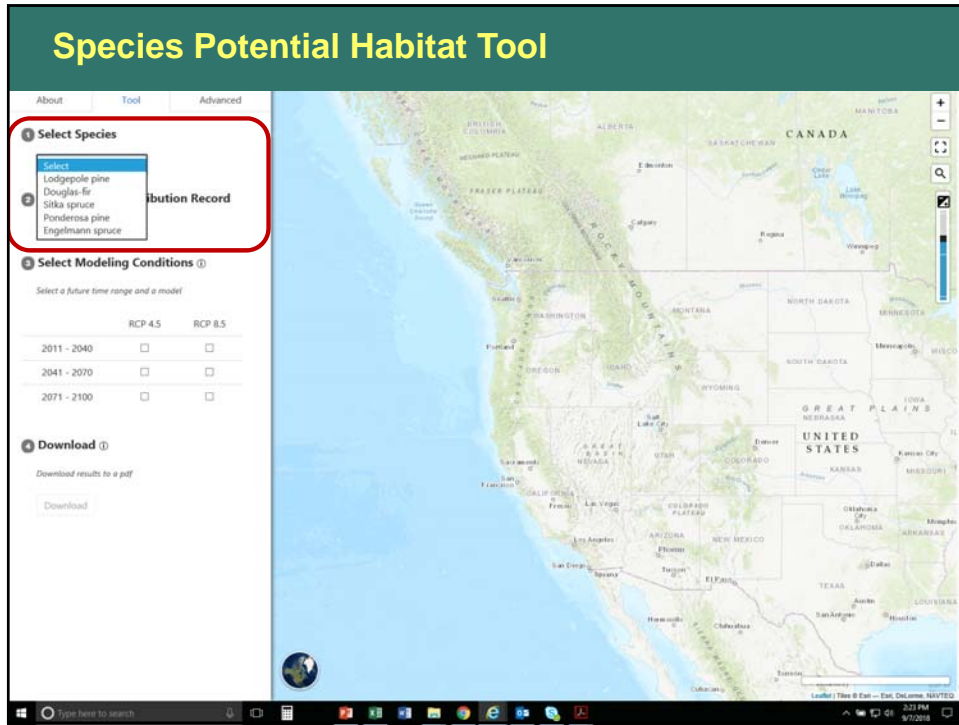
**Features:**

- Can zoom into areas of interest
- Can look at different time periods and RCPs
- Integrated with the Seedlot Selection Tool (can be used as a constraint)
- Can export as a GIS file

<https://specieshabitattool.org/spht/>

## Species Potential Habitat Tool





## Species Potential Habitat Tool

About | **Tool** | Advanced

**1 Select Species**

Sitka spruce

**2 Select Species Distribution Record**

1961 - 1990

**3 Select Modeling Conditions (i)**

Select a future time range and a model

	RCP 4.5	RCP 8.5
2011 - 2040	<input type="checkbox"/>	<input checked="" type="checkbox"/>
2041 - 2070	<input type="checkbox"/>	<input type="checkbox"/>
2071 - 2100	<input type="checkbox"/>	<input type="checkbox"/>

**4 Download (i)**

Download results to a pdf

Download

## Species Potential Habitat Tool

About | **Tool** | Advanced

**1 Select Species**

Sitka spruce

**2 Select Species Distribution Record**

1961 - 1990

**3 Select Modeling Conditions (i)**

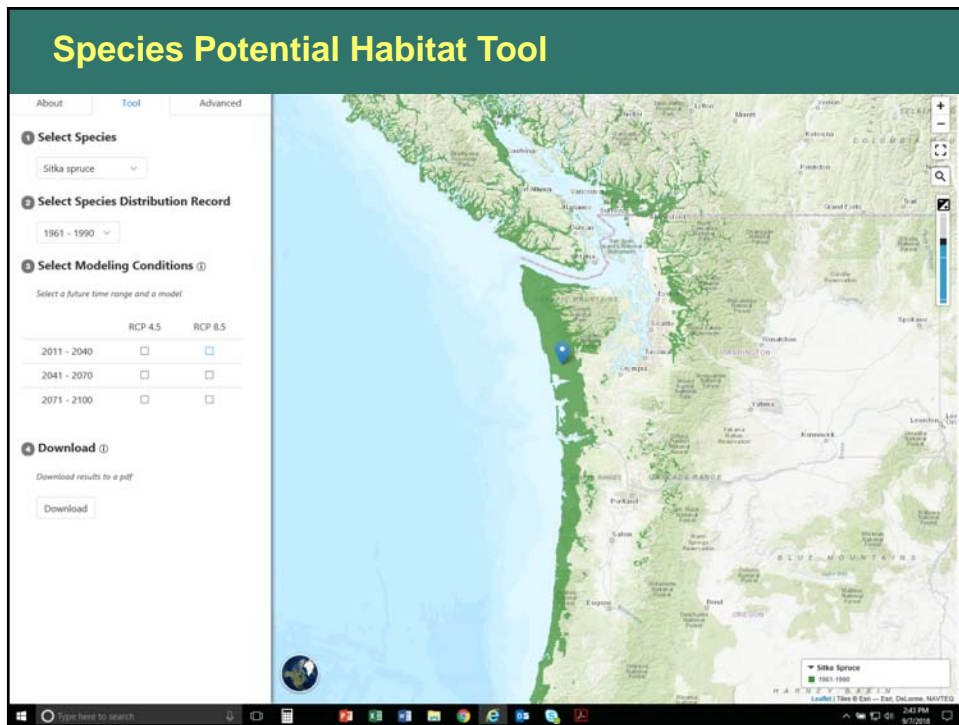
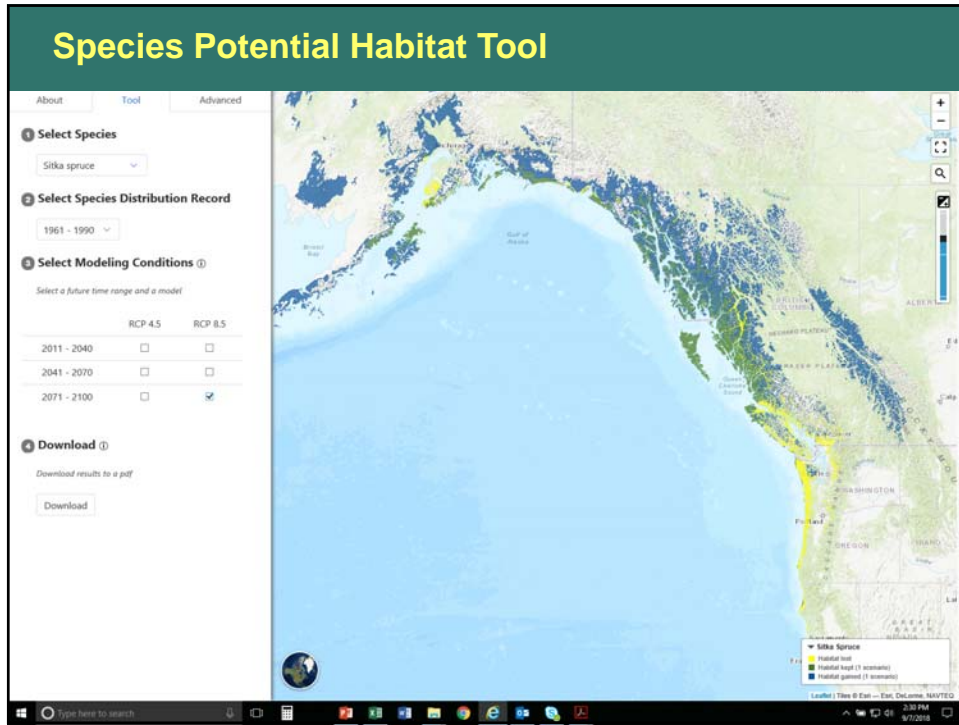
Select a future time range and a model

