Pacific Northwest Tree Improvement Research Cooperative

Annual Report

2018 - 2019

PNWTIRC

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

OREGON STATE UNIVERSITY COLLEGE OF FORESTRY

DEPARTMENT OF FOREST ECOSYSTEMS AND SOCIETY

2018-2019

Annual Report

Report editors
Glenn Howe *Jennifer Kling*
Anna Magnuson *Meridith McClure*
Cover photo by Anna Magnuson

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Pacific Northwest Tree Improvement Research Cooperative

Annual Report 2018-2019

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 Resources
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 2018-2019

- We completed development of the Axiom genotyping array for Douglas-fir and submitted the manuscript to BMC Genomics for publication. "Howe, G.T., Jayawickrama, K.J., Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S. 2019. A high-density Axiom genotyping array for Douglas-fir. BMC Genomics (submitted)."
- We published a study in collaboration with the US Forest Service that examines a century of lessons learned from the 1912 Douglas-fir heredity study. "St.Clair, J.B., Howe, G.T., and Kling, J.G. 2019. The 1912 Douglas-fir heredity study: Long-term effects of climatic transfer distance on growth and survival. Journal of Forestry (accepted)."
- Meridith McClure joined the PNWTIRC as a masters student and developed her research proposal, "Climate-based Seed Deployment Zones for the Pacific Northwest."
- PNWTIRC Program Manager Anna Magnuson was hired into the Research Coordinator position to replace Scott Kolpak, who took a job with the USFS.
- We're continuing to develop a reliable and cost-efficient low-density SNP genotyping system in Douglas-fir, building off of work that was done for the Axiom array.
- We're continuing to develop the genomic selection workplan in collaboration with the NWTIC.
- Margaret Banks of Stimson Lumber Co. was elected as the new Policy/Technical Committee Chair for the upcoming year.

MESSAGE FROM THE DIRECTOR

Last year was marked by new beginnings. Anna Magnuson, who had worked part-time as Program Manager for the PNWTIRC, replaced Scott Kolpak as our full-time Research Coordinator. During her first few months, she acquired new lab skills by working on a genotyping project for PNWTIRC members at Weyerhaeuser, Washington Department of Natural Resources, and Pope Resources. She used SSR markers developed by the PNWTIRC to validate Douglas-fir genotypes. This information was necessary to make decisions about the fate of high-value seed orchard and clone bank trees. During the upcoming year, Anna will assist with other PNWTIRC projects, such as developing a low-density SNP assay and building a SNP genetic map for Douglas-fir.

Meridith McClure settled into her Master's program and helped develop a PNWTIRC research proposal entitled, *Climate-based Seed Deployment Zones for the Pacific Northwest*. This proposal is aimed at developing a user-friendly system for climate-based seed transfer throughout the Pacific Northwest region. The proposal was accepted by PNWTIRC members at the annual meeting, so expect a progress report from Meridith at next year's annual meeting.

Jennifer Kling continues to work part-time on the genomic selection project. Last year, we completed a major milestone in the project—we took 5-year measurements on the genomic selection field test managed by Sara Lipow at Roseburg Resources. Jennifer analyzed and presented preliminary results from these measurements at the annual meeting. This project is a collaboration between the Northwest Tree Improvement Cooperative and the PNWTIRC with initial funding coming from the Northwest Advanced Renewables Alliance.

We'll have two important additions to the PNWTIRC next year. First, we'll hire a post-doc to help with the heavy lifting on the quantitative genetic analyses associated with our genomic selection and SNP genotyping projects. Second, we have a new Turkish Master's student, Zeynep Çiçekli, starting in January. Zeynep will be supported by a scholarship from the Turkish government.

Finally, we could not be successful without the active support of PNWTIRC members and collaborations with organizations such as the Northwest Tree Improvement Cooperative (Keith Jayawickrama, Director), Center for Intensive Planted-Forest Silviculture (Doug Maguire, Director), and Inland Empire Tree Improvement Cooperative (Marc Rust, Director). PNWTIRC members continue to play an active role in our research—by providing data, seed, and access to seed orchards, clone banks, and greenhouses. We greatly appreciate everyone's continued support and guidance.

Glenn Howe, PNWTIRC Director

Glenn Howe

AGENDA – TUESDAY OCTOBER 29, 2019

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

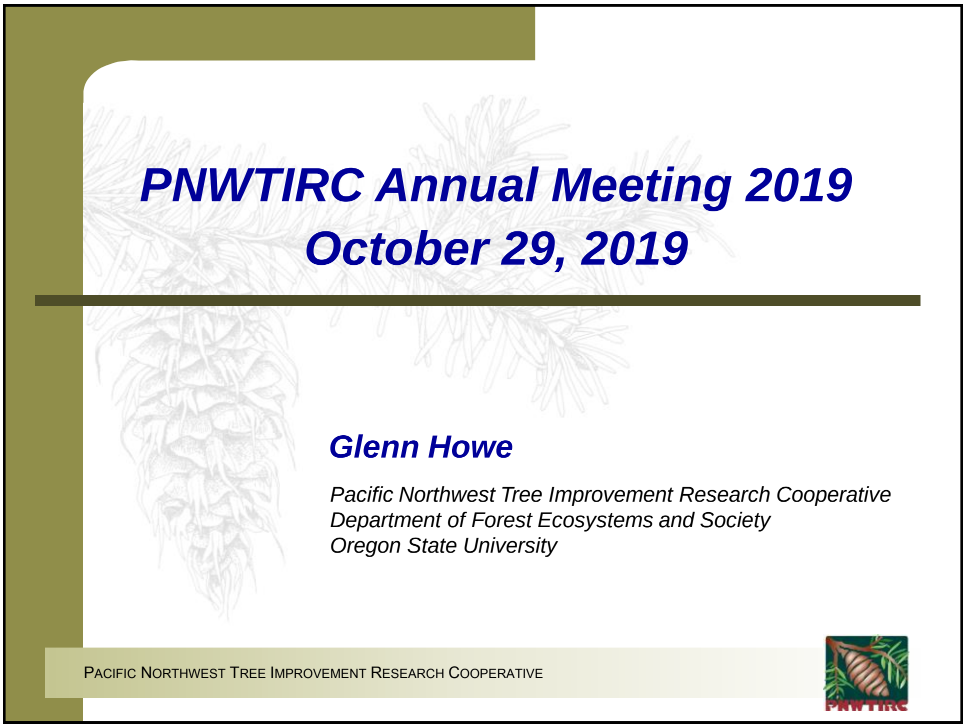
START TIME	9:00 AM for coffee; 9:30 AM for presentations
LOCATION	Clackamas Fire Station #3: Oak Grove 2930 SE Oak Grove Blvd, Milwaukie, OR
CONTACT TEL	541-730-3400 (Glenn); 503-475-7852 (Anna)
LOCATION TEL	503-742-2600
LUNCH	Lunch provided

Time	Topic	Responsibility
9:00-9:30	Coffee	
9:30-9:45	Welcome and introductions	Lauren Magalska
9:45-10:00	Overview • *PNWTIRC personnel* • *PNWTIRC accomplishments for 2018-19* • *PNWTIRC plans for 2019-20*	Glenn Howe
10:00-10:30	Why does Douglas-fir grow so much better in New Zealand?	Liam Gilson Doug Maguire
10:30-11:00	Routine marker genotyping in Douglas-fir: From SSRs to SNPs	Anna Magnuson Glenn Howe
11:00-11:15	Break	
11:15-11:30	Inland Empire Tree Improvement Cooperative	Marc Rust
11:30-12:00	PNWTIRC/NWTIC genomic selection research Preliminary results from the NARA plantation	Jennifer Kling
12:00-1:00	Lunch	
1:00-1:45	Climate-based seed deployment for the PNW PNWTIRC proposal	Meridith McClure Glenn Howe
1:45-2:00	Break	
2:00-2:15	Budget and other business • *Budget presentation and vote* • *Elect new Policy/Technical Committee Chair*	Glenn Howe Lauren Magalska
2:15-3:00	Climate-based seed deployment for the PNW: Member feedback	Glenn Howe
3:00	Wrap-up and adjourn	Glenn Howe

PNWTIRC annual meeting 2019

Glenn Howe

In this year's annual meeting, we highlighted work completed in 2018-19 and presented plans for continuing and future co-op research. We began by reviewing our mission, co-op structure, personnel changes, accomplishments, and collaborations. This introductory presentation concluded with an overview of the annual meeting, and how our research ties in with our long-term goals.



PNWTIRC Annual Meeting 2019
October 29, 2019

Glenn Howe

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

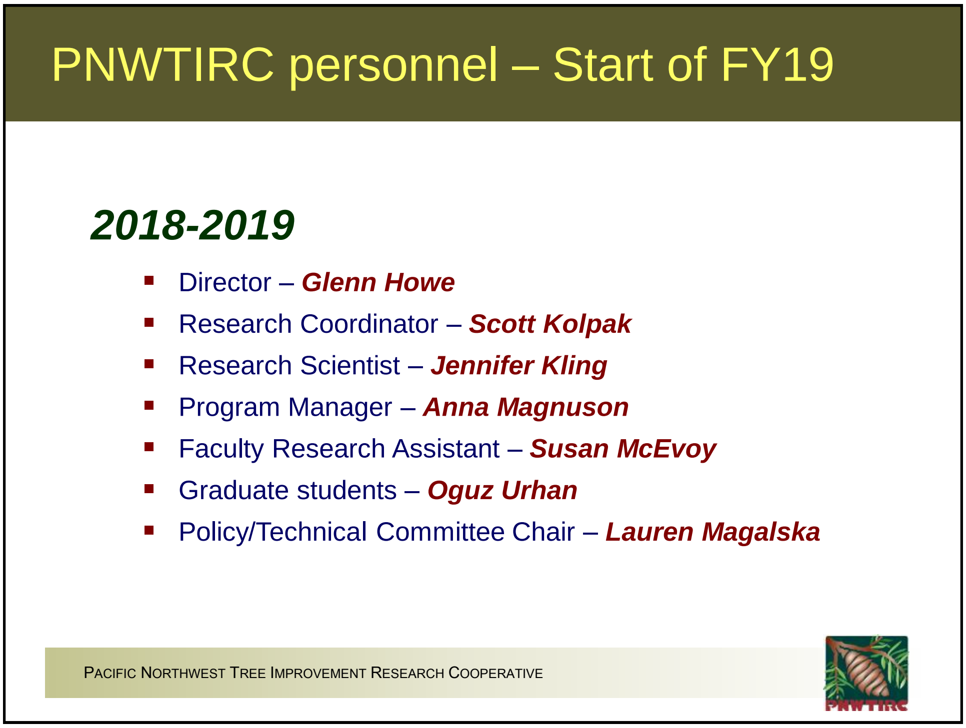
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

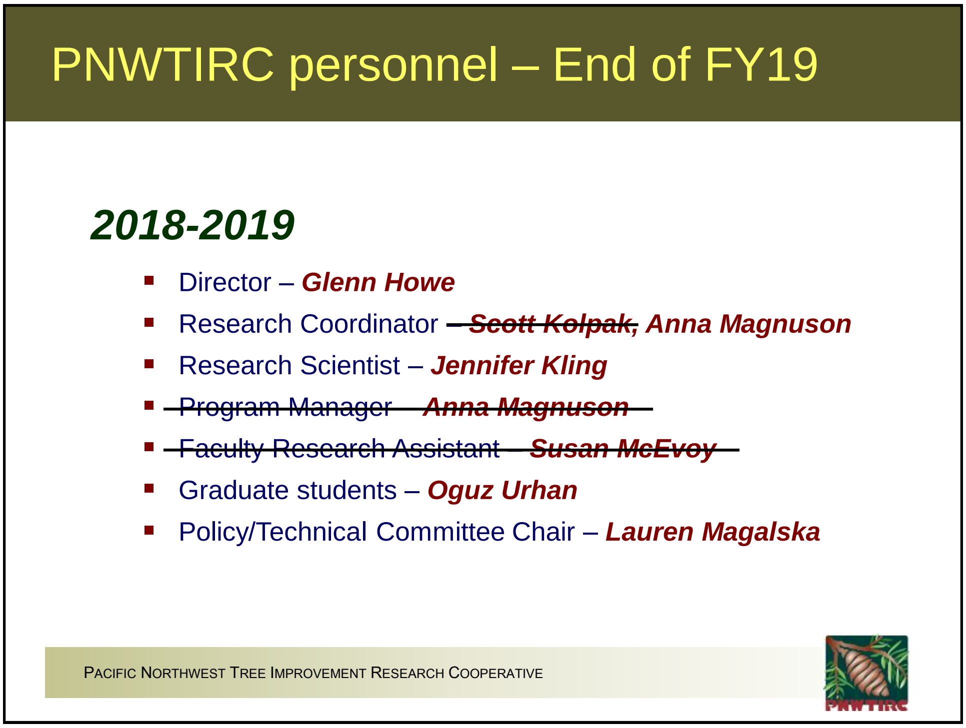


PNWTIRC personnel – Start of FY19

2018-2019

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

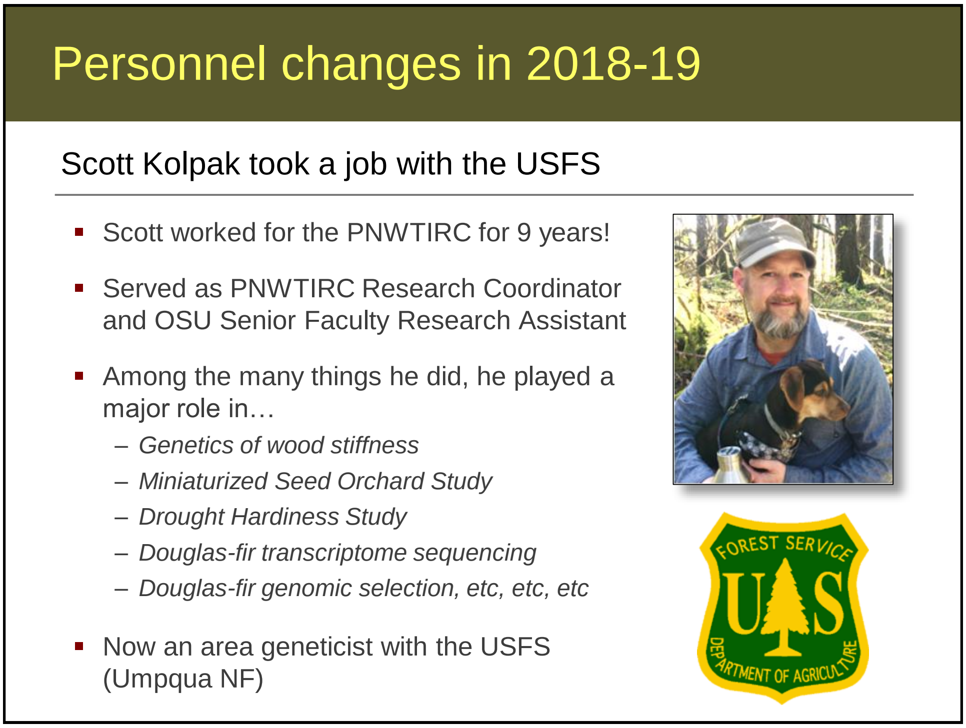


PNWTIRC personnel – End of FY19

2018-2019

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

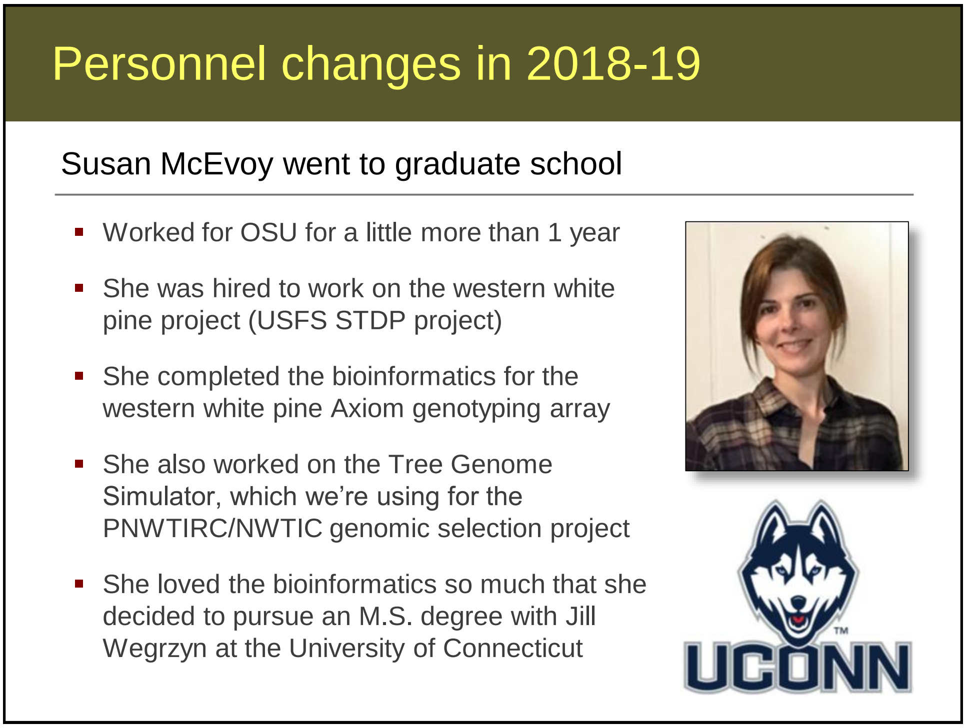
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Personnel changes in 2018-19

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 2018-19

Susan McEvoy went to 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

Activities in 2018-2019

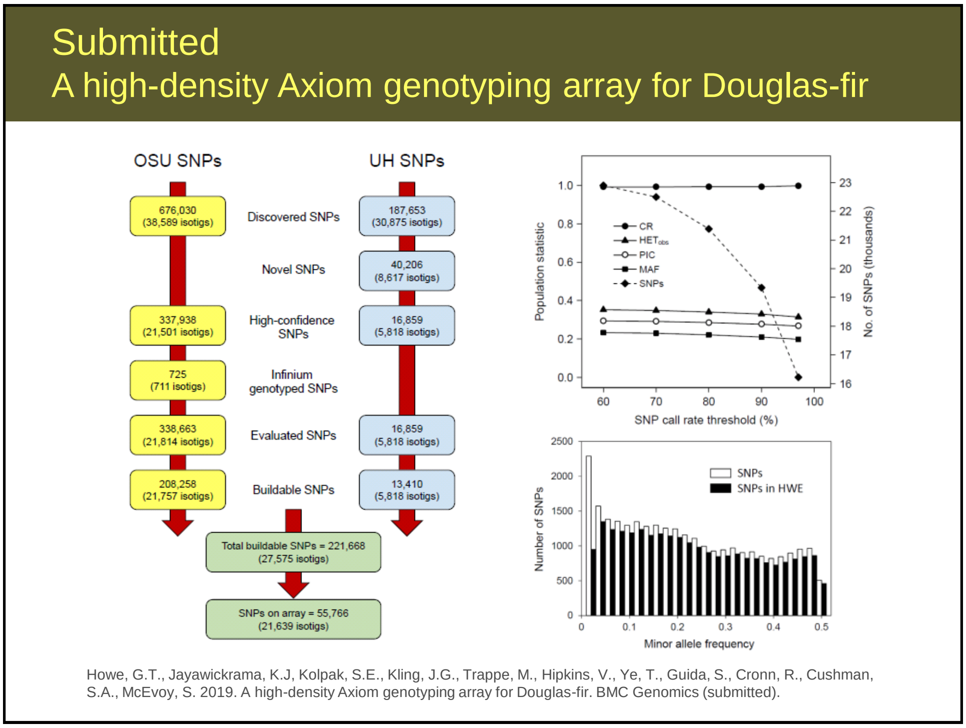
Collaborations and grants

- 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.