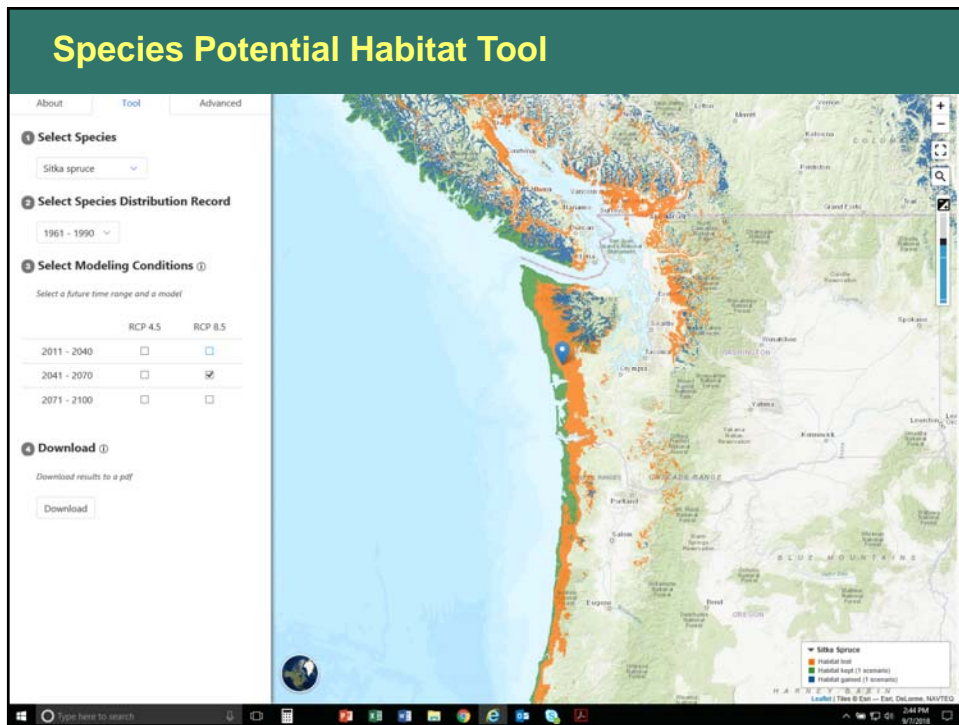
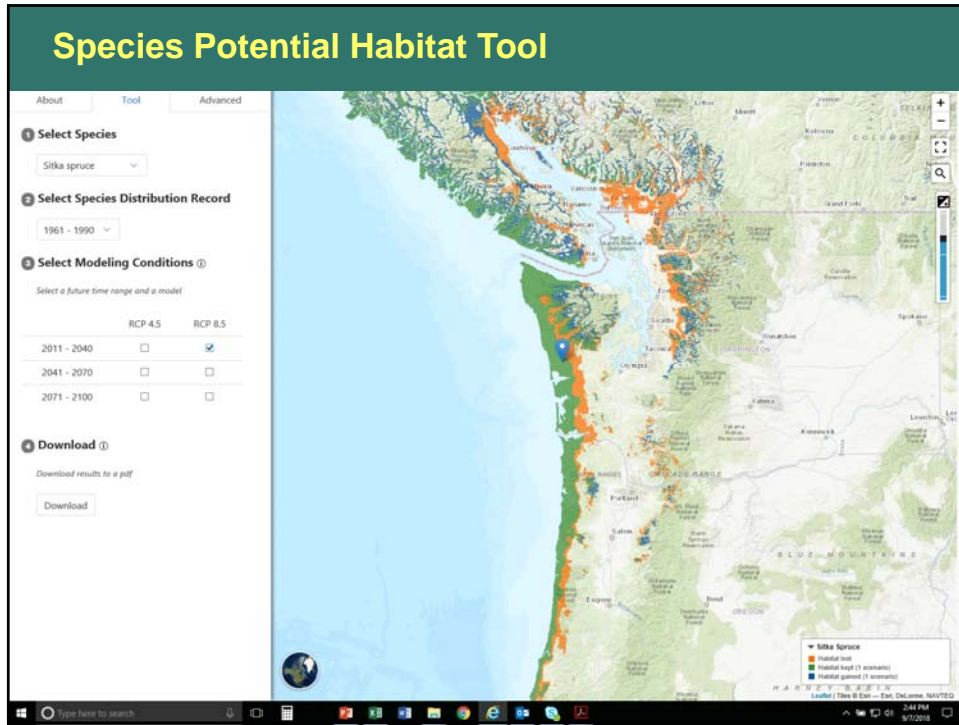
	RCP 4.5	RCP 8.5
2011 - 2040	<input type="checkbox"/>	<input type="checkbox"/>
2041 - 2070	<input type="checkbox"/>	<input checked="" type="checkbox"/>
2071 - 2100	<input type="checkbox"/>	<input type="checkbox"/>

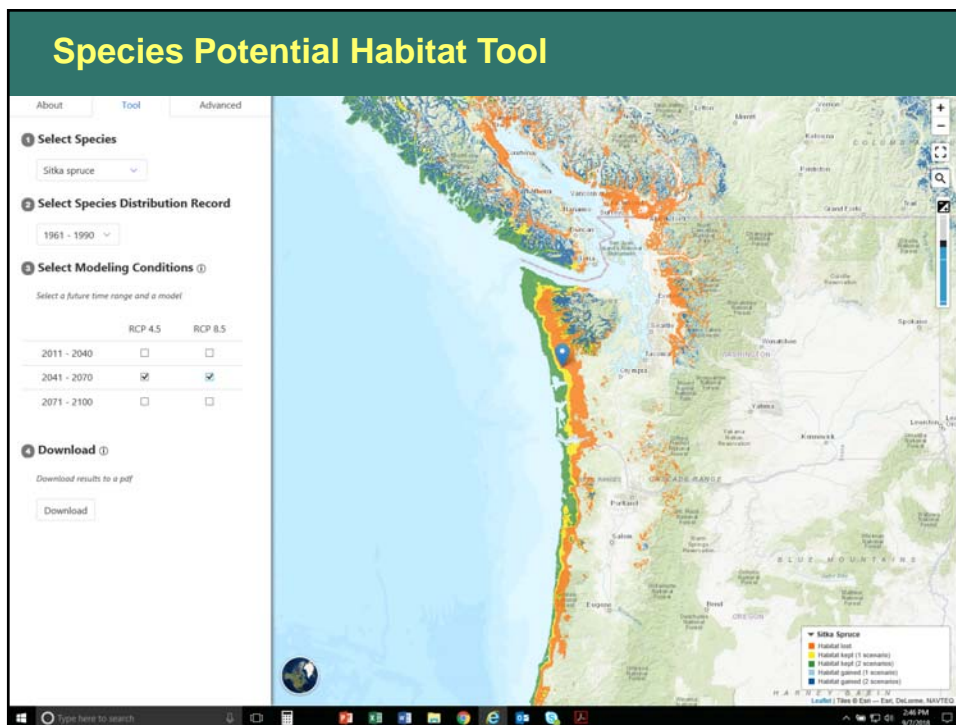
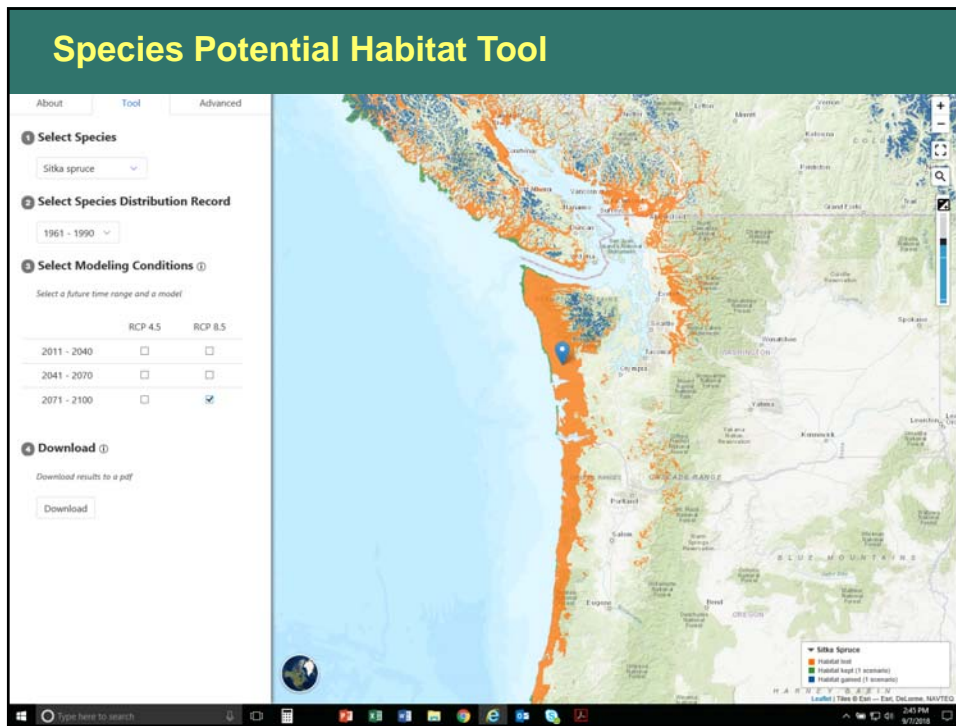
**4 Download (i)**

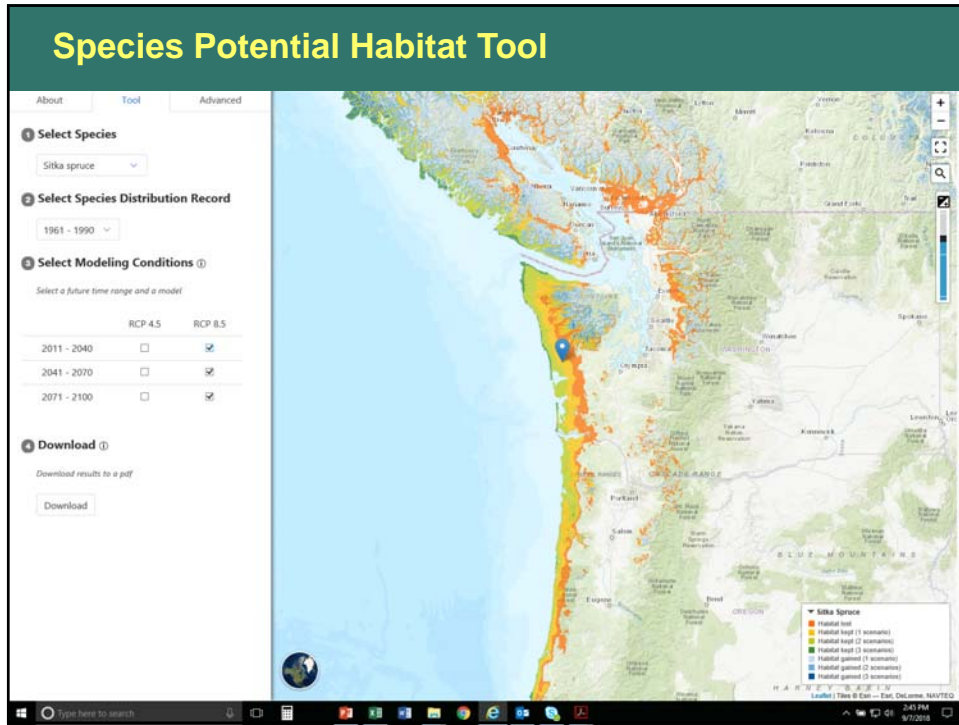
Download results to a pdf

Download









**Two questions:**

- 1. Are native populations adapted to current and future climates?**
- 2. If not, how far do we have to go to find populations adapted to a planting site (assisted migration)?**