Publications and posters

- Howe, G.T., Jayawickrama, K.J., Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S. 2019. A high-density Axiom genotyping array for Douglas-fir. BMC Genomics (submitted).
- St.Clair, J.B., Howe, G.T., and Kling, J.G. 2019. The 1912 Douglas-fir heredity study: Long-term effects of climatic transfer distance on growth and survival. Journal of Forestry (accepted).
- McClure, M., DeBell, J., and Howe, G.T. 2019. Climate-based seed deployment zones for the Pacific Northwest. Poster presented at: Northwest Climate Conference, 8 October, 2019, Portland, Oregon.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Submitted
A high-density Axiom genotyping array for Douglas-fir

OSU SNPs

UH SNPs

Discovered SNPs: 676,030 (38,589 isotigs) | 187,653 (30,875 isotigs)

Novel SNPs: 40,206 (8,617 isotigs)

High-confidence SNPs: 337,938 (21,501 isotigs) | 16,859 (5,818 isotigs)

Infinium genotyped SNPs: 725 (711 isotigs)

Evaluated SNPs: 338,663 (21,814 isotigs) | 16,859 (5,818 isotigs)

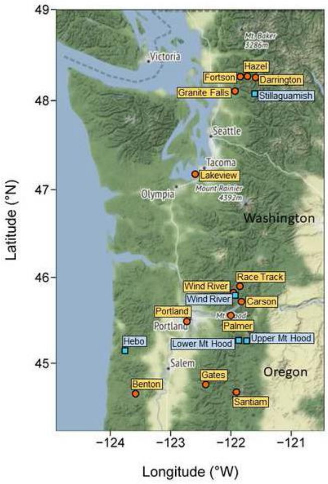
Buildable SNPs: 208,258 (21,757 isotigs) | 13,410 (5,818 isotigs)

Total buildable SNPs = 221,668 (27,575 isotigs)

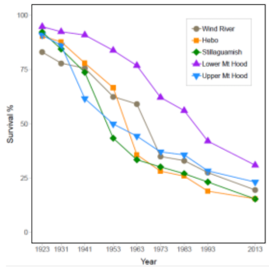
SNPs on array = 55,766 (21,639 isotigs)

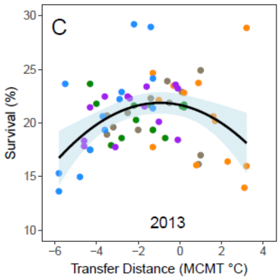
Howe, G.T., Jayawickrama, K.J, Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S. 2019. A high-density Axiom genotyping array for Douglas-fir. BMC Genomics (submitted).

Accepted
The 1912 Douglas-fir heredity study

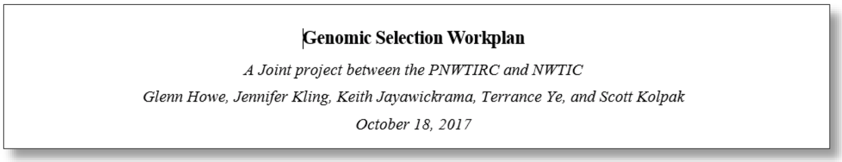


St.Clair, J.B., Howe, G.T., and Kling, J.G. 2019. The 1912 Douglas-fir heredity study: Long-term effects of climatic transfer distance on growth and survival. Journal of Forestry (accepted).





Plans for 2019-2020

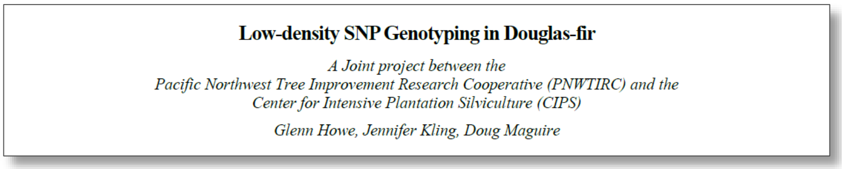


Genomic Selection Workplan

A Joint project between the PNWTIRC and NWTIC

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

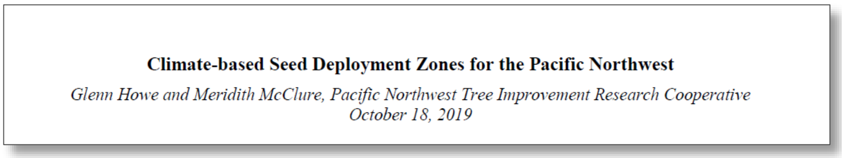
October 18, 2017



Low-density SNP Genotyping in Douglas-fir

A Joint project between the
Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and the
Center for Intensive Plantation Silviculture (CIPS)

Glenn Howe, Jennifer Kling, Doug Maguire



Climate-based Seed Deployment Zones for the Pacific Northwest

Glenn Howe and Meridith McClure, Pacific Northwest Tree Improvement Research Cooperative
October 18, 2019

Update: Why does Douglas-fir grow much better in New Zealand?

Liam Gilson and Doug Maguire

Many timber species, including Douglas-fir, are known to exhibit higher productivity when planted in exotic settings. Volume yields in New Zealand, for example, have been recorded as up to 50% higher than those in Oregon at a given age. The causes of this improved growth are not fully understood, though studies have identified seasonal precipitation patterns, temperatures, and summer vapor pressure deficits as potential drivers.

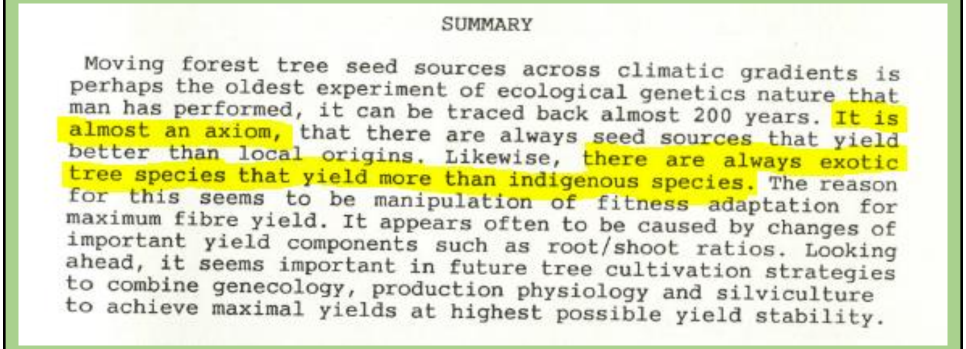
In this ongoing study, we identified Douglas-fir genetic material that was planted in both New Zealand and Oregon in 2004, and have taken on-site measurements of growth and various environmental factors. We hope to use our growth data and SNP genotyping of study trees to isolate meteorological and genetic effects on growth of Douglas-fir across this large environmental gradient.

Update: Why does Douglas-fir grow much better in New Zealand?

Liam Gilson
P.I.: Doug Maguire

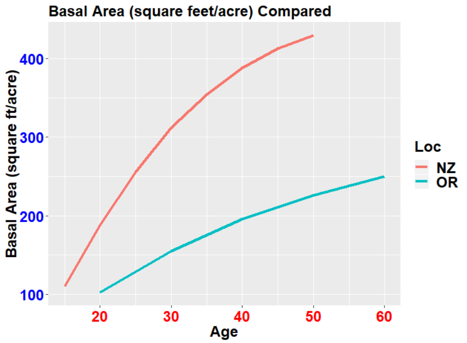
PNWTIRC Annual Meeting,
October 29, 2019

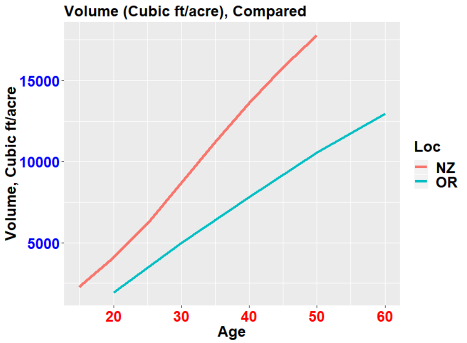
Oregon State University



SUMMARY

Moving forest tree seed sources across climatic gradients is perhaps the oldest experiment of ecological genetics nature that man has performed, it can be traced back almost 200 years. It is almost an axiom, that there are always seed sources that yield better than local origins. Likewise, there are always exotic tree species that yield more than indigenous species. The reason for this seems to be manipulation of fitness adaptation for maximum fibre yield. It appears often to be caused by changes of important yield components such as root/shoot ratios. Looking ahead, it seems important in future tree cultivation strategies to combine genecology, production physiology and silviculture to achieve maximal yields at highest possible yield stability.

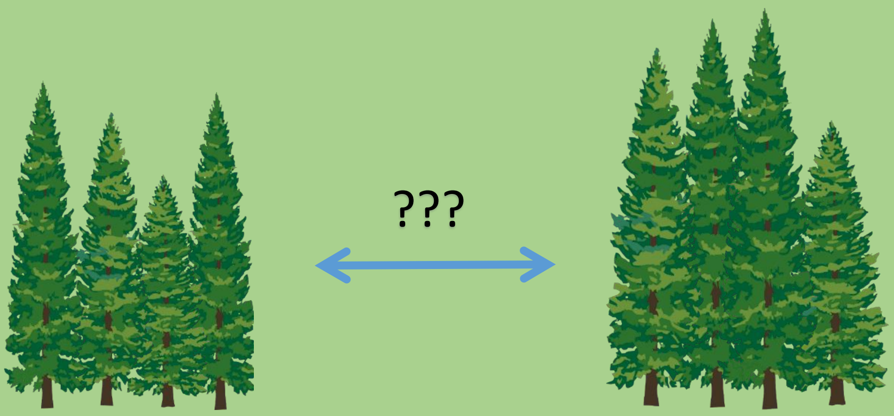

Basal Area (square feet/acre) Compared

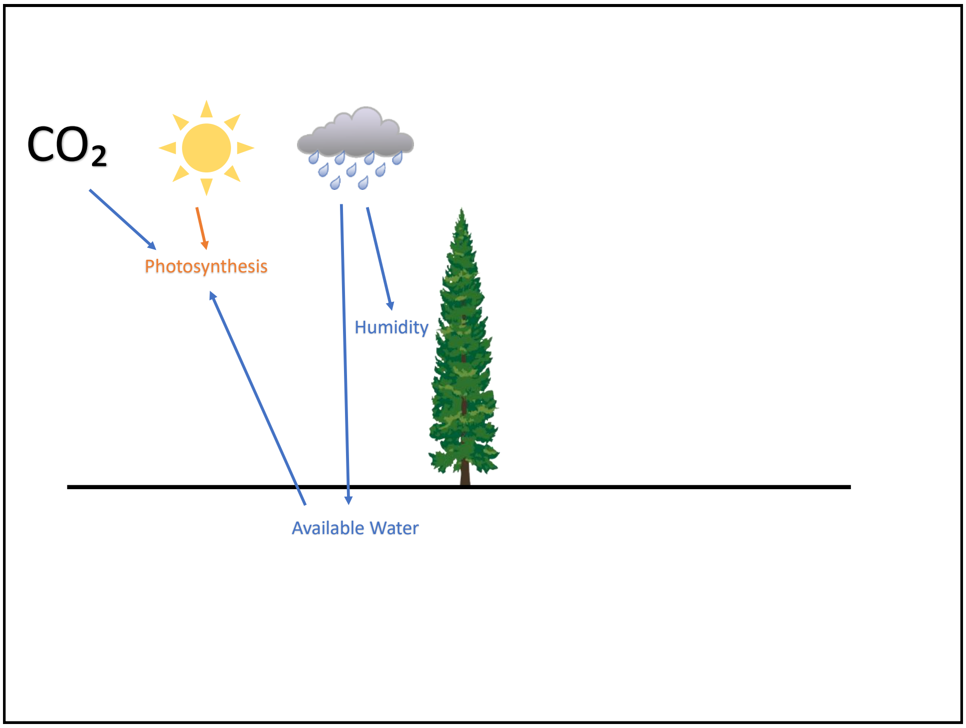

Volume (Cubic ft/acre), Compared

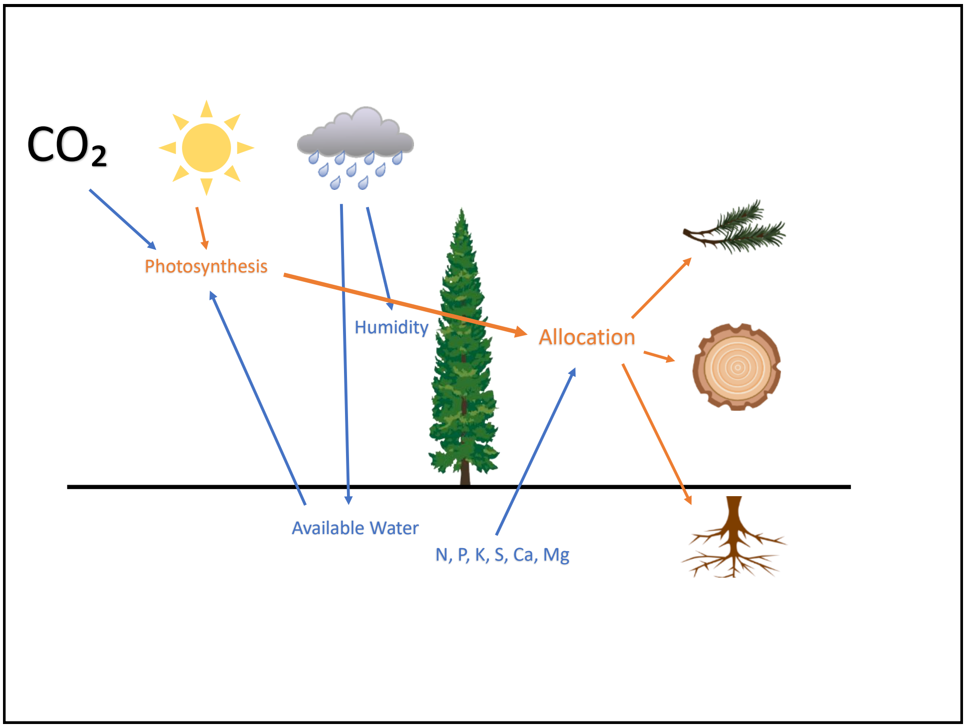
Data from:

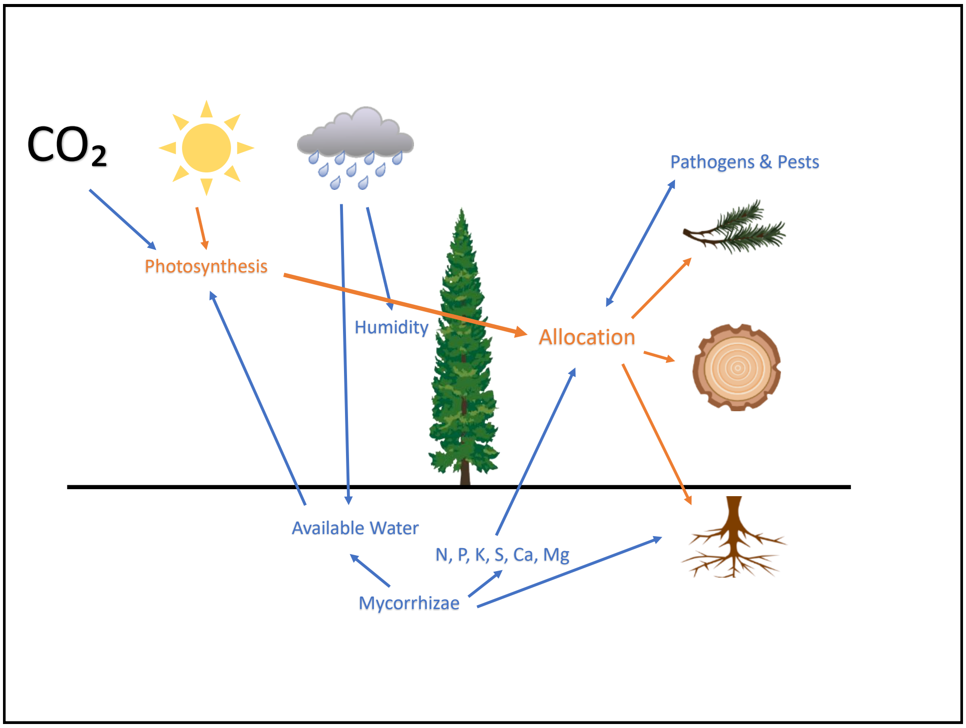
Spurr, S. H. (1963). Growth of Douglas fir in New Zealand. Forest Research Institute, New Zealand Forest Service, Rotorua, NZ. Technical Paper No. 43

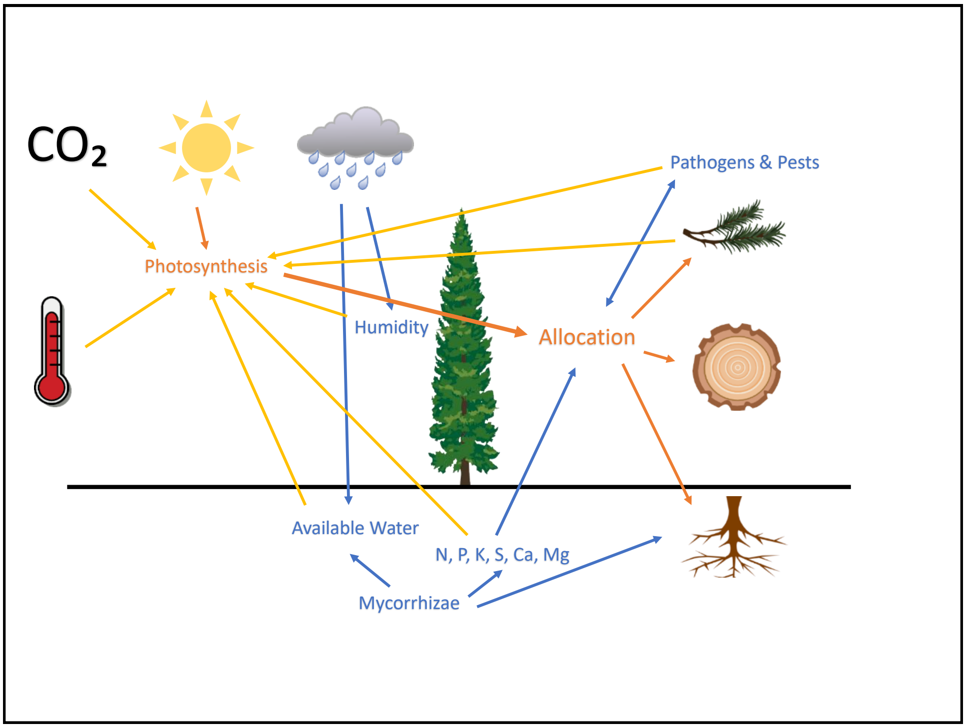
McArdle, R. E., Meyer, W. H., & Bruce, D. (1961). The Yield of Douglas Fir in the Pacific Northwest. *US Department of Agriculture. Technical Bulletin No. 201.*, (201), 82.