## Seedlot Selection Tool is a powerful tool for:

- Matching seedlots to planting sites
- Characterizing past, current, and future climates at a site
- Illustrating the potential concerns about climate change (when and where)
- Seed planning given climate change concerns
- Gene conservation given climate change concerns



## Can address two objectives:

***Given a planting site***  
***Which seedlot is well adapted today...or in the future?***



***Given a seedlot***  
***Where is it well adapted today...or in the future?***



## Douglas-fir in Oregon Cascades

The screenshot shows the "Seedlot Selection Tool" interface. On the left is a sidebar with several sections: "Select objective" (with "Find seedlots" and "Find planting sites" buttons), "Select planting site location" (with latitude and longitude input fields), "Select climate scenarios" (with dropdown menus for climate and timing), "Select transfer limit method" (with "Custom" and "Zone" buttons), "Select climate variables" (with "Units" and "Add a variable" options), and "Map your Results" (with "Run your Results", "Save Last Run", and "Export PDF" buttons). On the right is a map of the Pacific Northwest region, showing the Oregon Cascades. A blue location pin is placed on the map, and an inset photograph shows a field of young Douglas-fir trees, labeled "Douglas-Fir Seed Source Movement Study: Soda Test Site".

## Select location

**Select location by:**

- Clicking on map, or
- Entering the lat/long

## Select climate scenarios

**Select two climate scenarios:**

- Climate that seedlots are adapted to
- Climate of the planting site

## Select transfer limit method

**Transfer limit methods:**

- Custom, or
- Zone

## Use seed zones to define transfer limits

**When selecting zone method, the choices depends on prior inputs into the system:**

- Oregon and Washington have generic zones and species-specific zones
- The zone and elevation band of the site or seedlot are shown

Name	Center	Transfer limit (+/-)
MCMT	1.9 °C	2.80 °C
MAP	2130 mm	1000 mm

## Select climate variables

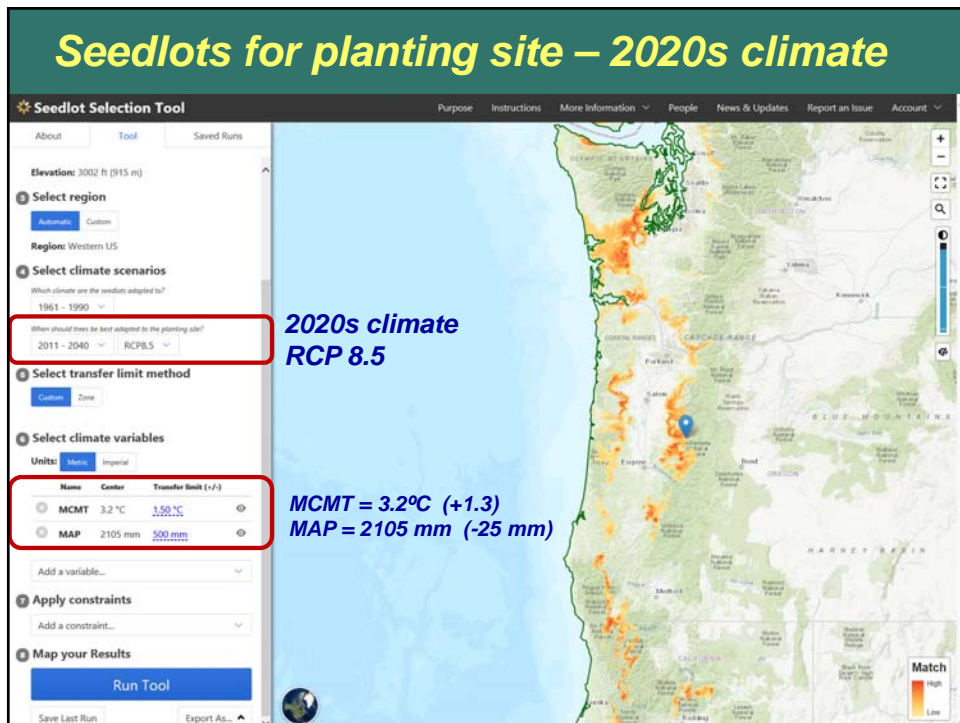
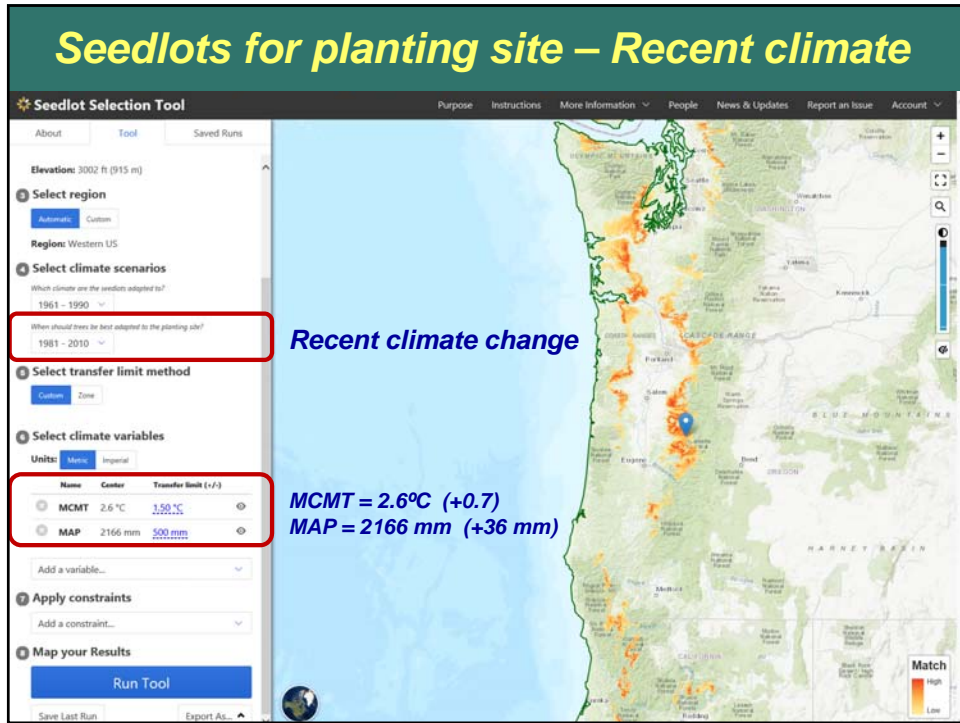
**Add climate variables and adjust transfer limits:**

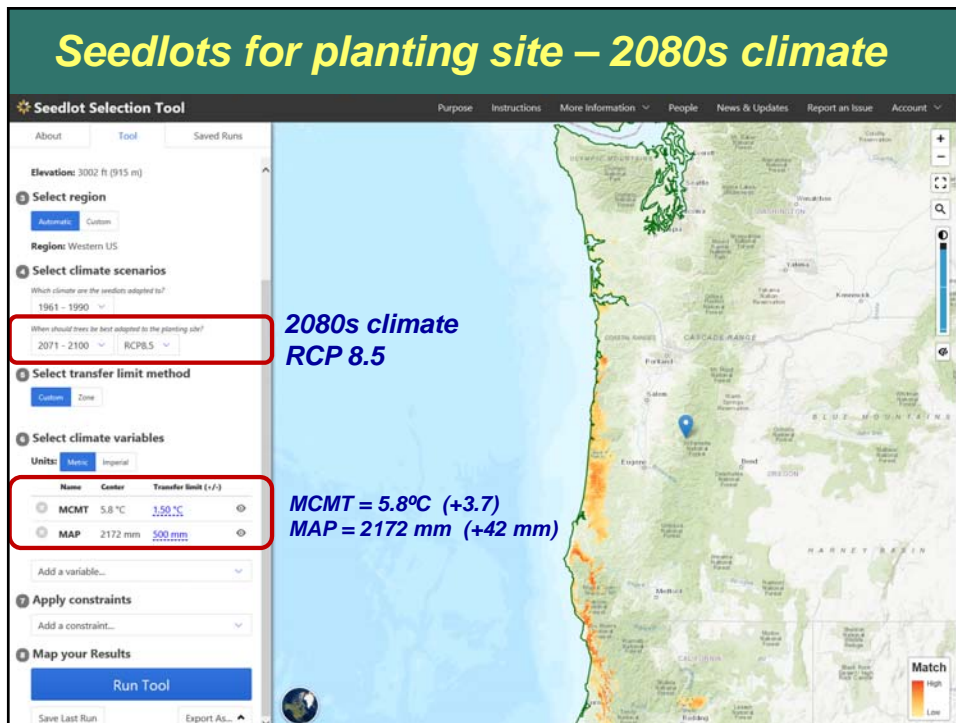
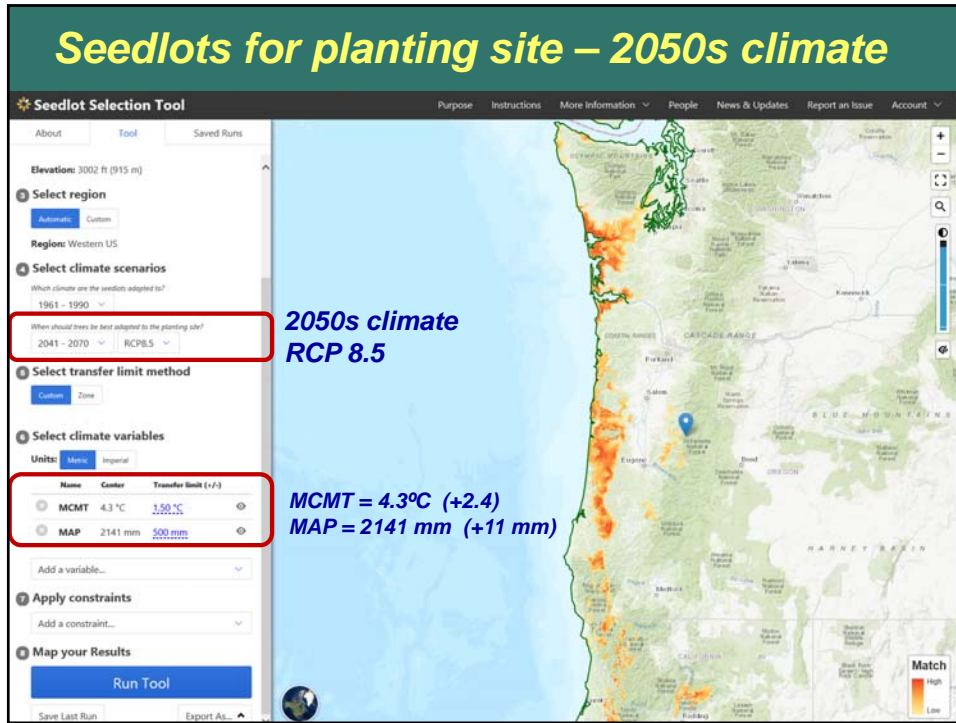
- Winter minimum temperature =  $1.9\text{ }^{\circ}\text{C} \pm 1.5\text{ }^{\circ}\text{C}$
- Annual precipitation =  $2130\text{ mm} \pm 500\text{ mm}$