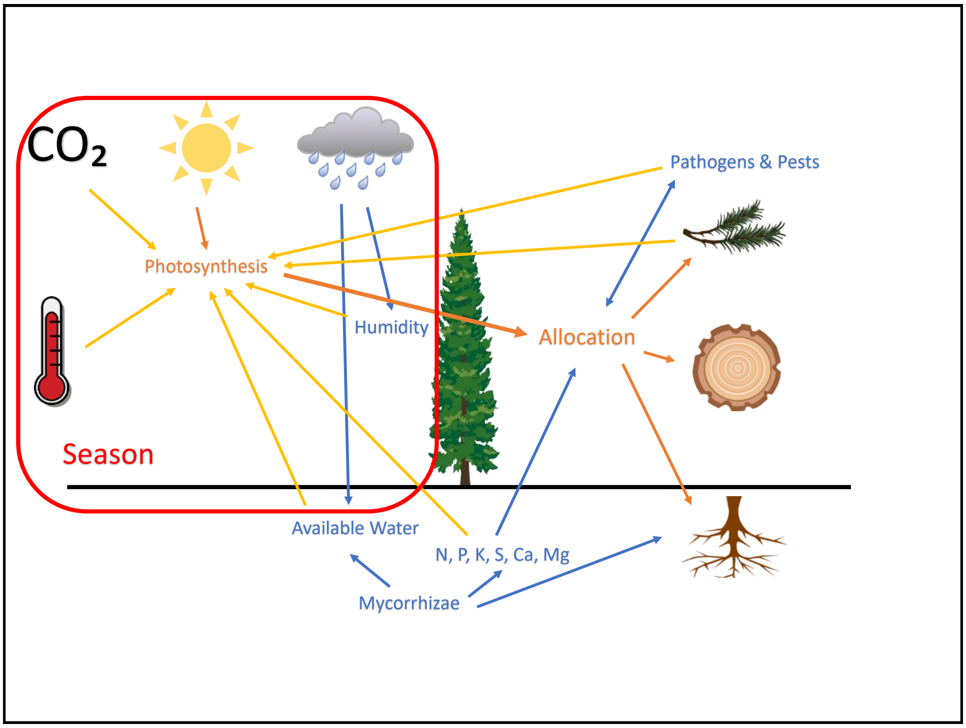


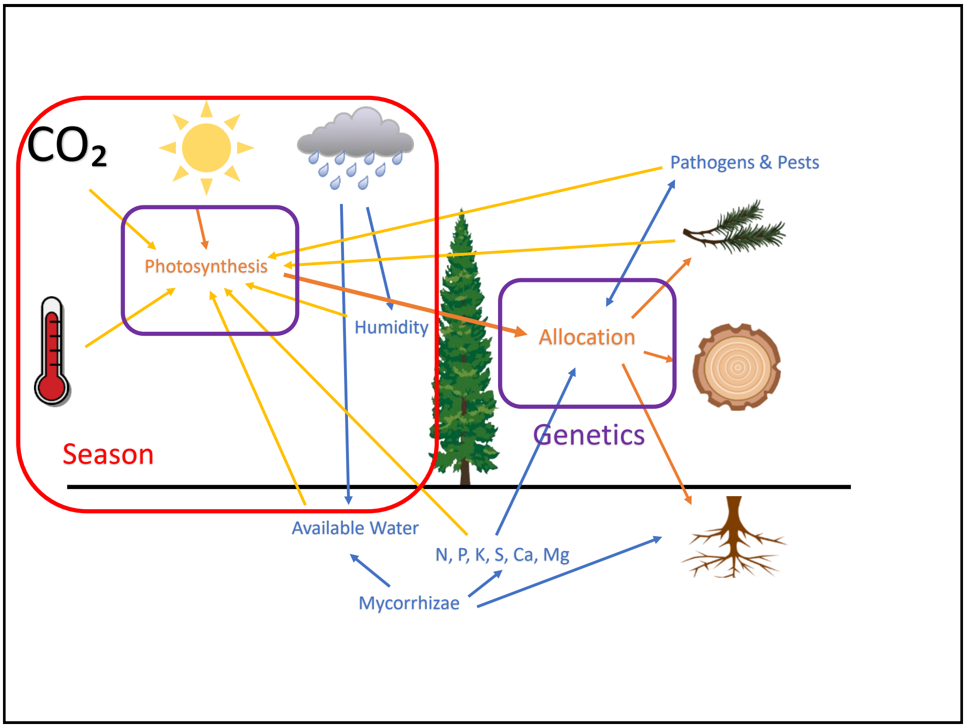

CO_2
Photosynthesis
Humidity
Available Water


CO_2
Photosynthesis
Humidity
Allocation
Available Water
N, P, K, S, Ca, Mg


CO_2
Photosynthesis
Humidity
Allocation
Pathogens & Pests
Available Water
N, P, K, S, Ca, Mg
Mycorrhizae


CO_2
Photosynthesis
Humidity
Allocation
Pathogens & Pests
Available Water
N, P, K, S, Ca, Mg
Mycorrhizae


CO_2
Pathogens & Pests
Photosynthesis
Humidity
Allocation
Season
Available Water
N, P, K, S, Ca, Mg
Mycorrhizae


CO_2
Pathogens & Pests
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Genetics
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Available Water
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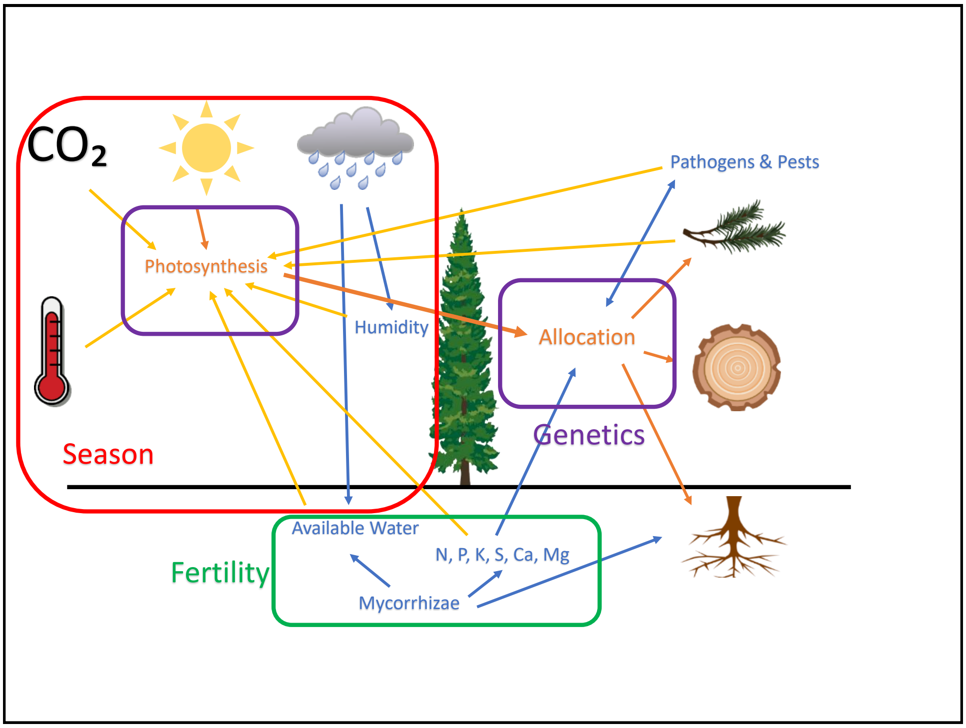
Mycorrhizae



CO_2

Photosynthesis

Humidity

Pathogens & Pests

Allocation

Genetics

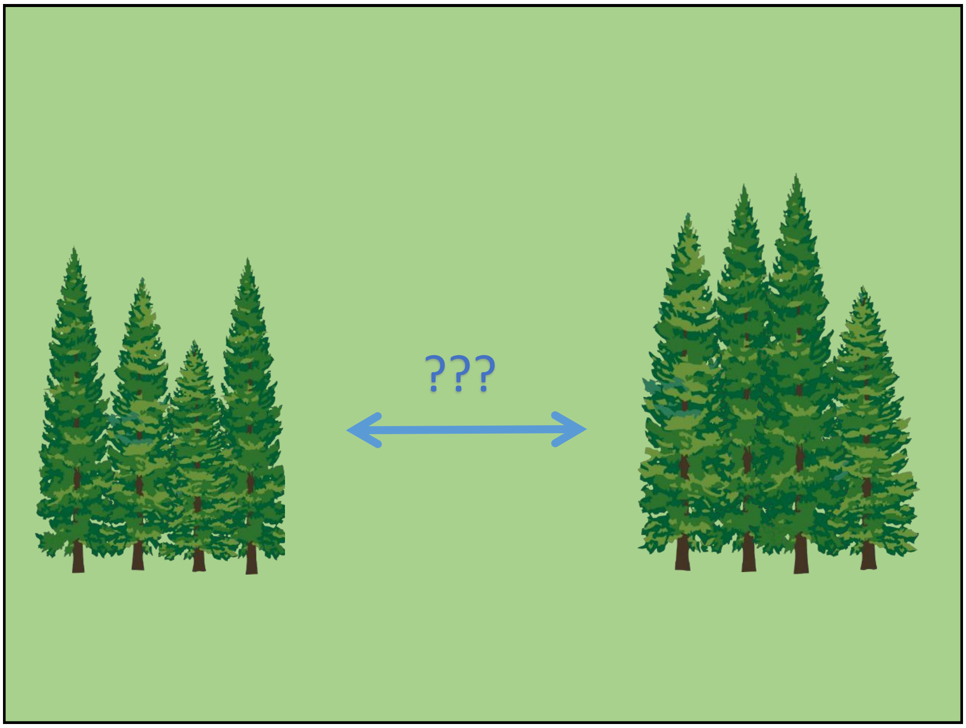
Season

Available Water

N, P, K, S, Ca, Mg

Mycorrhizae

Fertility



Why is the productivity of Douglas-fir higher in New Zealand than in its native range in the Pacific Northwest, USA?

Richard Waring[a,*], Alan Nordmeyer[b], David Whitehead[c], John Hunt[c], Michael Newton[a], Christoph Thomas[a], James Irvine[a]

[a] Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA
[b] PO Box 63, Woodend 7641, New Zealand
[c] Landcare Research PO Box 40, Lincoln 7640, New Zealand

and precipitation were similar at all sites, as was solar irradiance, the much higher yields recorded in New Zealand can be attributed to differences in temperature extremes recorded in Oregon during the summer growing season. These higher temperatures lead to higher daytime air humidity deficits which result in a reduction in stomatal conductance and hence photosynthesis. The water use efficiency (carbon gain per unit of water transpired) is much higher during summer months in New Zealand, averaging 7.0 g C m^{-2}/mm water between November and March compared with

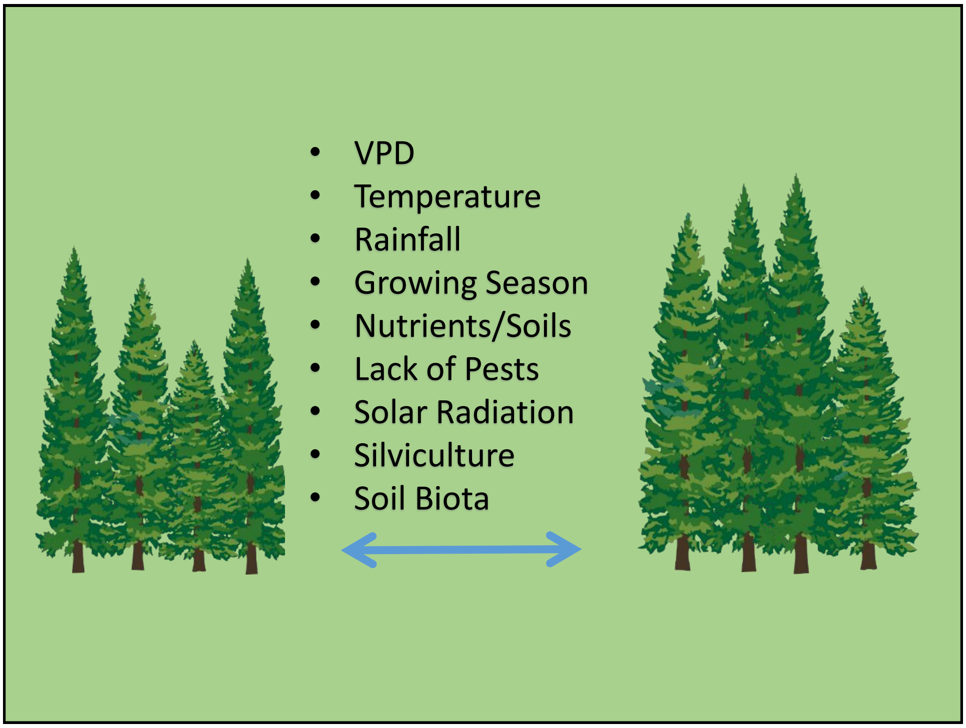
A common garden experiment examining light use efficiency and heat sum to explain growth differences in native and exotic *Pinus taeda*

Timothy J. Albaugh[a,*], Thomas R. Fox[b], Chris A. Maier[c], Otávio C. Campoe[d], Rafael A. Rubilar[e], Rachel L. Cook[f], Jay E. Raymond[a], Clayton A. Alvares[g], Jose L. Stape[h,i]

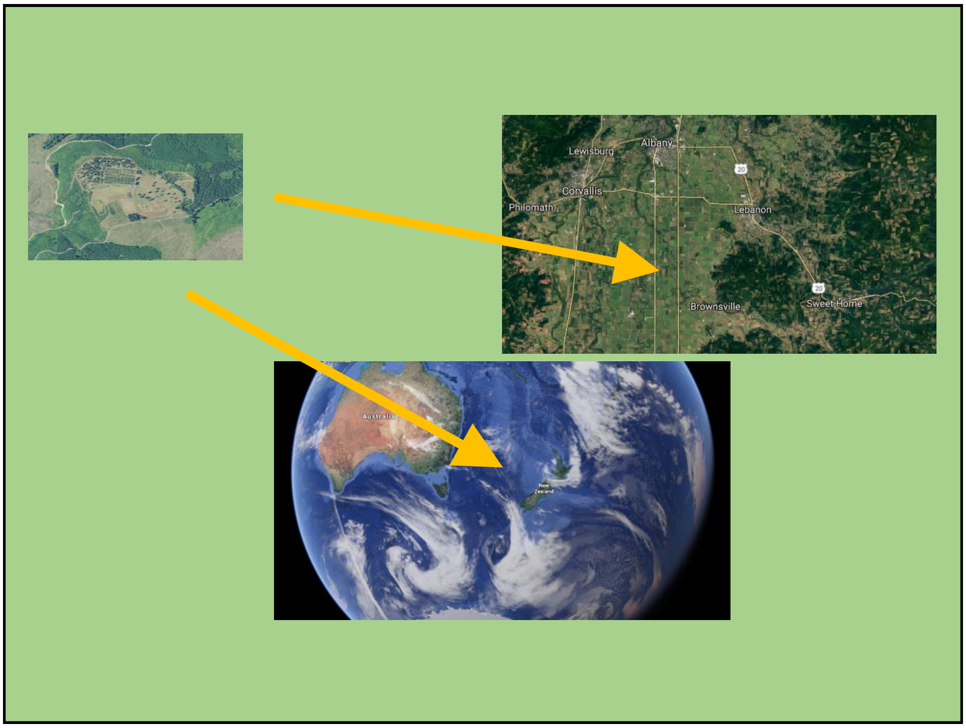
[a] Virginia Tech, Department of Forest Resources and Environmental Conservation, 228 Cheatham Hall, Blacksburg, VA 24061, USA
[b] Forest Productivity and Sustainability, Forest Research Center, Rayonier Inc., Yulee, Fl. 32097, USA
[c] USDA Forest Service, 3041 Cornwallis Road, Research Triangle Park, NC, USA
[d] Federal University of Santa Catarina, Curitibanos, SC, Brazil
[e] Cooperativa de Productividad Forestal, Facultad de Ciencias Forestales, Universidad de Concepción, Victoria 631, Casilla 160-C, Concepción, Chile
[f] Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695-8008, USA
[g] Forestry Science and Research Institute – IPEF, Piracicaba, SP 13400-970, Brazil
[h] Department of Forest Science, Sao Paulo State University and Botucatu, SP, Brazil
[i] Department of Forest Sciences, University of Sao Paulo, Piracicaba, SP, Brazil

still a site effect where site ranking of volume growth per unit of cumulative degree hours was BR > VA > NC. These results indicate that whereas more favorable weather may play a part in explaining the better growth observed in exotic plantations, there may be something else that contributes to the better exotic growth and thus would account for the site differences in volume growth per unit degree hour.

lowing nights where nighttime temperatures were less than 0 °C. This analysis showed greater growth per unit of degree hours for the BR site indicating that something other than these factors was causing the observed difference in growth. However, additional factors including respiration and extreme climatic conditions may also contribute to the differences in growth per unit degree hour and including these differences in the analysis would require a more detailed modeling effort to



- VPD
- Temperature
- Rainfall
- Growing Season
- Nutrients/Soils
- Lack of Pests
- Solar Radiation
- Silviculture
- Soil Biota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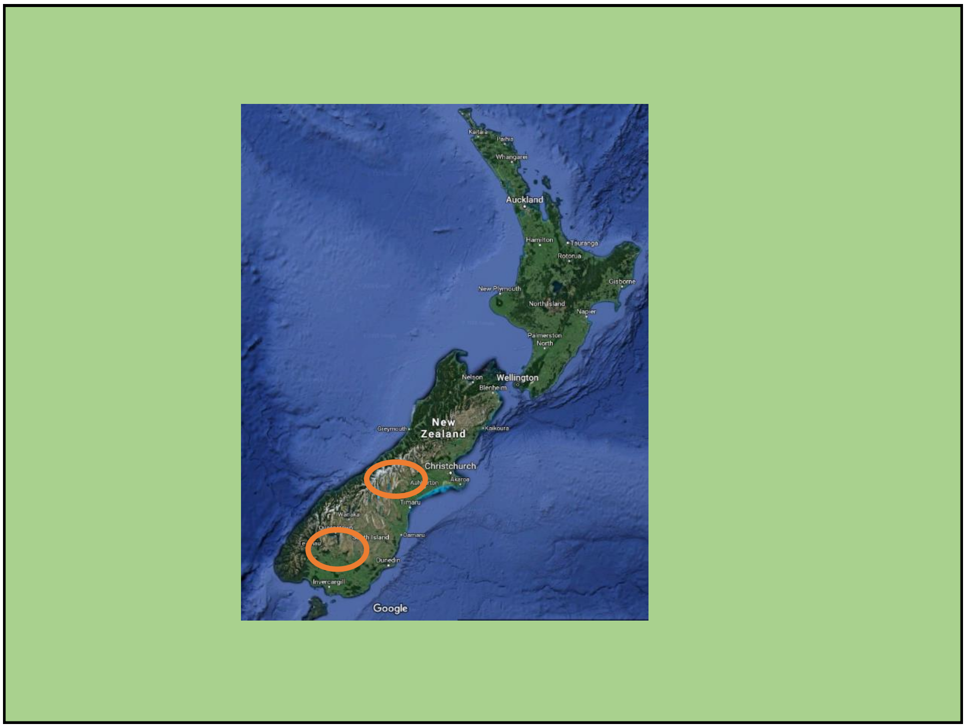
Corvallis
Philomath
Lebanon
20
Brownsville
Sweet Home
Australia
New Zealand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Paihia
Whangarei
Auckland
Hamilton
Tauranga
Rotorua
New Plymouth
Gisborne
North Island
Napier
Palmerston North
Nelson
Wellington
Blenheim
Greymouth
New Zealand
Kaikoura
Christchurch
Akaroa
Timaru
Wanaka
Oamaru
Dunedin
Invercargill
Google


Pegasus Bay
Rangiora
New Zealand
South Island
Christchurch
Canterbury
Ashburton
Canterbury Bight

2008


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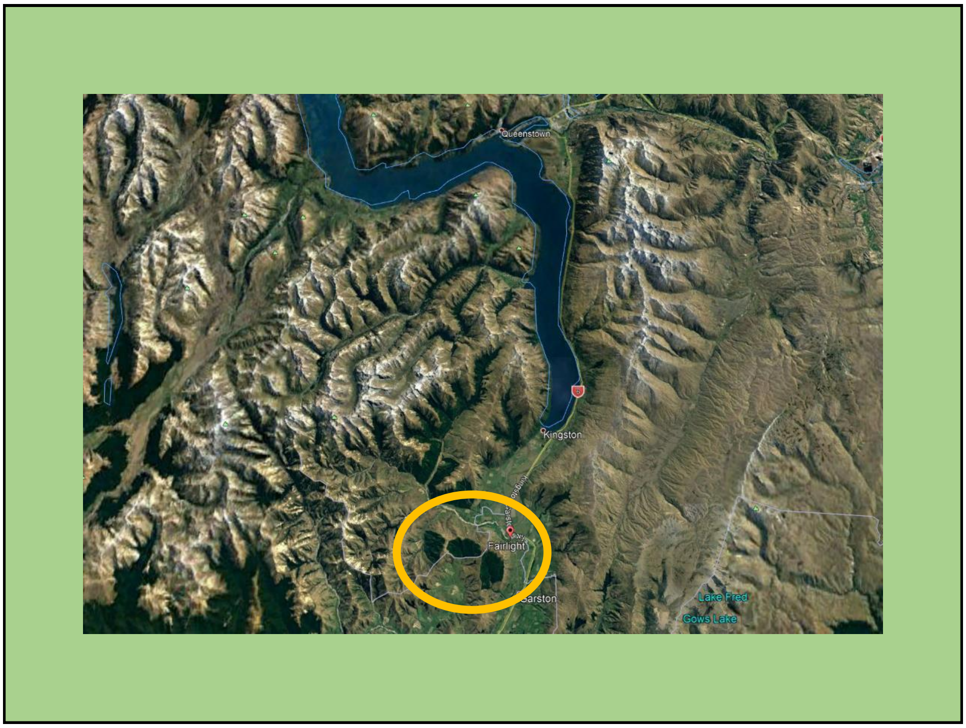
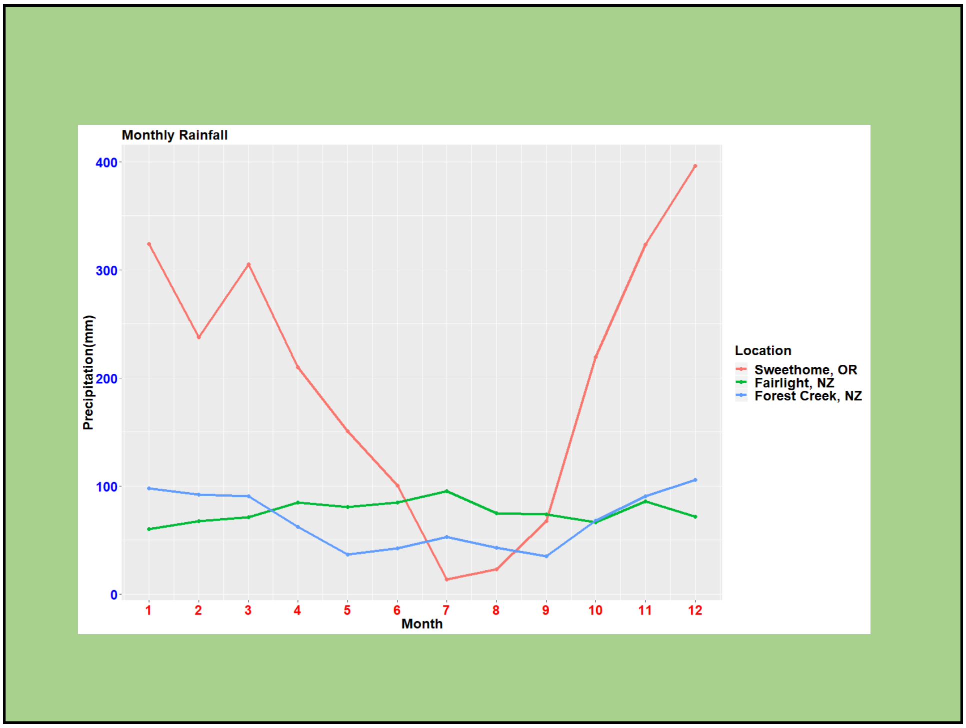
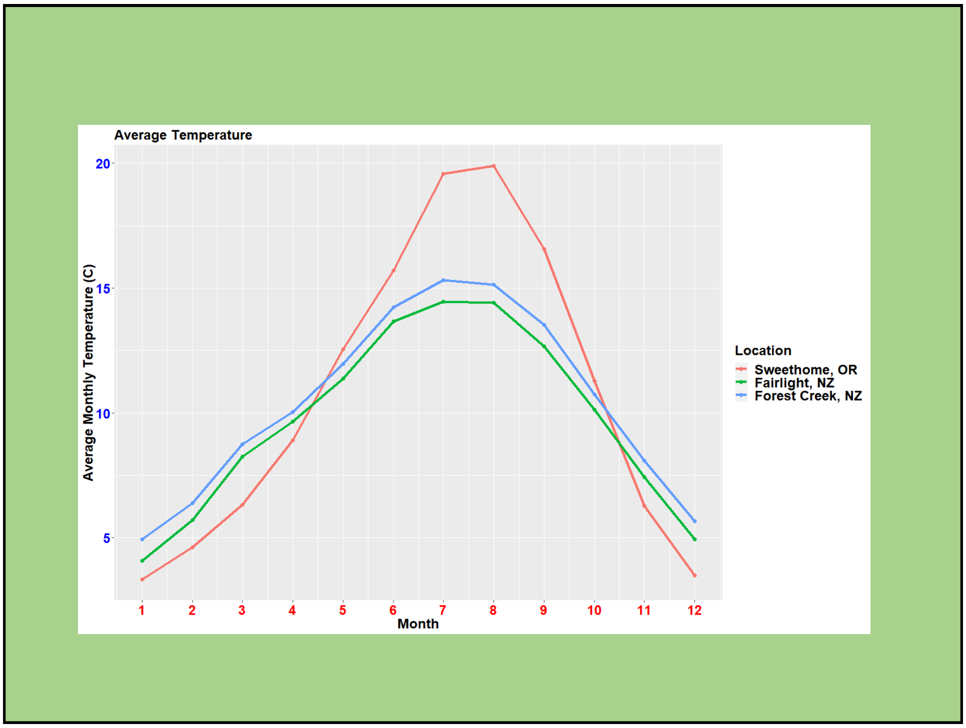
Google Earth


Queenstown
Kingston
Fairlight
Garston
Lake Fred
Gows Lake

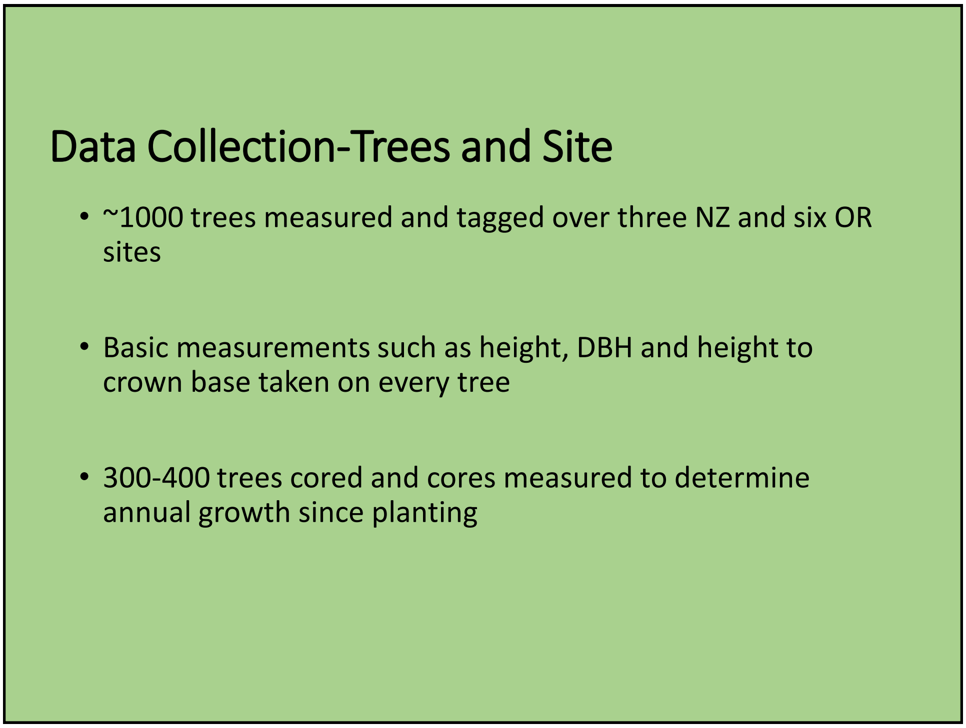

2008
2018
Fairlight
Google Earth


Monthly Rainfall
Precipitation(mm)
0
100
200
300
400
1
2
3
4
5
6
7
8
9
10
11
12
Month
Location
Sweethome, OR
Fairlight, NZ
Forest Creek, NZ


Average Temperature
Average Monthly Temperature (C)
5
10
15
20
1
2
3
4
5
6
7
8
9
10
11
12
Month
Location
Sweethome, OR
Fairlight, NZ
Forest Creek, NZ

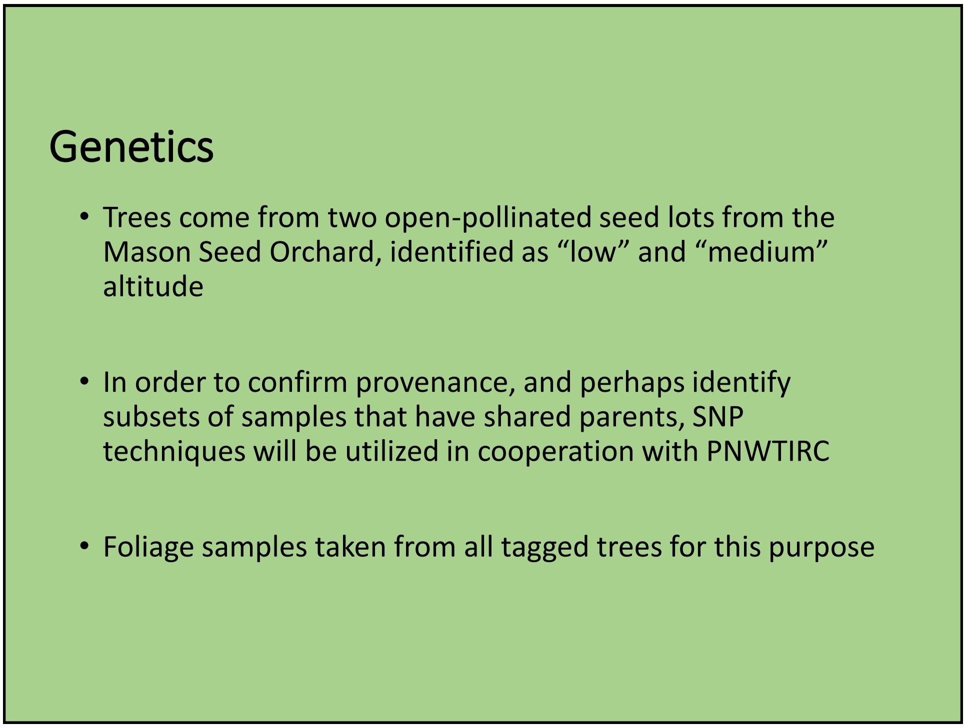
Instrumentation

- Six weather stations and soil probes installed at sites in NZ and OR
- Intended to confirm or correct interpolated weather data, and provide information about soil water levels during the growing season



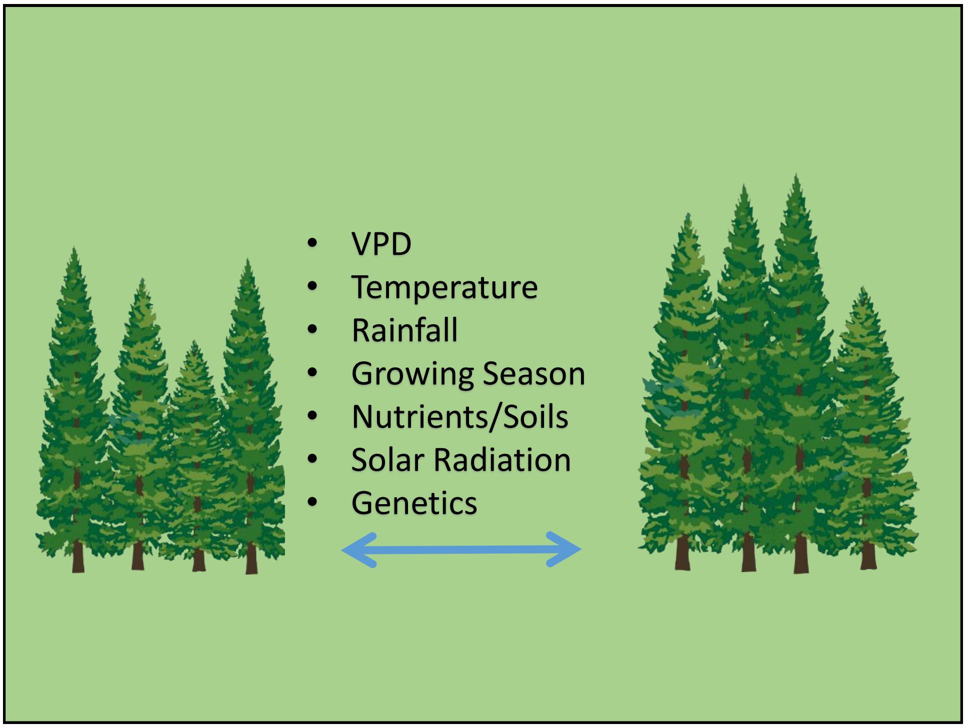
Data Collection-Trees and Site

- ~1000 trees measured and tagged over three NZ and six OR sites
- Basic measurements such as height, DBH and height to crown base taken on every tree
- 300-400 trees cored and cores measured to determine annual growth since planting



Genetics

- Trees come from two open-pollinated seed lots from the Mason Seed Orchard, identified as “low” and “medium” altitude
- In order to confirm provenance, and perhaps identify subsets of samples that have shared parents, SNP techniques will be utilized in cooperation with PNWTIRC
- Foliage samples taken from all tagged trees for this purpose



- VPD
- Temperature
- Rainfall
- Growing Season
- Nutrients/Soils
- Solar Radiation
- Genetics

Questions?

Acknowledgements

CIPS: Doug Mainwaring, Sukhyun Joo, Martín Uranga, Jake Putney

PNWTIRC: Glenn Howe

OSU Staff: Chelsey Durling, Madison Dudley, Rebecca Currier, Glenn Folkert

USFS: Richard Cronn

Josh Cairns, Southern Forests NZ, Ltd.

Bill Marshall, Cascade Timber Consulting, Inc

Ryu Barrett, field assistant

SOUTHERN FORESTS NZ LTD
Forest Managers | Logging Managers | Consultants

CASCADE
Timber Consulting, Inc.

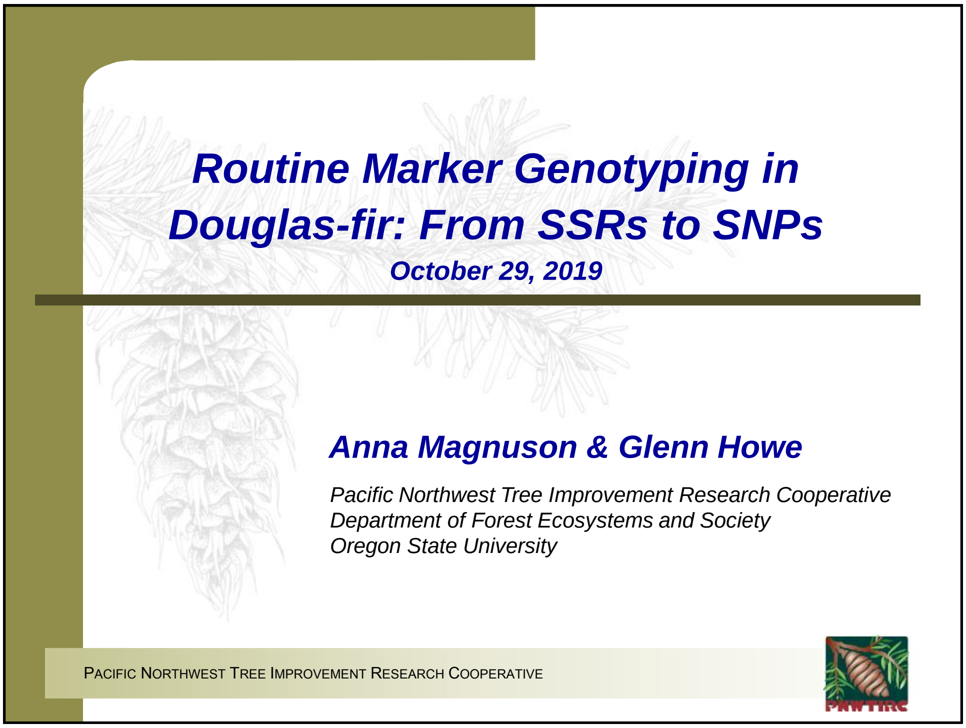
Routine marker genotyping in Douglas-fir: From SSRs to SNPs

Anna Magnuson and Glenn Howe

SSRs, which are also called simple sequence repeats or microsatellite markers, have been the go-to method for routine genotyping in Douglas-fir tree breeding programs. Their genetic variability and modest cost make them particularly suitable for validating genotypes, inferring parents, and measuring pollen contamination in seed orchards. However, the technology does not lend itself to high-throughput automation, and genotypes often need to be scored 'by hand.' Thus, it is difficult to process very large numbers of samples quickly. Instead of relying on SSRs, we are developing routine methods for genotyping Douglas-fir using SNPs. SNPs, or single nucleotide polymorphisms, are the next generation of genotyping technology. SNPs are single letter changes in the genetic code that can be measured efficiently on many samples and scored using automated computer programs.

Early this year, we undertook a small-scale project to use SSRs to confirm identities of high-value Douglas-fir clones belonging to PNWTIRC members. We obtained needle tissue from trees belonging to the Washington Department of Natural Resources, Weyerhaeuser, and Pope Resources. Twenty known ramets or ortets were compared to 29 unknown ramets or composite samples. Due to the small scale of the project and short timeline, we chose to use proven SSR markers developed by the PNWTIRC (Slavov et al. 2004). Ultimately, we were able to confirm 6 genotype matches and 19 suspected mismatches, but 4 comparisons remained ambiguous. Although this project was mostly successful, it also highlighted the need for more robust and higher-throughput genotyping methods.

The PNWTIRC recently developed a high-density Axiom array for Douglas-fir that can be used to genotype about 28,000 SNPs (Howe et al. 2020). Although this is a great resource for many applications, the Axiom array is not cheap, and can be 'overkill' for applications such as genotype validation and parental assignment. Thus, this year, we will develop and test a low-density genotyping array consisting of 5,000 or fewer SNPs. Our aim is to develop a lower cost, outsourced approach suitable for routine genotyping by PNWTIRC members and for research. For example, we will use this approach to develop a framework SNP genetic map, and for collaborative research with the Center for Intensive Planted-Forest Silviculture.



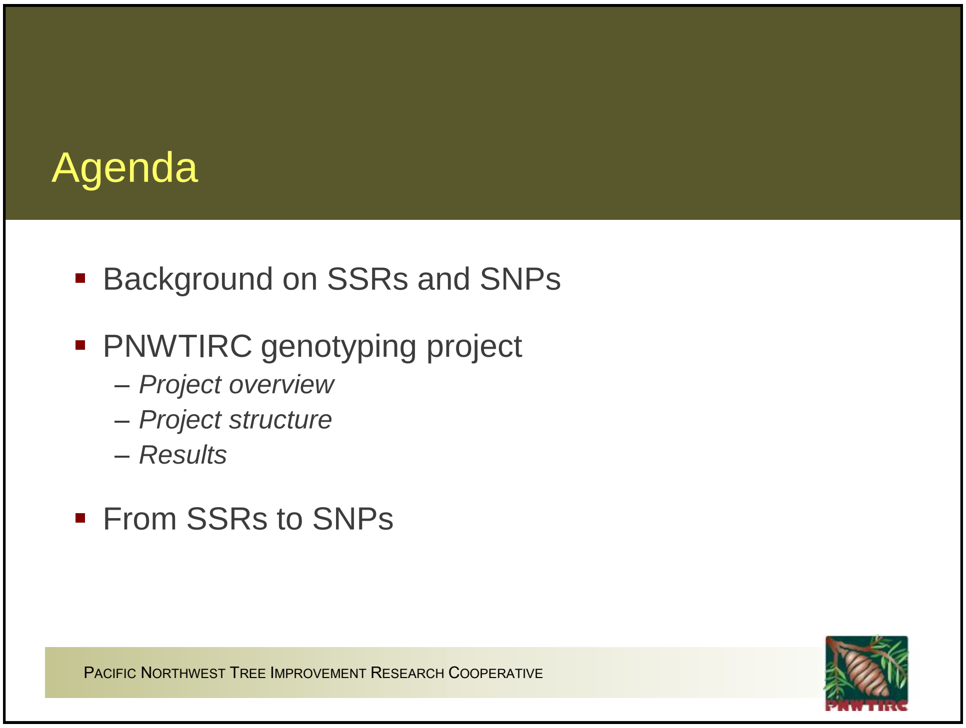
Routine Marker Genotyping in Douglas-fir: From SSRs to SNPs

October 29, 2019

Anna Magnuson & Glenn Howe

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

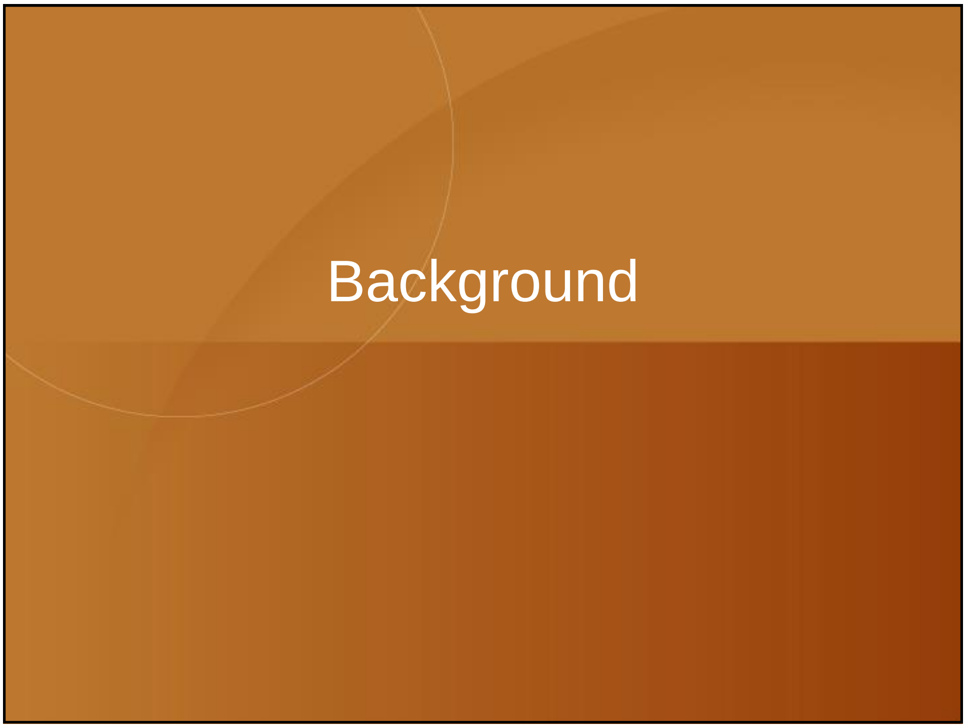
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