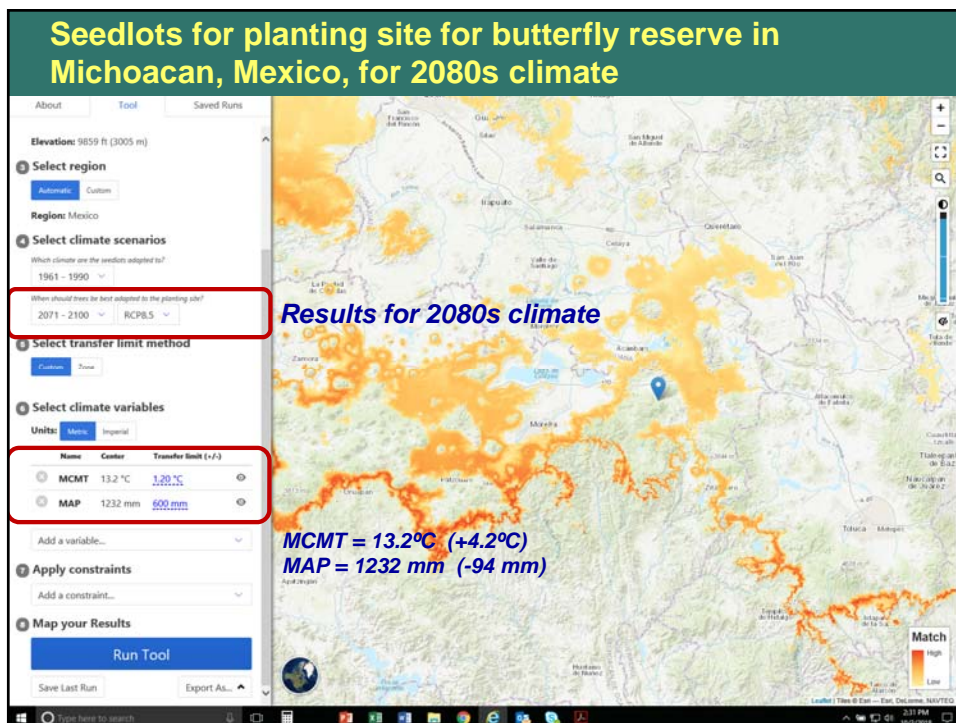
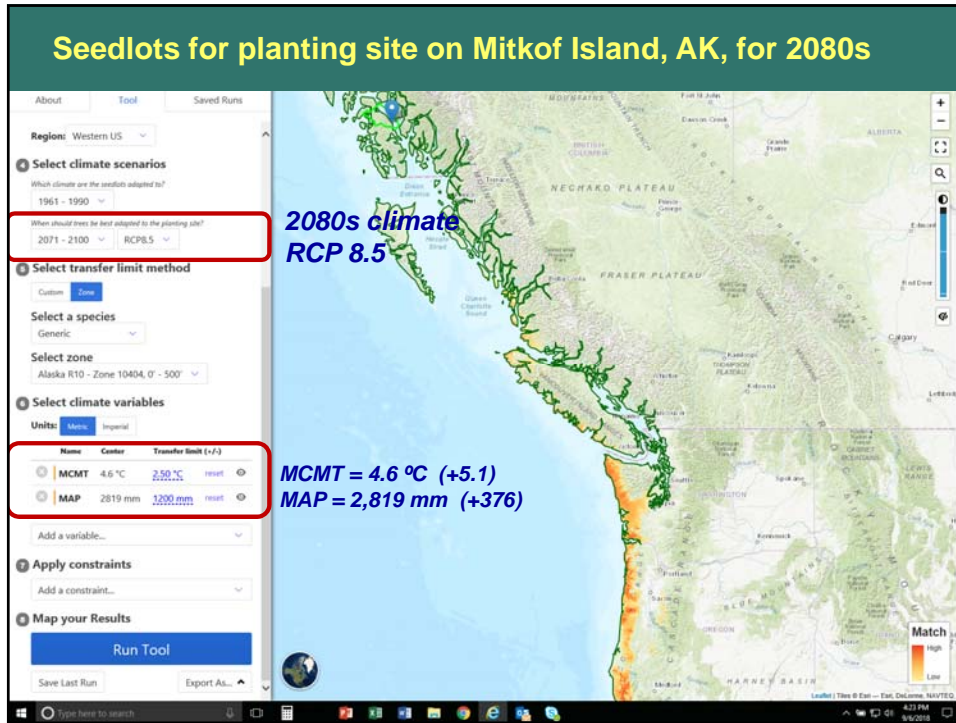
## Map your results: Ignoring climate change

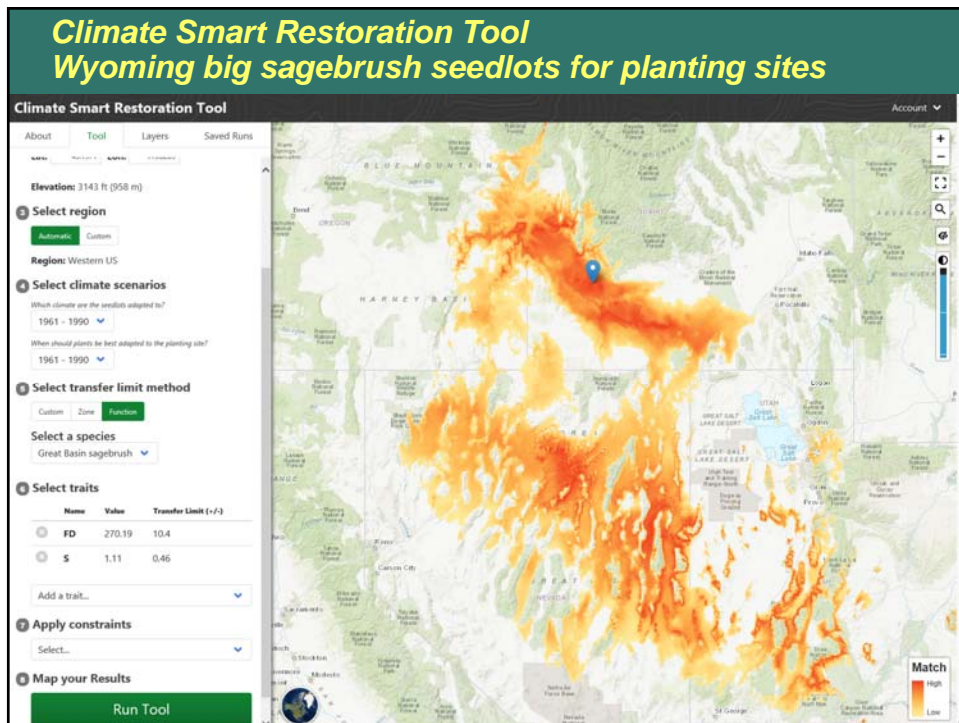
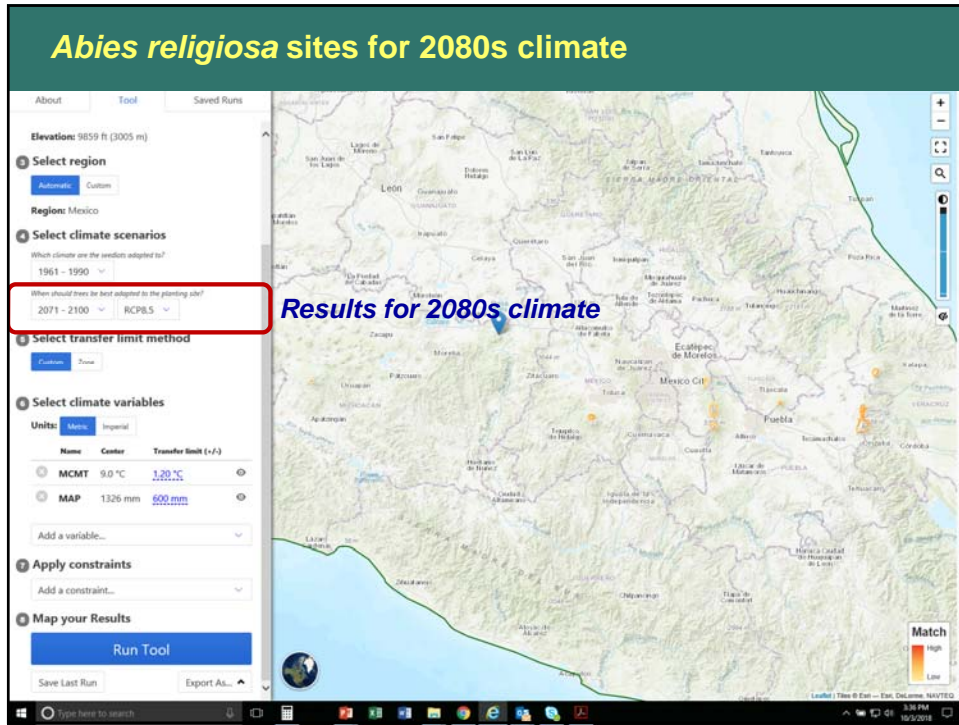
**Results with no climate change**