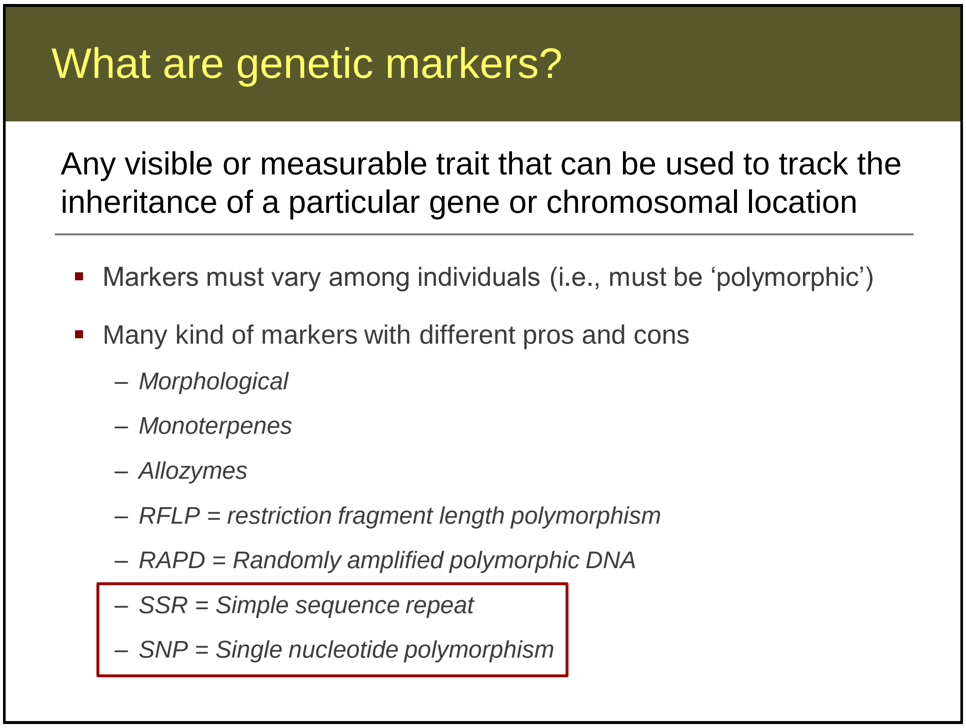
Agenda

- Background on SSRs and SNPs
- PNWTIRC genotyping project
    - *Project overview*
    - *Project structure*
    - *Results*
- From SSRs to SNPs

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



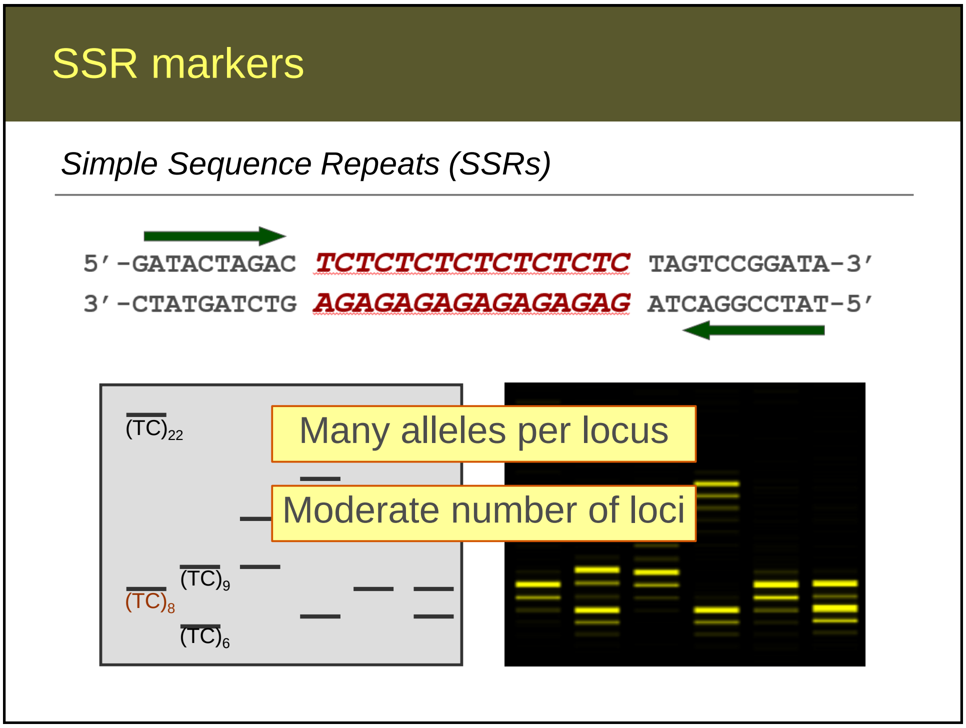
Background



What are genetic markers?

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

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



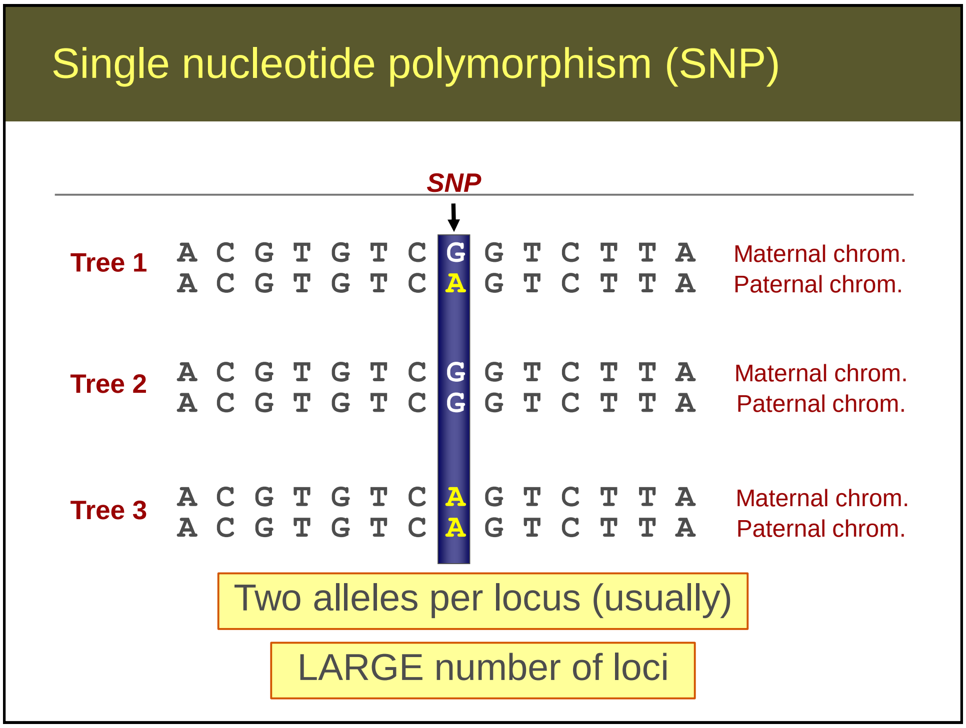
SSR markers

Simple Sequence Repeats (SSRs)

5′-GATACTAGAC ***TCTCTCTCTCTCTCTC*** TAGTCCGGATA-3′
3′-CTATGATCTG ***AGAGAGAGAGAGAGAG*** ATCAGGCCTAT-5′

Many alleles per locus

Moderate number of loci



Single nucleotide polymorphism (SNP)

Tree 1 A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.

Tree 2 A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C G G T C T T A Paternal chrom.

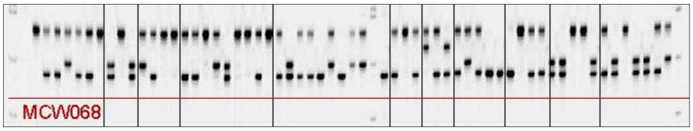
Tree 3 A C G T G T C A G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.

Two alleles per locus (usually)

LARGE number of loci

Markers in tree improvement

- Check the identity of genotypes (fingerprinting) and validate pedigrees
- Measure relatedness (paternity analysis and pedigree reconstruction)
  - *Polymix breeding with paternity analysis*
  - *Improve estimates of breeding values via BLUP*
- Seed orchard management (paternity or parental analysis)
  - *What is the level of pollen or seed contamination?*
  - *Which parents are represented in orchard seedlots (mating systems)?*
  - *What is the success of CMP or SMP?*
- Management of genetic diversity including inbreeding



NFGEL has provided SSR genotyping

Forest Service Home > USDA Forest Service National Forest Genetics Laboratory (NFGEL)

National Forest Genetics Laboratory (NFGEL)

...put science to work to solve on-the-ground problems.

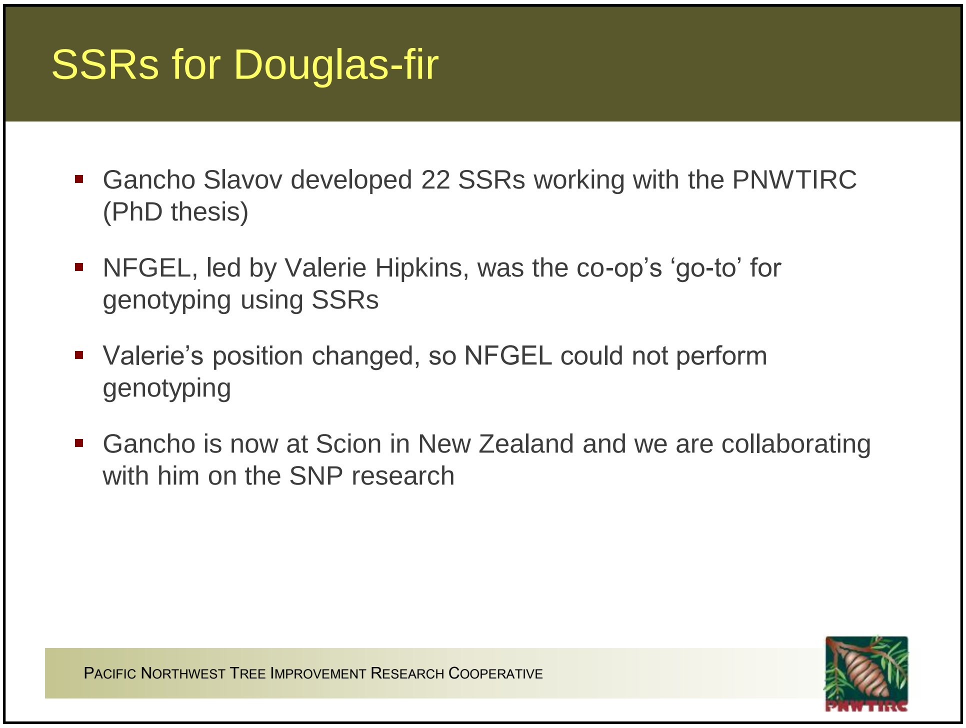
USDA Forest Service
National Forest Genetics
Laboratory (NFGEL)
2480 Carson Road
Placerville, CA 95667

Ponderosa Pine Evolutionary History and Genetic Variation

A genetic database to address conservation and management of ponderosa pine.

Mitochondrial DNA Haplotype Distribution Patterns in *Pinus Ponderosa* (Pinaceae): Range-Wide Evolutionary History and Implications For Conservation

The National Forest Genetics Laboratory (NFGEL) provides genetic testing and information for integrated solutions to on-the-ground problems faced by natural

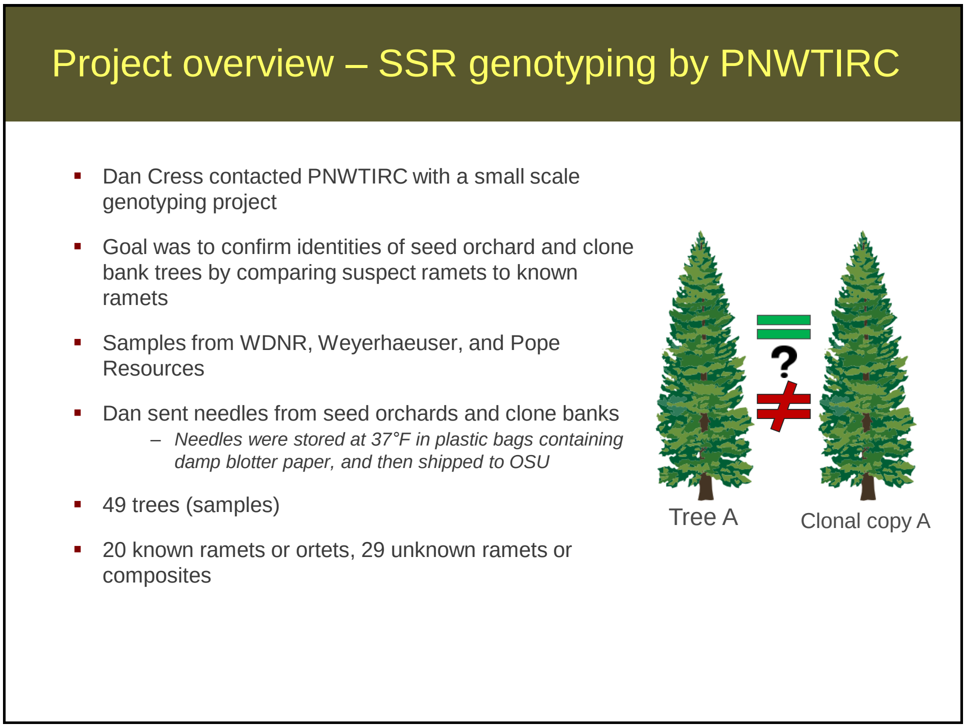


SSRs for Douglas-fir

- Gancho Slavov developed 22 SSRs working with the PNWTIRC (PhD thesis)
- NFGEL, led by Valerie Hipkins, was the co-op's 'go-to' for genotyping using SSRs
- Valerie's position changed, so NFGEL could not perform genotyping
- Gancho is now at Scion in New Zealand and we are collaborating with him on the SNP research

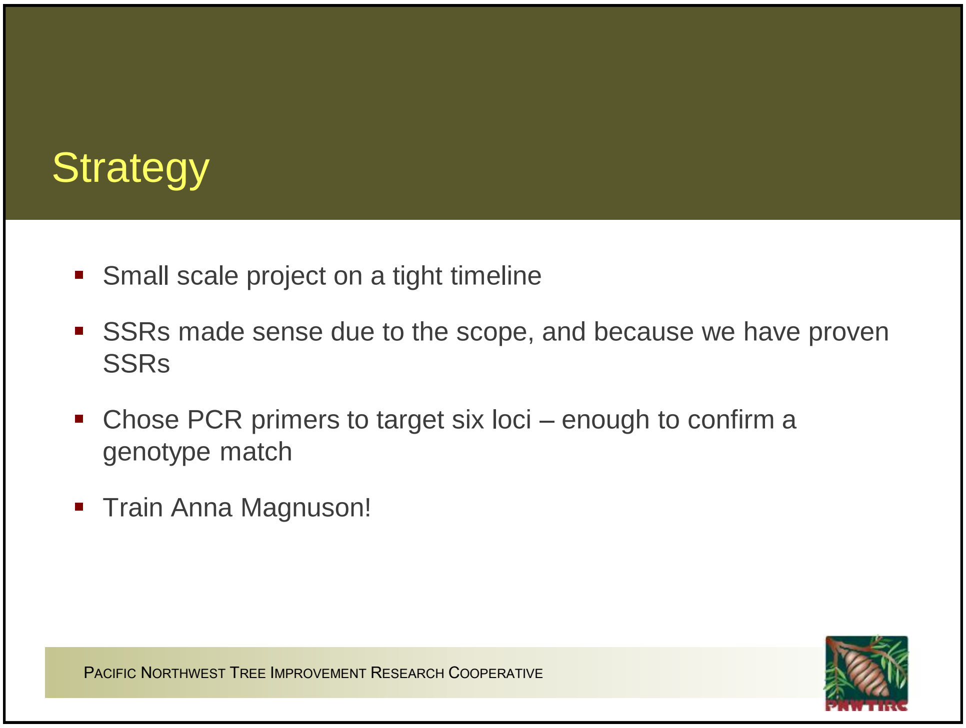
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Project overview



Project overview – SSR genotyping by PNWTIRC

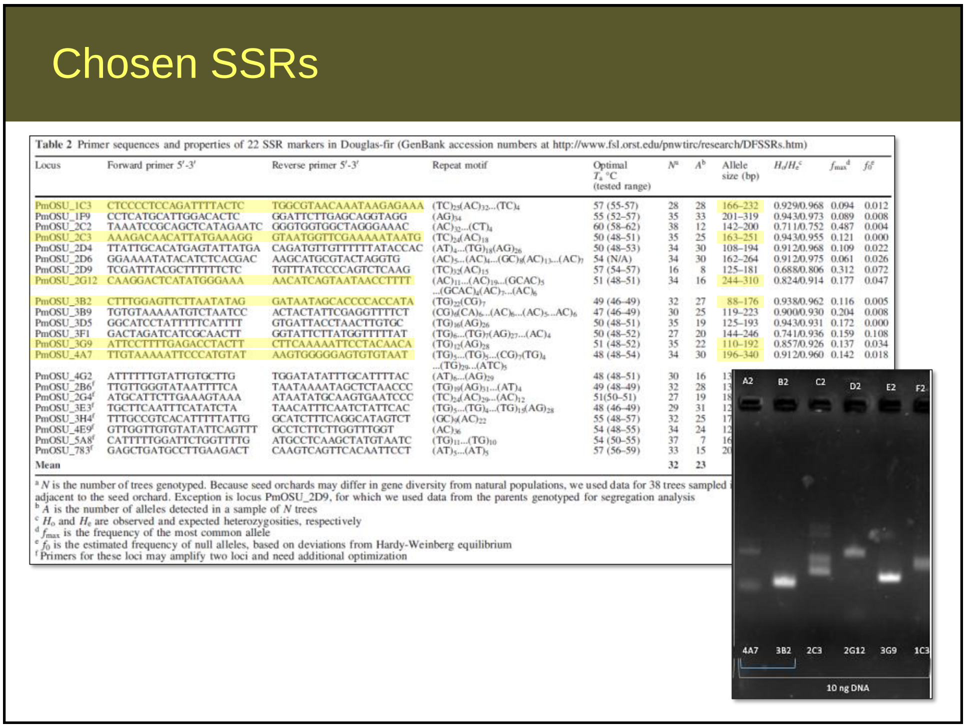
- Dan Cress contacted PNWTIRC with a small scale genotyping project
- Goal was to confirm identities of seed orchard and clone bank trees by comparing suspect ramets to known ramets
- Samples from WDNR, Weyerhaeuser, and Pope Resources
- Dan sent needles from seed orchards and clone banks
  - *Needles were stored at 37°F in plastic bags containing damp blotter paper, and then shipped to OSU*
- 49 trees (samples)
- 20 known ramets or ortets, 29 unknown ramets or composites



Strategy

- Small scale project on a tight timeline
- SSRs made sense due to the scope, and because we have proven SSRs
- Chose PCR primers to target six loci – enough to confirm a genotype match
- Train Anna Magnuson!

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Chosen SSRs

Table 2 Primer sequences and properties of 22 SSR markers in Douglas-fir (GenBank accession numbers at http://www.fsl.orst.edu/pnwtirc/research/DFSSRs.htm)

Locus	Forward primer 5′-3′	Reverse primer 5′-3′	Repeat motif	Optimal T_a °C (tested range)	N^a	A^b	Allele size (bp)	H_o/H_e^c	f_{max}^d	f_0^e
PmOSU_1C3	CTCCCCTCCAGATTTTACTC	TGGCGTAACAAATAAGAGAAA	$(TC)_{25}(AC)_{32}\ldots(TC)_4$	57 (55-57)	28	28	166–232	0.929/0.968	0.094	0.012
PmOSU_1F9	CCTCATGCATTGGACACTC	GGATTCTTGAGCAGGTAGG	$(AG)_{34}$	55 (52–57)	35	33	201–319	0.943/0.973	0.089	0.008
PmOSU_2C2	TAAATCCGCAGCTCATAGAATC	GGGTGGTGGCTAGGGAAAC	$(AC)_{32}\ldots(CT)_4$	60 (58–62)	38	12	142–200	0.711/0.752	0.487	0.004
PmOSU_2C3	AAAGACAACATTATGAAAGG	GTAATGGTTCGAAAAATAATG	$(TC)_{24}(AC)_{18}$	50 (48–51)	35	25	163–251	0.943/0.955	0.121	0.000
PmOSU_2D4	TTATTGCACATGAGTATTATGA	CAGATGTTGTTTTTTATACCAC	$(AT)_4\ldots(TG)_{18}(AG)_{26}$	50 (48–53)	34	30	108–194	0.912/0.968	0.109	0.022
PmOSU_2D6	GGAAAATATACATCTCACGAC	AAGCATGCGTACTAGGTG	$(AC)_5\ldots(AC)_4\ldots(GC)_8(AC)_{13}\ldots(AC)_7$	54 (N/A)	34	30	162–264	0.912/0.975	0.061	0.026
PmOSU_2D9	TCGATTTACGCTTTTTTCTC	TGTTTATCCCCAGTCTCAAG	$(TC)_{32}(AC)_{15}$	57 (54–57)	16	8	125–181	0.688/0.806	0.312	0.072
PmOSU_2G12	CAAGGACTCATATGGGAAA	AACATCAGTAATAACCTTTT	$(AC)_{11}\ldots(AC)_{19}\ldots(GCAC)_5$ $\ldots(GCAC)_4(AC)_7\ldots(AC)_6$	51 (48–51)	34	16	244–310	0.824/0.914	0.177	0.047
PmOSU_3B2	CTTTGGAGTTCTTAATATAG	GATAATAGCACCCCACCATA	$(TG)_{22}(CG)_7$	49 (46–49)	32	27	88–176	0.938/0.962	0.116	0.005
PmOSU_3B9	TGTGTAAAAATGTCTAATCC	ACTACTATTCGAGGTTTTCT	$(CG)_6(CA)_6\ldots(AC)_6\ldots(AC)_5\ldots AC)_6$	47 (46–49)	30	25	119–223	0.900/0.930	0.204	0.008
PmOSU_3D5	GGCATCCTATTTTTCATTTT	GTGATTACCTAACTTGTGC	$(TG)_{16}(AG)_{26}$	50 (48–51)	35	19	125–193	0.943/0.931	0.172	0.000
PmOSU_3F1	GACTAGATCATCGCAACTT	GGTATTCTTATGGTTTTTAT	$(TG)_6\ldots(TG)_7(AG)_{27}\ldots(AC)_4$	50 (48–52)	27	20	144–246	0.741/0.936	0.159	0.108
PmOSU_3G9	ATTCCTTTTGAGACCTACTT	CTTCAAAAATTCCTACAACA	$(TG)_{12}(AG)_{28}$	51 (48–52)	35	22	110–192	0.857/0.926	0.137	0.034
PmOSU_4A7	TTGTAAAAATTCCCATGTAT	AAGTGGGGGAGTGTGTAAT	$(TG)_5\ldots(TG)_5\ldots(CG)_7(TG)_4$ $\ldots(TG)_{29}\ldots(ATC)_5$	48 (48–54)	34	30	196–340	0.912/0.960	0.142	0.018
PmOSU_4G2	ATTTTTTGTATTGTGCTTG	TGGATATATTTGCATTTTAC	$(AT)_6\ldots(AG)_{29}$	48 (48–51)	30	16	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_2B6[f]	TTGTTGGGTATAATTTTCA	TAATAAAATAGCTCTAACCC	$(TG)_{19}(AG)_{31}\ldots(AT)_4$	49 (48–49)	32	28	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_2G4[f]	ATGCATTCTTGAAAGTAAA	ATAATATGCAAGTGAATCCC	$(TC)_{24}(AC)_{29}\ldots(AC)_{12}$	51(50–51)	27	19	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_3E3[f]	TGCTTCAATTTCATATCTA	TAACATTTCAATCTATTCAC	$(TG)_5\ldots(TG)_4\ldots(TG)_{15}(AG)_{28}$	48 (46–49)	29	31	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_3H4[f]	TTTGCCGTCACATTTTTATTG	GCATCTTTCAGGCATAGTCT	$(GC)_9(AC)_{22}$	55 (48–57)	32	25	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_4E9[f]	GTTGGTTGTGTATATTCAGTTT	GCCTCTTCTTGGTTTGGT	$(AC)_{36}$	54 (48–55)	34	24	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_5A8[f]	CATTTTTGGATTCTGGTTTTG	ATGCCTCAAGCTATGTAATC	$(TG)_{11}\ldots(TG)_{10}$	54 (50–55)	37	7	[illegible]	[illegible]	[illegible]	[illegible]
PmOSU_783[f]	GAGCTGATGCCTTGAAGACT	CAAGTCAGTTCACAATTCCT	$(AT)_5\ldots(AT)_5$	57 (56–59)	33	15	[illegible]	[illegible]	[illegible]	[illegible]
Mean					32	23				

[a] N is the number of trees genotyped. Because seed orchards may differ in gene diversity from natural populations, we used data for 38 trees sampled
adjacent to the seed orchard. Exception is locus PmOSU_2D9, for which we used data from the parents genotyped for segregation analysis

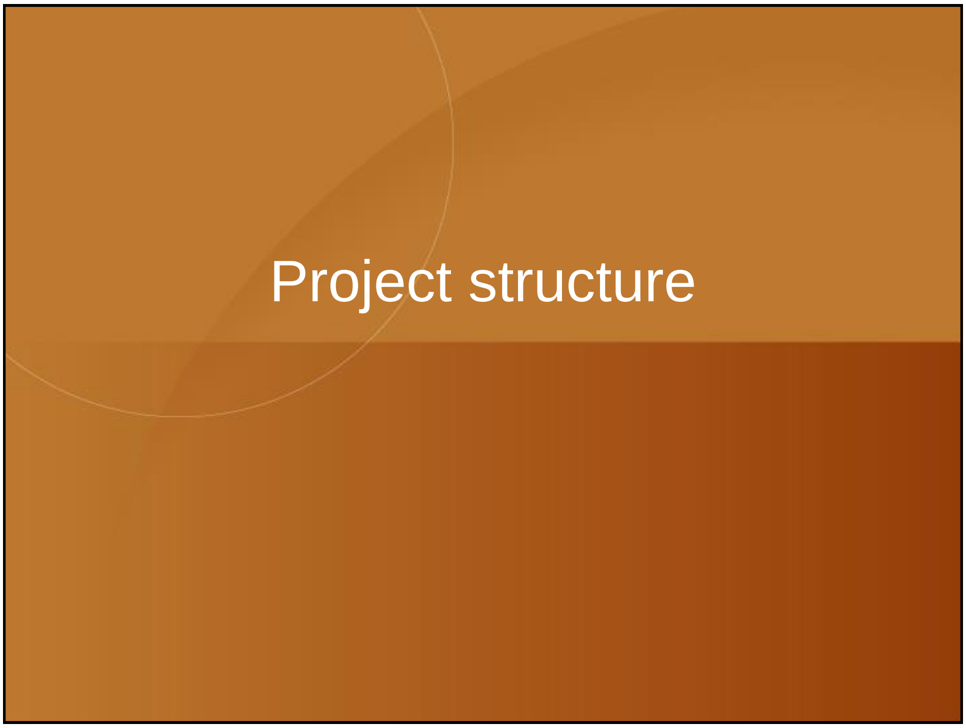
[b] A is the number of alleles detected in a sample of N trees

[c] H_o and H_e are observed and expected heterozygosities, respectively

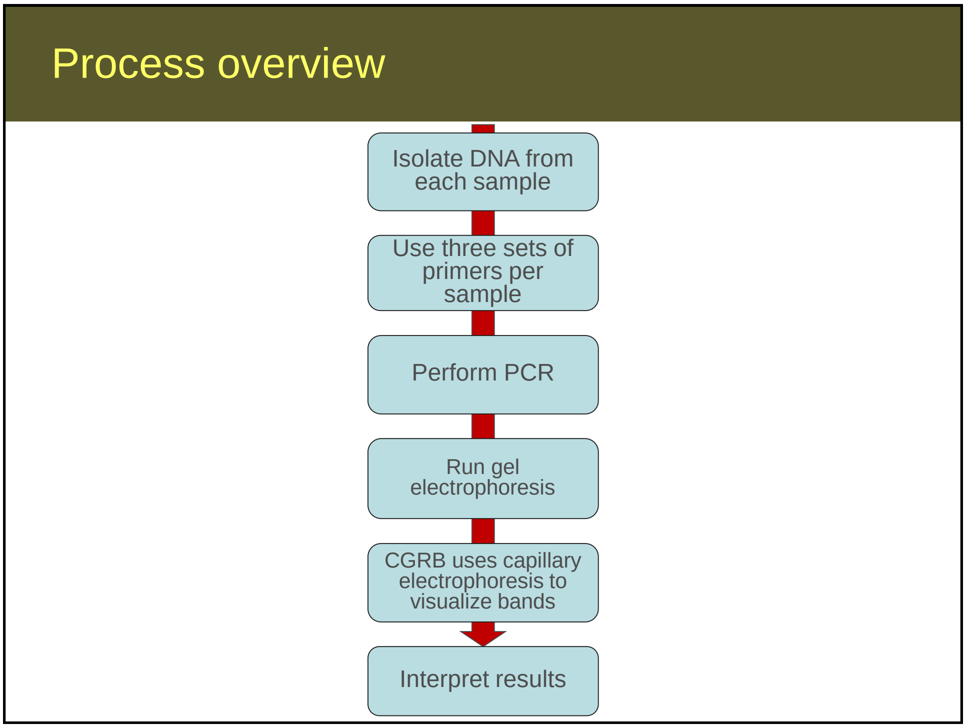
[d] f_{max} is the frequency of the most common allele

[e] f_0 is the estimated frequency of null alleles, based on deviations from Hardy-Weinberg equilibrium

[f] Primers for these loci may amplify two loci and need additional optimization



Project structure



Process overview

Isolate DNA from each sample

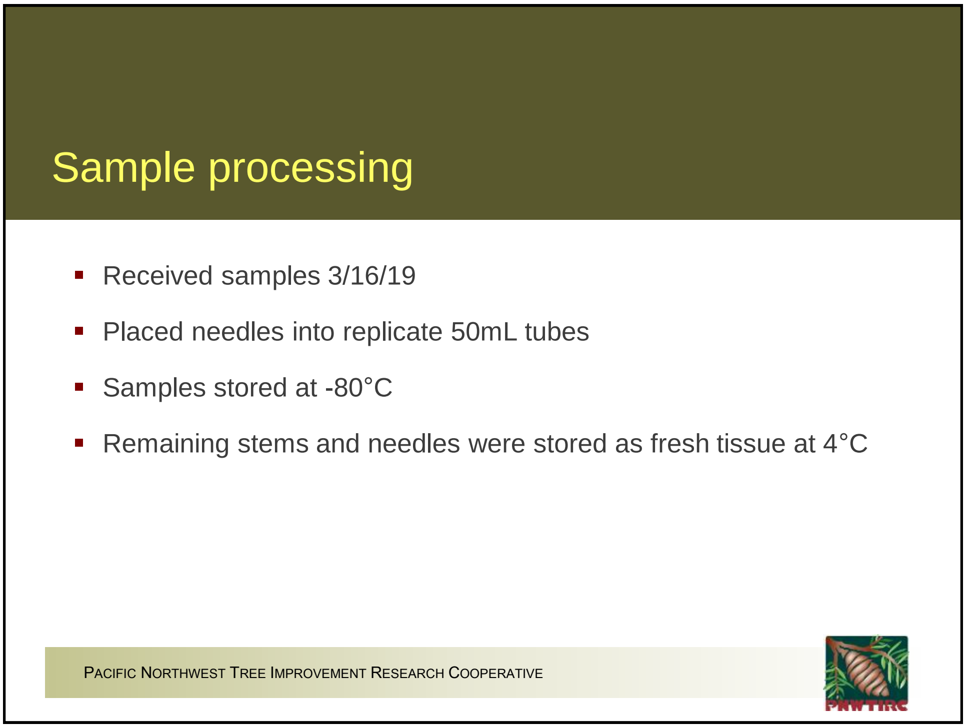
Use three sets of primers per sample

Perform PCR

Run gel electrophoresis

CGRB uses capillary electrophoresis to visualize bands

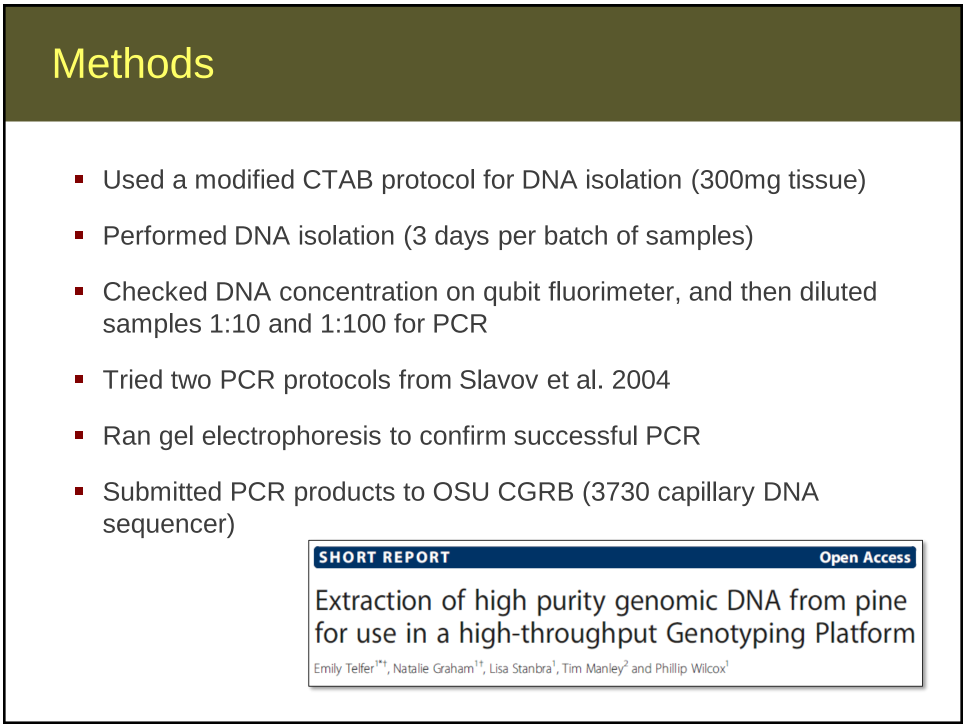
Interpret results



Sample processing

- Received samples 3/16/19
- Placed needles into replicate 50mL tubes
- Samples stored at -80°C
- Remaining stems and needles were stored as fresh tissue at 4°C

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Methods

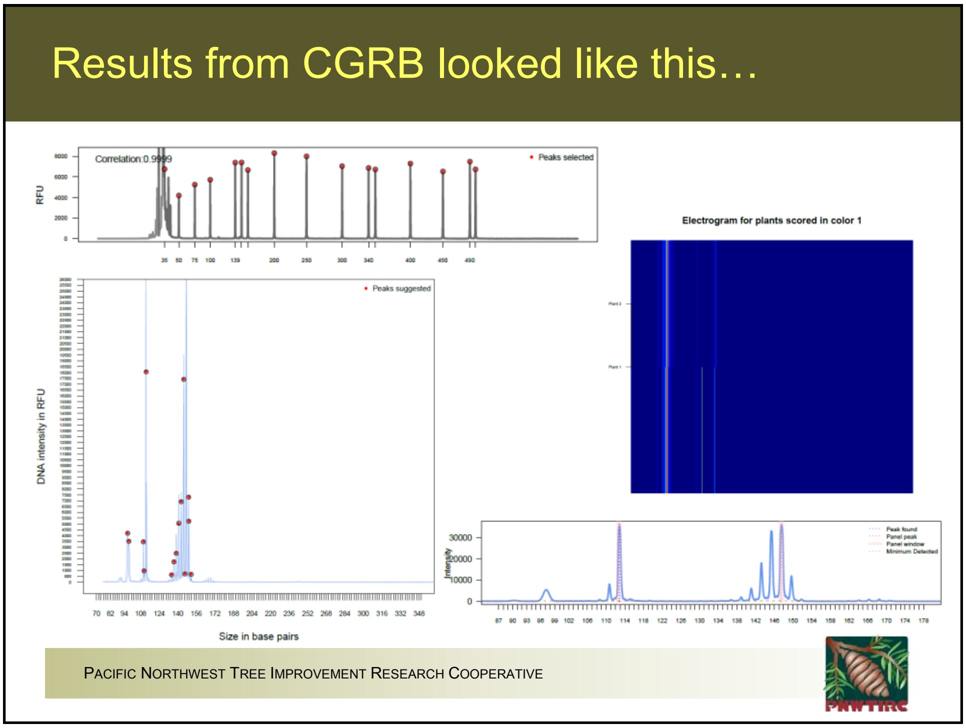
- Used a modified CTAB protocol for DNA isolation (300mg tissue)
- Performed DNA isolation (3 days per batch of samples)
- Checked DNA concentration on qubit fluorimeter, and then diluted samples 1:10 and 1:100 for PCR
- Tried two PCR protocols from Slavov et al. 2004
- Ran gel electrophoresis to confirm successful PCR
- Submitted PCR products to OSU CGRB (3730 capillary DNA sequencer)

SHORT REPORT **Open Access**

Extraction of high purity genomic DNA from pine for use in a high-throughput Genotyping Platform

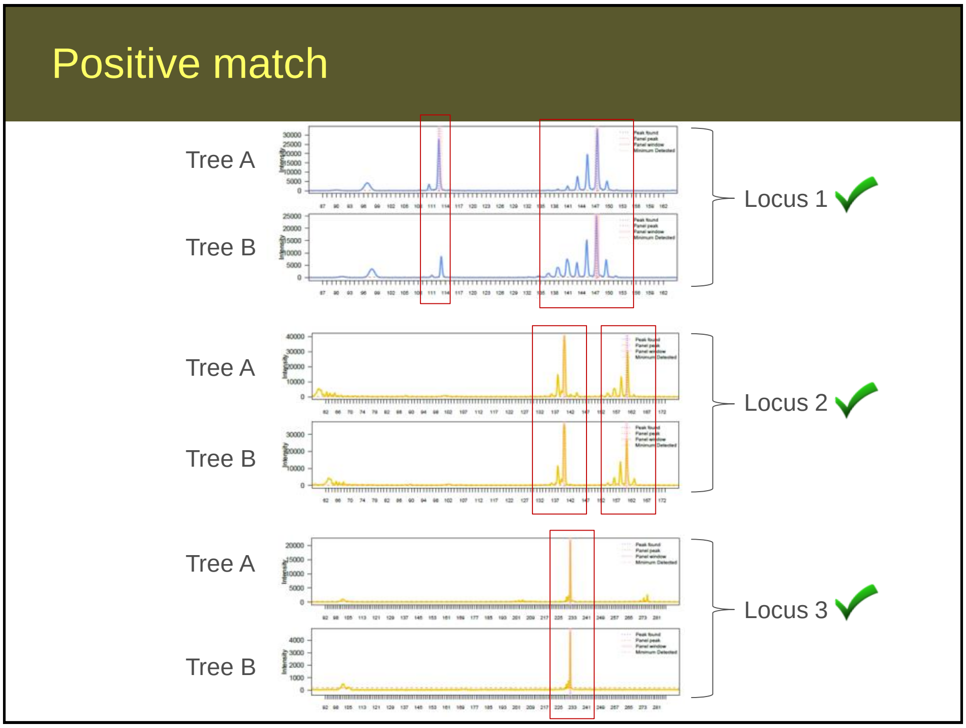
Emily Telfer[1*†], Natalie Graham[1†], Lisa Stanbra[1], Tim Manley[2] and Phillip Wilcox[1]