**MGMT =  $1.9\text{ }^{\circ}\text{C}$**   
**MAP =  $2130\text{ mm}$**











# People and Funding

**Glenn Howe – Co-Principal Investigator**  
 Oregon State University, Corvallis, Oregon  
[glenn.howe@oregonstate.edu](mailto:glenn.howe@oregonstate.edu)

**Brad St.Clair – Co-Principal Investigator**  
 Pacific Northwest Research Station  
 USDA Forest Service, Corvallis, Oregon, USA  
[bstclair@fs.fed.us](mailto:bstclair@fs.fed.us)

**Nikolas Stevenson-Molnar – Software Engineer**  
 Conservation Biology Institute, Corvallis, Oregon  
[nik.molnar@consbio.org](mailto:nik.molnar@consbio.org)

**Brendan Ward – Project Manager**  
 Conservation Biology Institute, Corvallis, Oregon  
[bward@consbio.org](mailto:bward@consbio.org)

**Tongli Wang – Climatic niche models**  
 University of British Columbia, Vancouver, BC  
[tongli.wang@ubc.ca](mailto:tongli.wang@ubc.ca)



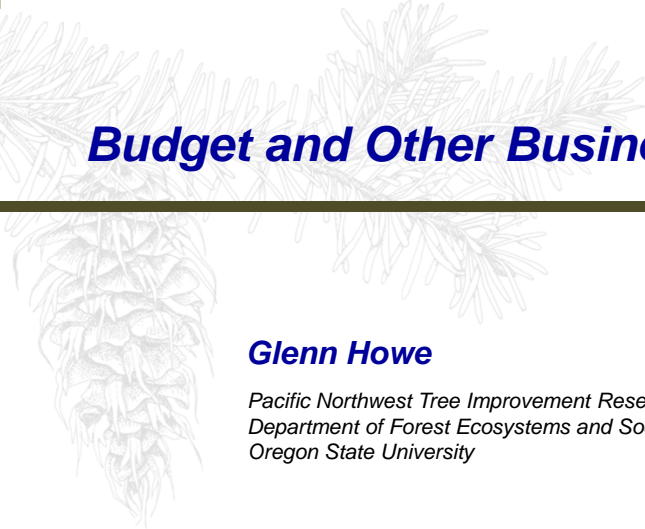
[consbio.org/products/webinars/climate-smart-seedlot-selection-tool](http://consbio.org/products/webinars/climate-smart-seedlot-selection-tool)



## **Budget and Other Business**

By Glenn Howe

Glenn Howe presented last year's budget (FY2017-2018) and the proposed budget for next year (FY2018-2019). During this portion of the annual meeting, we also elected a new Policy/Technical Committee Chair and 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 and other business**

***Vote on budget***

***Elect new Policy/Technical Committee Chair***

***Other business?***

## APPENDIX I

**Collaborations and Grants  
2017-2018**

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, 2012-2018, \$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.

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 II

**Annual Meeting Minutes**

October 18, 2018, North Willamette Research and Extension Center, Aurora, OR

**Attendees**

Richard Sniezko – USFS, Dorena Genetic Resources	Meridith McClure – PNWTIRC, OSU
Michael Crawford – Bureau of Land Management	Anna Magnuson – PNWTIRC, OSU
David Barker – Rayonier Forest Resources	Brianna McTeague – Weyerhaeuser
Estefania Elorriaga – TBGRC, OSU	Brian Murray – Cascade Timber Consulting
Florian Deisenhofer – Hancock Forest Management	Oguz Urhan – PNWTIRC, OSU
Jeremy Johnson, USFS, Dorena Genetic Resources	Lauren Magalska – Port Blakely Tree Farms
Terrance Ye – NWTIC, OSU	Josh Sherrill – Rayonier Forest Resources
Dan Cress – Olympic Resource Management	Sara Lipow – Roseburg Forest Products
Katy Kavanagh – College of Forestry, OSU	Margaret Banks – Stimson Lumber Co.
Brad St.Clair – PNW Research Station, USFS	Jeff DeBell – Washington State DNR
Glenn Howe – PNWTIRC, OSU	Brian Baltunis – Weyerhaeuser

**I. Welcome.** Lauren Magalska, PNWTIRC Policy/Technical Chair, called the meeting to order at 9:30 am.

**II. PNWTIRC highlights for 2017-2018.** Glenn Howe presented an overview of major accomplishments for 2017-2018

**1. Administration and members**

- Director - Glenn Howe
- Research Coordinator – Scott Kolpak
- Research Scientist – Jennifer Kling
- Program Manager – Anna Magnuson
- Graduate Student – Oguz Urhan
- Policy/Technical Committee Chair – Lauren Magalska

**2. Significant activities during 2017-2018**

- Scott Kolpak took a job as an area geneticist with the USFS (Umpqua NF)
- Susan McEvoy left for graduate school
- Jennifer Kling reduced her hours substantially during 2017-2018, but will continue working for the PNWTIRC
- We continued genomic selection analyses in Douglas-fir
- 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.

**3. Collaborations and grants during 2017-2018**

- **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, 2012-2018, \$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.

- **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.

### III. PNWTIRC plans for 2018-2019.

Glenn Howe presented plans for 2018-2019.

- We will continue with the research described in the Genomic Selection Work Plan (2017). The goal of this research is to understanding how to implement genomic selection in Douglas-fir.
- We will work toward developing a genetic map of our SNP genetic markers for Douglas-fir.
- A new CAFS Phase III project is being proposed, which will be led by Jeff Hatten, a soil scientist in the Department of Forest Engineering and Resource Management. The OSU cooperatives involved in the new proposal will be the Center for Planted-forest Silviculture (CIPS; Doug Maguire, Director) and the Vegetation Management Research Cooperative (VMRC; Carlos Gonzalez-Benecke, Director). The University of Maine will be the lead institution with a potential focus on lidar applications in forestry.

### IV. PNWTIRC research presentations

1. ***Breeding for resistance to white pine blister rust in western white pine.*** Oguz Urhan
2. ***Axiom genotyping array for western white pine.*** Glenn Howe
3. ***PNWTIRC/NWTIC genomic selection research.*** Glenn Howe
4. ***Update - Seedlot Selection Tool/Species Potential Habitat Tool.*** Brad St.Clair

### V. Research needs – Breakout groups and discussion.

Josh Sherrill led a breakout session and discussion on PNWTIRC research needs. The results are reported in the minutes (Appendix).

### VI. Budget.

Glenn Howe presented the budget for FY 2017-2018. The proposed budget for FY 2018-2019 was also presented. A motion to approve the budgets was offered by Josh Sherrill. The motion was seconded and approved by unanimous voice vote.

### VII. PNWTIRC Policy/Technical Committee Chair.

Lauren Magalska was nominated to continue as the Policy/Technical Committee Chair by Brian Baltunis. The nomination was seconded and approved by unanimous voice vote.

### VIII. PNWTIRC annual meeting.

Next year's meeting will be held **Tuesday, October 29, 2019**. The location of this year's meeting (OSU North Willamette Research and Extension Center) is generally preferred.

### IX. Other presentations

1. ***Katy Kavanagh, OSU College of Forestry Associate Dean for Research.*** Katy updated PNWTIRC members on College of Forestry (COF) activities and perspectives. She emphasized that the COF is a strong supporter of research cooperatives, and there are close connections between the COF and the forest industry at all levels. Katy described how OSU calculates indirect cost rates, and emphasized that the generation of new knowledge is an important goal of the university. She also mentioned that fostering collaboration among research cooperatives is one of her objectives.

**2. Brad St.Clair, USFS Pacific Northwest Research Station.** Brad gave a short presentation on the Pacific Northwest Research Station (PNWRS). Rich Cronn prepared the slides, but he was unable to attend the meeting. Brad emphasized the need for interactions and collaboration among the PNWTIRC, OSU COF, and USFS PNWRS silviculture and genetics teams.

**X. Meeting adjourned.** The meeting adjourned about 3:00 pm.

## APPENDIX III

**PNWTIRC Research needs**

**Procedure.** Josh Sherill led a brainstorming session to learn about PNWTIRC research needs. Attendees at the 2018 PNWTIRC annual meeting gathered into groups of 4-5 people for discussion of research needs. Ideas were written on Post-it notes, and these were posted to the white-board at the front of the room. Each attendee was given three votes to cast for their highest priority topics. The (sometimes cryptic) phrases on the Post-it notes were edited for clarity, re-framed as questions, and organized into categories by Glenn Howe. For each topic, the original number of votes (PNWTIRC members only) are indicated with asterisks.

**Genetics of drought hardiness**

- What is the best way to test for drought hardiness in breeding programs? \*\*\*\*\*
  - Short-term nursery tests?
  - Longer-term field tests?
  - Greenhouse tests?
  - Rainfall exclusion tests?
- What is the best way to characterize population vulnerability to drought across the landscape?

**Genomics and genomic selection (GS)**

- Can we use genetic markers to select for traits we don't currently measure? \*\*\*\*\*
- Can we develop a realistic plan for cooperatives and industry to implement applied genomics? \*\*\*
- Can we implement genomic selection or other genomic approaches operationally? \*\*
  - Resistance to animal browse or differences in terpene levels? \*
  - Bark thickness or stem taper in relation to useable stem volume?
- Can we use two-stage selection with genomics to enhance tree breeding? \*
- Can we use population genomics to understand maladaptation of seed sources and predict the effects of climate change?
- Can we develop a range of array options (e.g., high- to low-density) to optimize genomic selection?
- Can we study results from crop species to better understand how genomic selection will work in Douglas-fir?
- Is there a way to integrate genetics and growth modeling using genomics?

**Wide crossing/Testing**

- How can deployment be optimized using wide-crossing and wide-testing? \*\*\*\*\*
  - Integration into third-cycle testing?
  - Integration into genetic gain trials?

**Disease and insects**

- How will climate change affect forest diseases? \*\*\*
- How can the PNWTIRC cooperate with the Swiss Needle Cast Cooperative to understand genetic resistance to SNC disease?
- Can we use genomic selection to improve resistance to Swiss needle cast disease?



- Can outreach activities increase support for research on disease resistance?
- Is it possible to use genetic engineering to increase resistance to white pine blister rust?
  - Will it be possible to use genetic engineering (e.g., CRISPR) to improve Douglas-fir in the future? \*
- Can we develop new tools (like the Seedlot Selection Tool) that informs land managers about climate change effects on insects and disease?
- How will climate change (e.g., increased drought or increased rainfall) affect leaf blight in Pacific madrone?

### **Deployment and climate change**

- How much genetic variation should be deployed in operational plantations? What is the appropriate tradeoff between genetic gain and genetic diversity (risk)? \*
- How can we practice assisted migration today?
- What can the population genetic structure of alleles (i.e., population genomics), tell us about how to manage forests for climate change?
- What are the climatic niches of the breeding materials used in NWTIC cooperatives?
- What are appropriate climate transfer distances, considering both growth and survival?

### **Competition and genotype by spacing interactions**

- Do competitive interactions among trees have an important genetic component (i.e., do ideotypes exist)? Should genetic differences in competitive effects be incorporated into growth models? \*
- How should GxE effects be used to design optimal breeding zones (i.e., how much G x E is too much G x E)?
- Genotype x spacing interactions: Should genotype x spacing interactions be considered in designing the optimal spacing of operational plantations?

### **Phenotyping and selection**

- Can we use high-throughput (mass) phenotyping to improve the efficiency of tree breeding programs?
- Can we use lidar to measure tree heights in progeny tests?
- Which traits should be the focus of selection in minor species (e.g., hemlock, noble fir, etc.)?
- What is the economic impact of genetically controlled stem defects (i.e., forks, ramicorn branches, stem sinuosity)? \*\*\*
- Can we practice early selection against stem defects by selecting for less second flushing?

### **Seed orchards**

- Can we scale-up controlled mass pollination so that it can be used operationally? \*\*\*
- Can we control vegetation in seed orchards without herbicides? \*
- What are the best watering regimes to obtain optimal seed ripeness?
- Can we develop ways to manage seed pests without pesticides?
  - Can we use heat traps to attract seed bugs in seed orchards?

## APPENDIX IV

## Financial Statement 2017-2018

### PNWTIRC Financial Support for Fiscal Year 2017-2018

Regular members <sup>1</sup>	120,000
Associate members <sup>1</sup>	5,000
Contracts	2,500
Forest Research Laboratory, Oregon State University <sup>2</sup>	122,711
<b>Total</b>	<b>250,211</b>

<sup>1</sup> Each Regular Member contributed \$10,000 and each Associate Member contributed \$5,000 excluding in-kind contributions of labor, supplies, etc.

<sup>2</sup> The contribution from Oregon State University includes salaries, facility costs, and administrative support.