Results



Results from CGRB looked like this…

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Positive match

Tree A

Tree B

Locus 1 ✓

Tree A

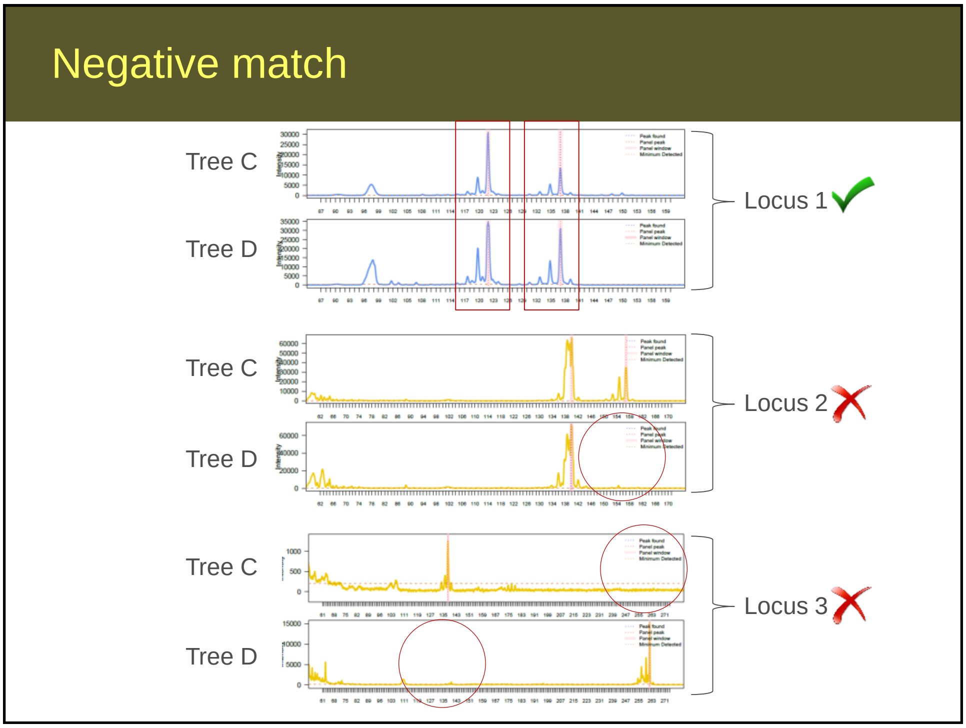
Tree B

Locus 2 ✓

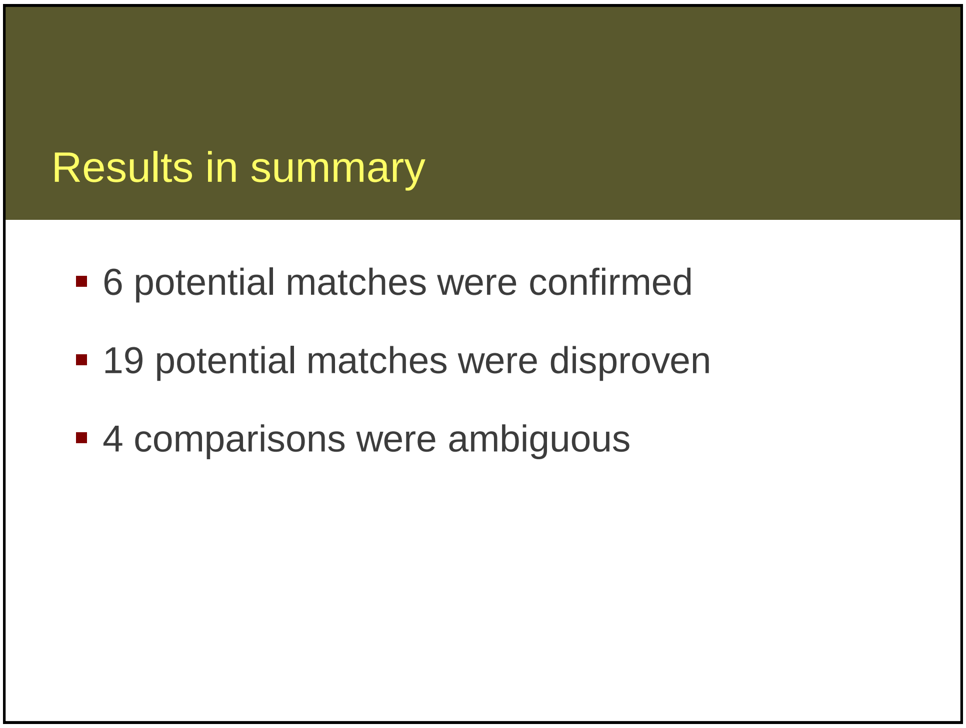
Tree A

Tree B

Locus 3 ✓

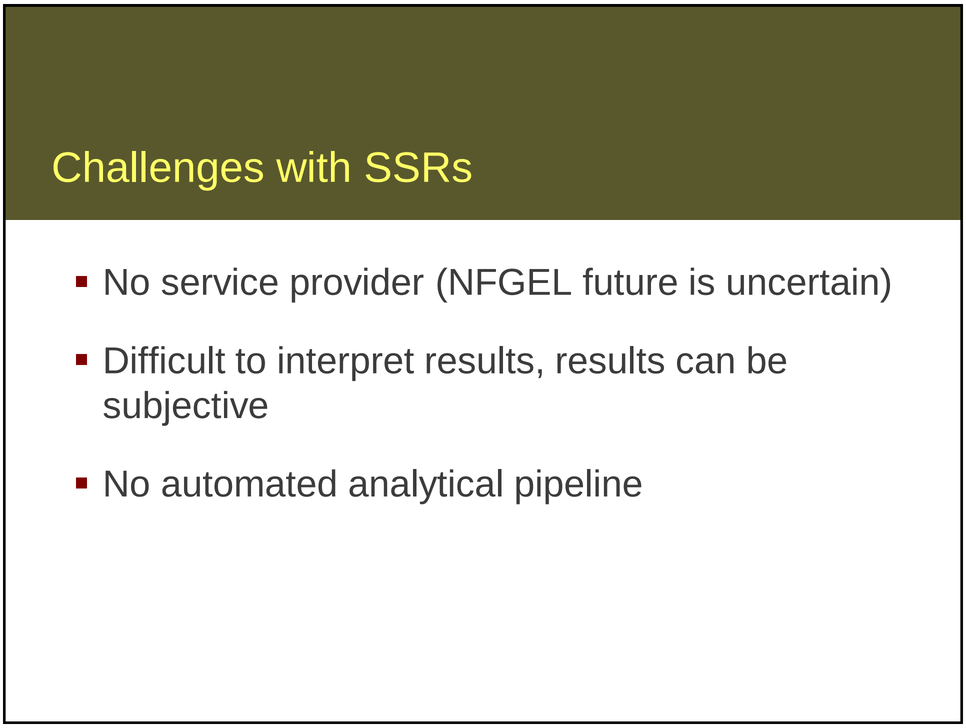


Negative match



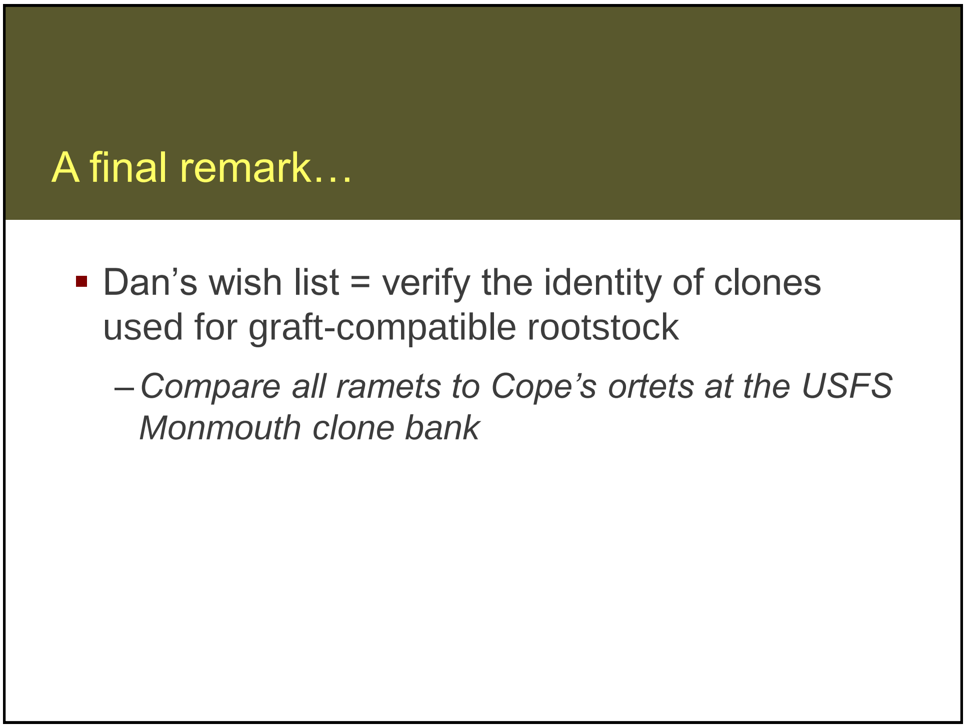
Results in summary

- 6 potential matches were confirmed
- 19 potential matches were disproven
- 4 comparisons were ambiguous



Challenges with SSRs

- No service provider (NFGEL future is uncertain)
- Difficult to interpret results, results can be subjective
- No automated analytical pipeline



A final remark…

- Dan’s wish list = verify the identity of clones used for graft-compatible rootstock
  - *Compare all ramets to Cope’s ortets at the USFS Monmouth clone bank*

From SSRs to SNPs

Advantages of ‘low-density’ SNP genotyping

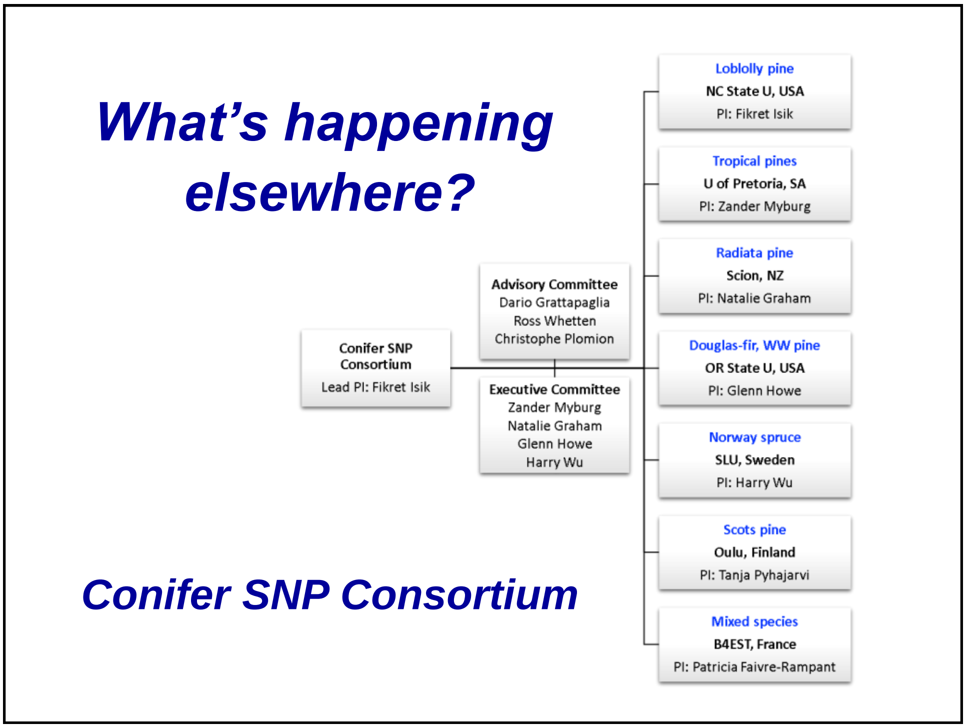
Advantages of SNPs

- Many more genetic markers are available
- Many options for out-sourcing (e.g., not just NFGEL)
- Software pipelines can be used for analysis (no ‘hands-on’ interpretation needed)

Advantages of ‘low-density’ SNP genotyping

- Axiom array is high-density genotyping = 28K SNPs
- Low-density genotyping = fewer SNPs
- We’re looking closely at AgriSeq genotyping of 5K SNPs

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



What's happening elsewhere?

Conifer SNP Consortium
Lead PI: Fikret Isik

Advisory Committee
Dario Grattapaglia
Ross Whetten
Christophe Plomion

Executive Committee
Zander Myburg
Natalie Graham
Glenn Howe
Harry Wu

Loblolly pine
NC State U, USA
PI: Fikret Isik

Tropical pines
U of Pretoria, SA
PI: Zander Myburg

Radiata pine
Scion, NZ
PI: Natalie Graham

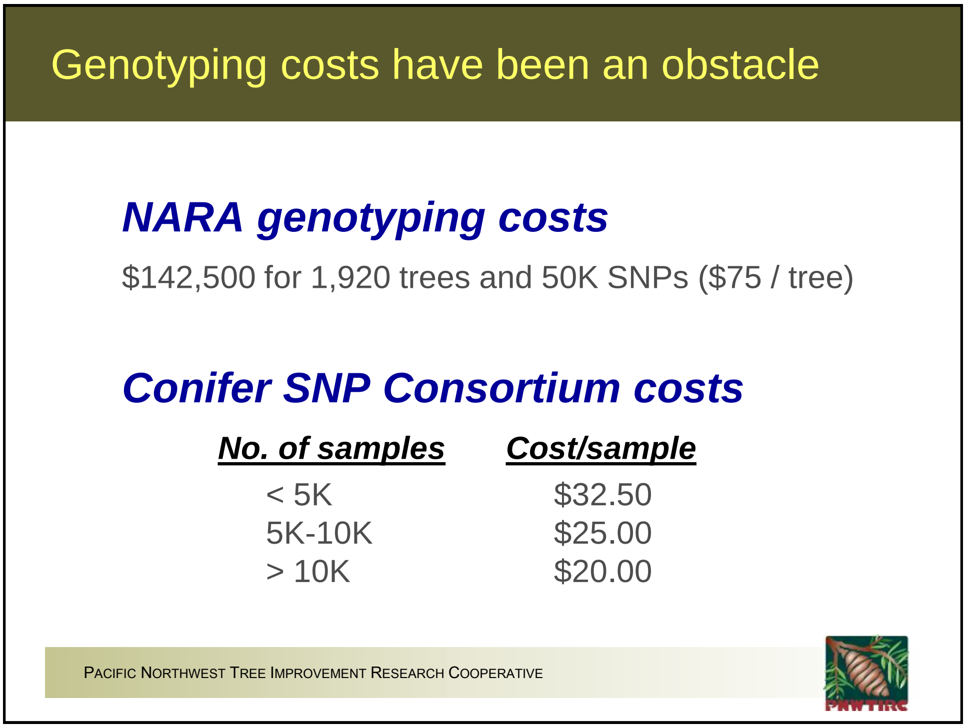
Douglas-fir, WW pine
OR State U, USA
PI: Glenn Howe

Norway spruce
SLU, Sweden
PI: Harry Wu

Scots pine
Oulu, Finland
PI: Tanja Pyhajarvi

Mixed species
B4EST, France
PI: Patricia Faivre-Rampant

Conifer SNP Consortium



Genotyping costs have been an obstacle

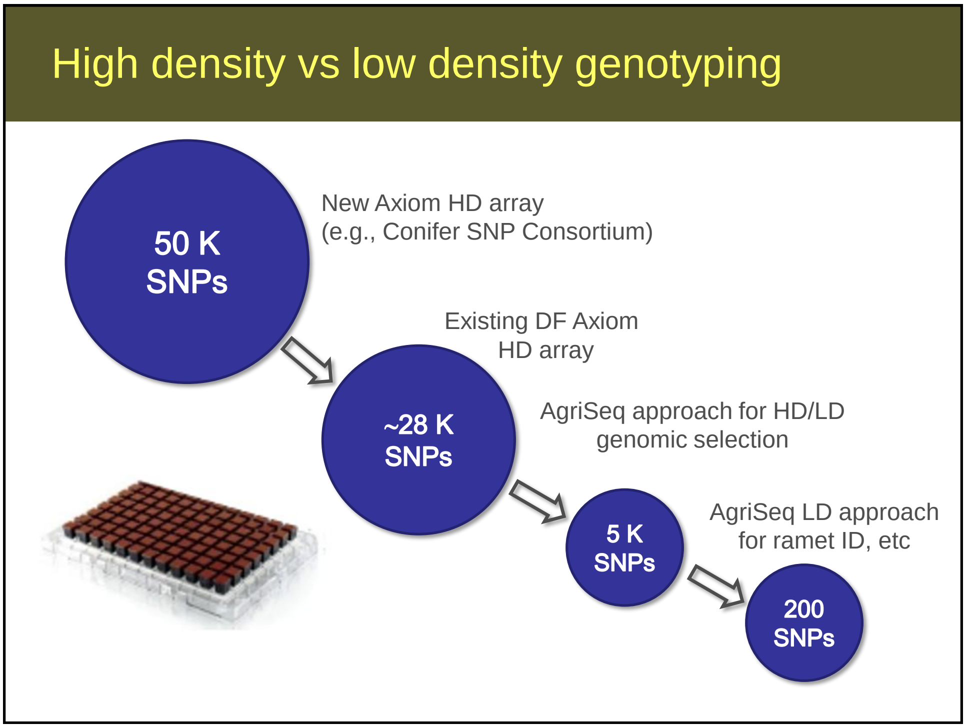
NARA genotyping costs

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

Conifer SNP Consortium costs

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



High density vs low density genotyping

50 K SNPs

New Axiom HD array
(e.g., Conifer SNP Consortium)

Existing DF Axiom
HD array

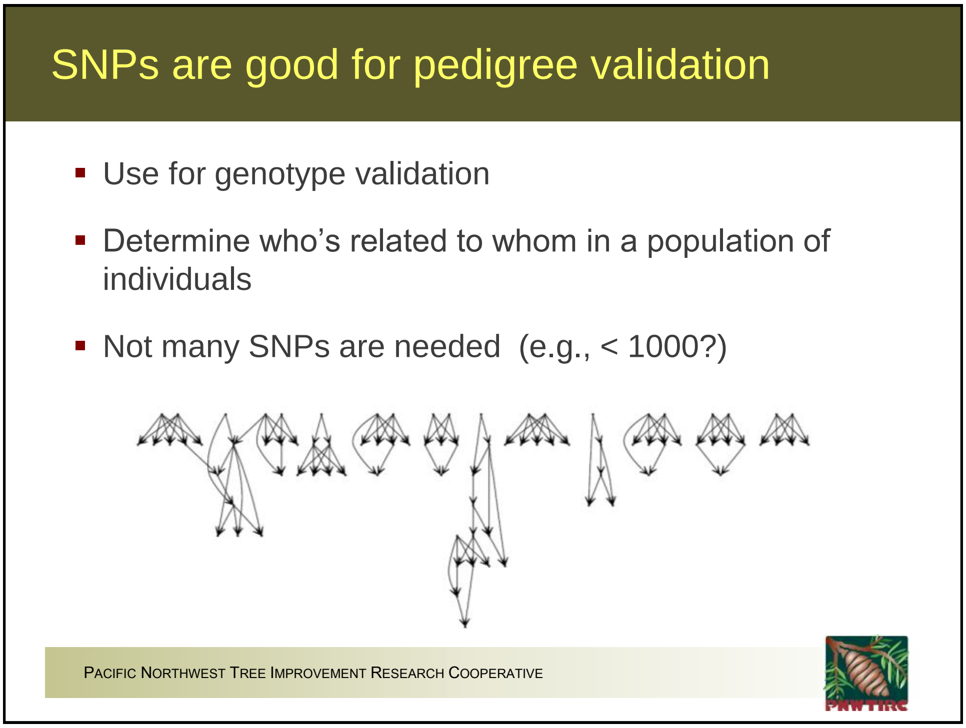
~28 K SNPs

AgriSeq approach for HD/LD
genomic selection

5 K SNPs

AgriSeq LD approach
for ramet ID, etc

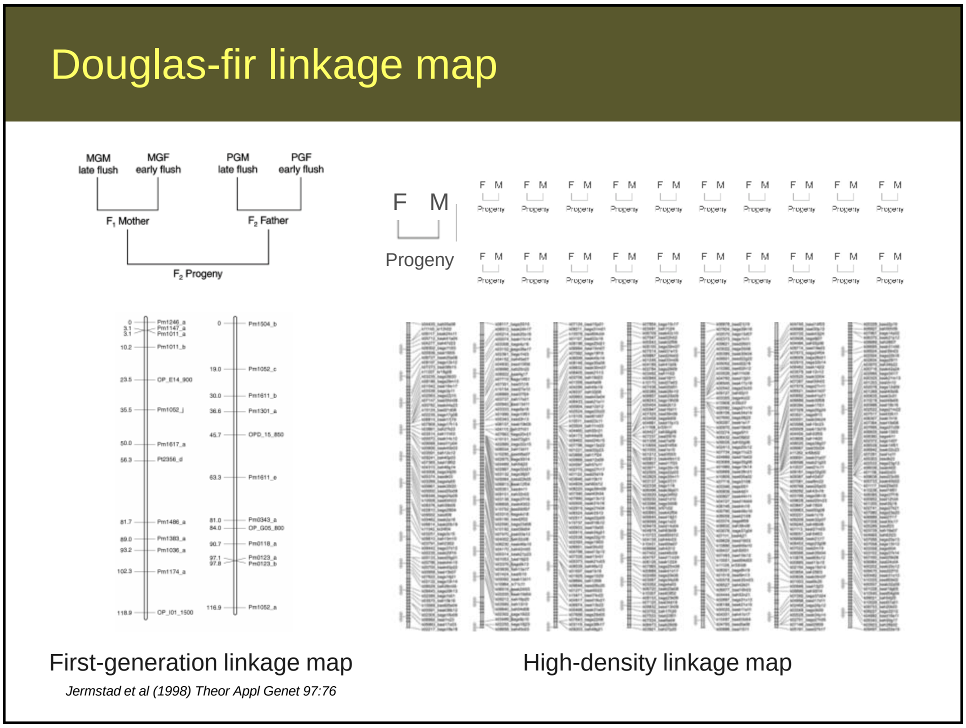
200 SNPs



SNPs are good for pedigree validation

- Use for genotype validation
- Determine who’s related to whom in a population of individuals
- Not many SNPs are needed (e.g., < 1000?)

Pacific Northwest Tree Improvement Research Cooperative

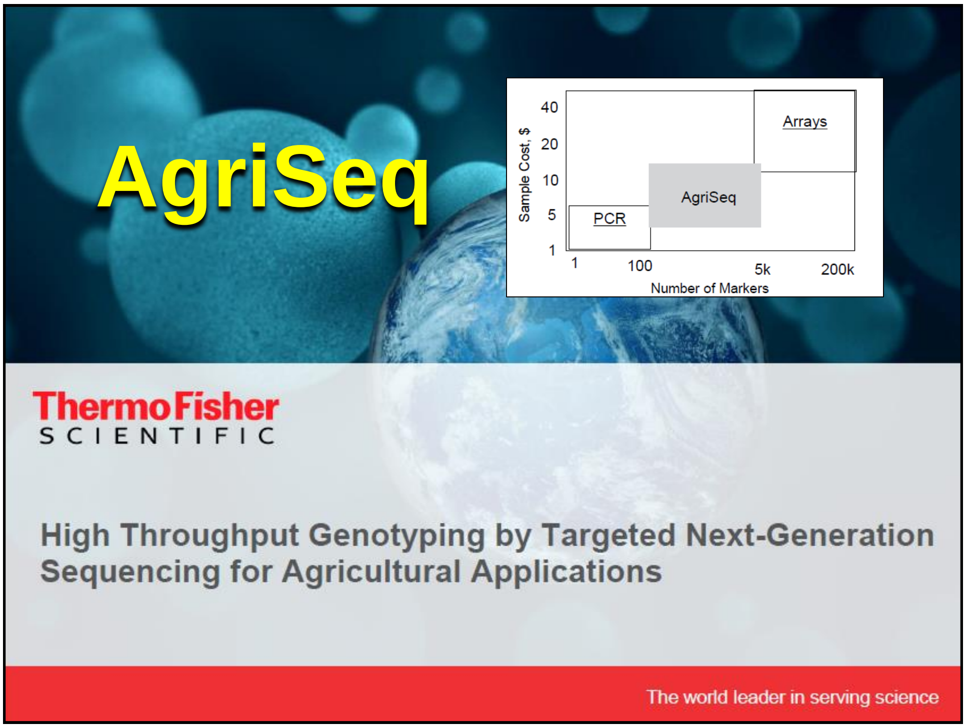


Douglas-fir linkage map

First-generation linkage map

Jermstad et al (1998) Theor Appl Genet 97:76

High-density linkage map



AgriSeq

ThermoFisher SCIENTIFIC

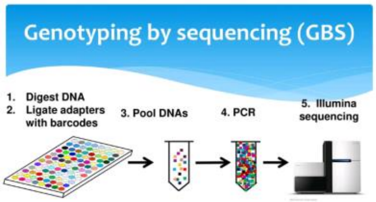
High Throughput Genotyping by Targeted Next-Generation Sequencing for Agricultural Applications

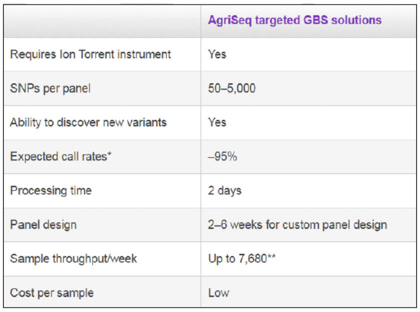
The world leader in serving science

AgriSeq characteristics

Axiom is array-based genotyping

AgriSeq involves genotyping-by-sequencing





	AgriSeq targeted GBS solutions
Requires Ion Torrent instrument	Yes
SNPs per panel	50–5,000
Ability to discover new variants	Yes
Expected call rates*	~95%
Processing time	2 days
Panel design	2–6 weeks for custom panel design
Sample throughput/week	Up to 7,680**
Cost per sample	Low

https://www.thermofisher.com/us/en/home/life-science/agricultural-biotechnology/agrigenomics/targeted-genotyping-sequencing.html?kui=e7RBWHiFjaw_xpOIKuCOLQ#_ts=1558125264812

Proposed collaboration with CIPS

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

College of Forestry

Center for Intensive Planted-forest Silviculture

News

Northwest forests are becoming denser and more vulnerable to fire

Deschutes Collaborative Forest Project shows targeted thinning, burns can save homes

Extension program makes a difference for women forest managers

Mission

To understand and quantify the interactive effects of silvicultural activities and site conditions on maintaining and improving the productivity, health, and sustainability of intensively managed, planted forests in the Pacific Northwest.

Member Login

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

New Zealand Douglas-fir

Regime 1250-1650 stems/ha

Thin to waste @15m to 800 stems/ha

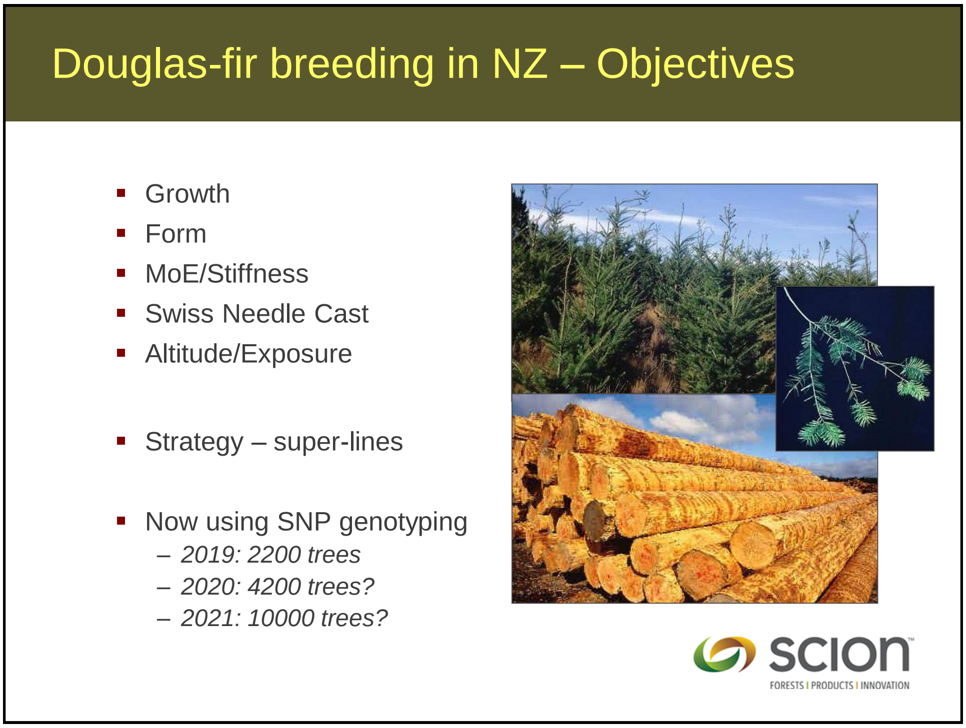
Production thin at later ages where appropriate

Rotation 40-60 years

MAI up to 40 m^3/ha/year

NZ average 18.4 m^3/ha/year

Best CAI over 50 m^3/ha/year



Douglas-fir breeding in NZ – Objectives

- Growth
- Form
- MoE/Stiffness
- Swiss Needle Cast
- Altitude/Exposure

- Strategy – super-lines

- Now using SNP genotyping
  - *2019: 2200 trees*
  - *2020: 4200 trees?*
  - *2021: 10000 trees?*

scion
FORESTS | PRODUCTS | INNOVATION

Objectives

Low-density SNP Genotyping in Douglas-fir

A Joint project between the
Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and the
Center for Intensive Plantation Silviculture (CIPS)

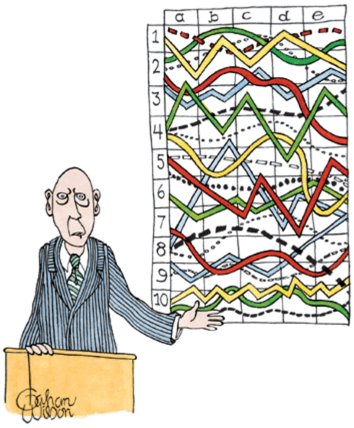
Glenn Howe, Jennifer Kling, Doug Maguire

Our specific objectives are to…

- Develop a new, low-cost genotyping approach that can be used by Douglas-fir breeders (PNWTIRC)
- Develop a framework genetic map to augment our existing mapping data (PNWTIRC)
- Use SNP genotyping to help compare growth of Douglas-fir in Oregon and New Zealand (CIPS)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Thank you!



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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

The Inland Empire Tree Improvement Cooperative

Marc L. Rust

The Inland Empire Tree Improvement Cooperative (IETIC) includes 21 member organizations representing private industry, federal and state agencies, tribal councils, universities, and other agencies in eastern Washington, northern Idaho, and western Montana. The IETIC applies classical breeding methods to develop genetically improved tree seed of important native conifer species for commercial reforestation and ecosystem restoration.

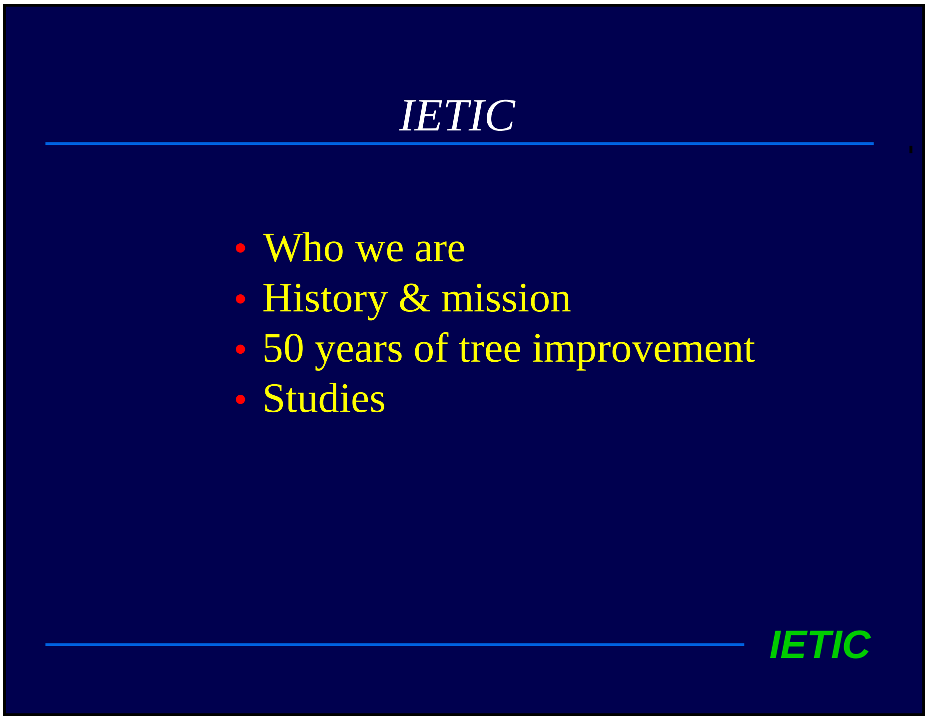
Since its establishment in 1974, IETIC has focused on ponderosa pine, western larch, western white pine, Douglas-fir, and lodgepole pine. Earlier tree improvement initiatives in the region were developed for individual tree species. The introduction of white pine blister rust in 1910 provided the impetus for establishing the USFS white pine genetic improvement program in 1950. The Ponderosa Pine Tree Improvement Committee (PPTIC) began as a grass-roots initiative in 1968, and later expanded to become the IETIC.

First-generation progeny tests were established from collections of open-pollinated seed from about 200 plus tree selections in each zone. Zones were based on J. Rehfeldt's genecology studies. Typically, progeny trials were planted at three test sites in each zone. For western white pine, the primary selection criterion was resistance to white pine blister rust. For all other species, selections were made for rapid growth based on volume measurements. The best families were used to establish seed and breeding orchards.

Breeding for white pine blister rust by the USDA Forest Service in Coeur d'Alene, Idaho has been a major focus of the IETIC. Evaluation of inoculated seedlings of second-generation crosses from the program are currently underway. For Douglas-fir and western larch, full-sib crosses have been made to develop second-generation progeny for evaluation. The scheme is similar to that employed by the NWTIC, where each selection is mated three times. Although all IETIC members have access to improved genetic materials for grafting, seed orchards were initially developed only by larger members. Since 2007, a cost sharing strategy has been used to establish additional orchards and ensure that all members have access to improved seed.

IETIC is technically an operational tree improvement cooperative, and does not have a designated budget for research. Studies have focused primariy on seed production and seed protection issues. In recent years, CAFS funding has created additional research opportunities. Topics include efficacy of systemic insecticides to control cone/seed insects, genomic studies in western white pine, a genetic study in western larch, and use of endophytes to enhance blister rust resistance in improved western white pine.

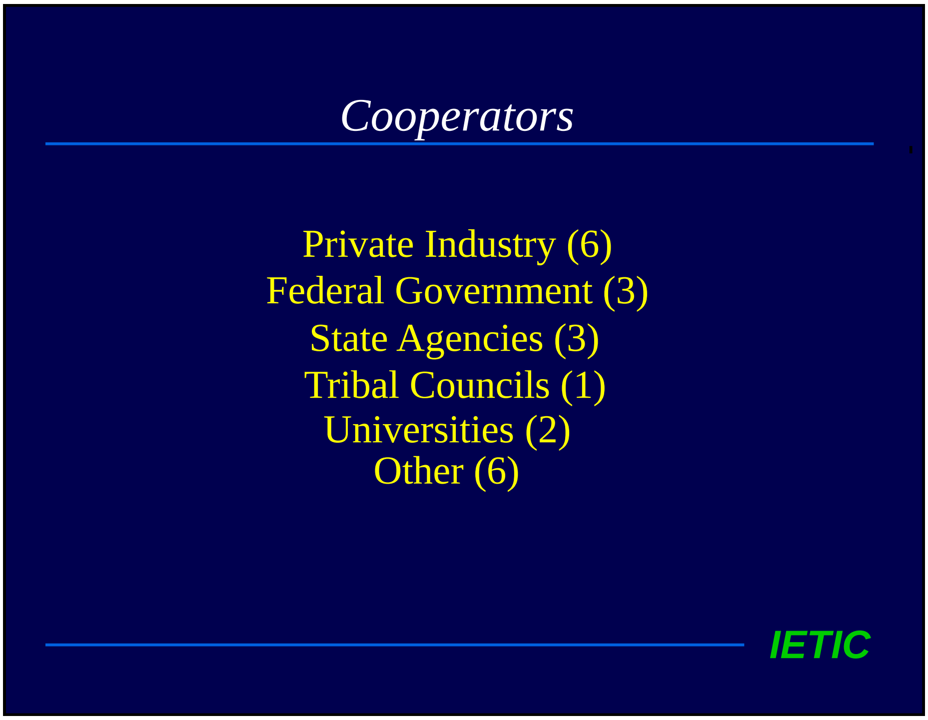
The Inland Empire
Tree Improvement Cooperative



IETIC

- Who we are
- History & mission
- 50 years of tree improvement
- Studies

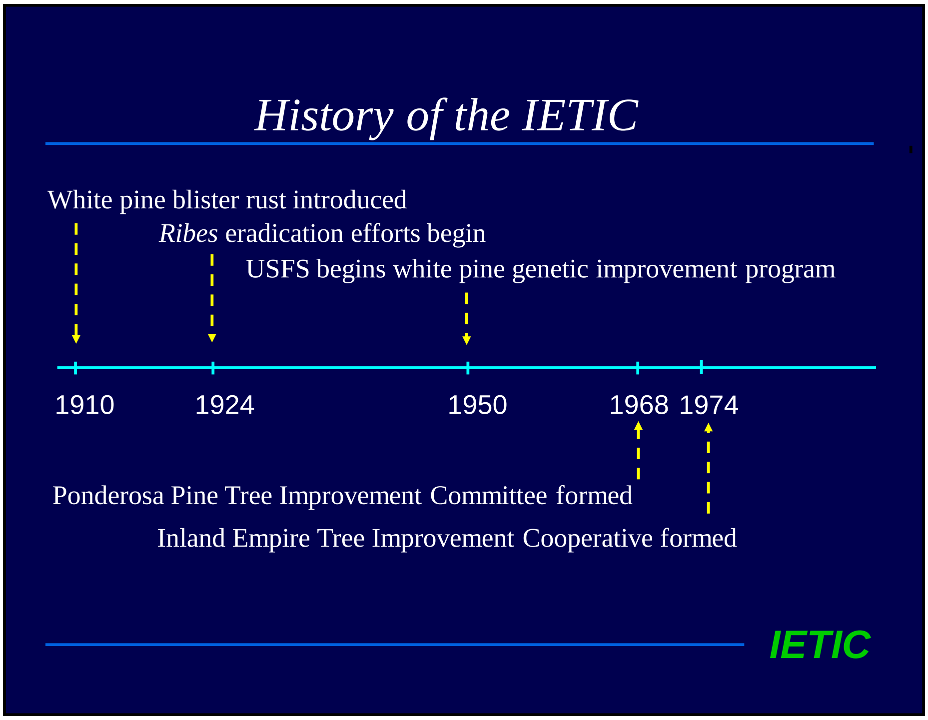
IETIC



Cooperators

Private Industry (6)
Federal Government (3)
State Agencies (3)
Tribal Councils (1)
Universities (2)
Other (6)

IETIC



History of the IETIC

White pine blister rust introduced
Ribes eradication efforts begin
USFS begins white pine genetic improvement program

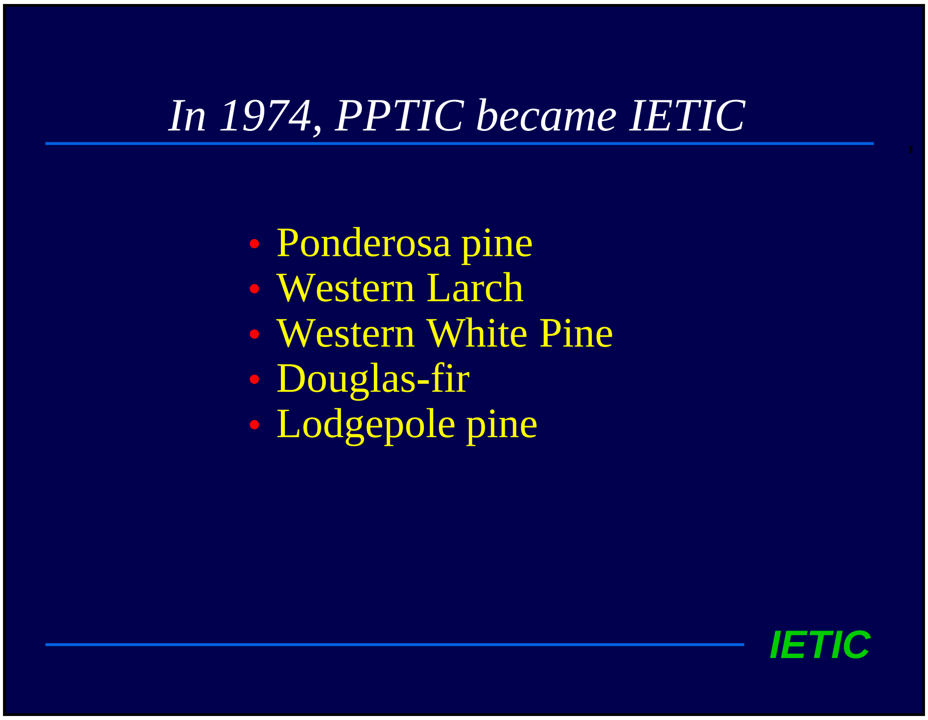
Ponderosa Pine Tree Improvement Committee formed
Inland Empire Tree Improvement Cooperative formed

IETIC

History of the IETIC

- Established 1968 as the Ponderosa Pine Tree Improvement Committee
- Apply classical techniques to improve native species
- Ensure healthy, productive forests for the future

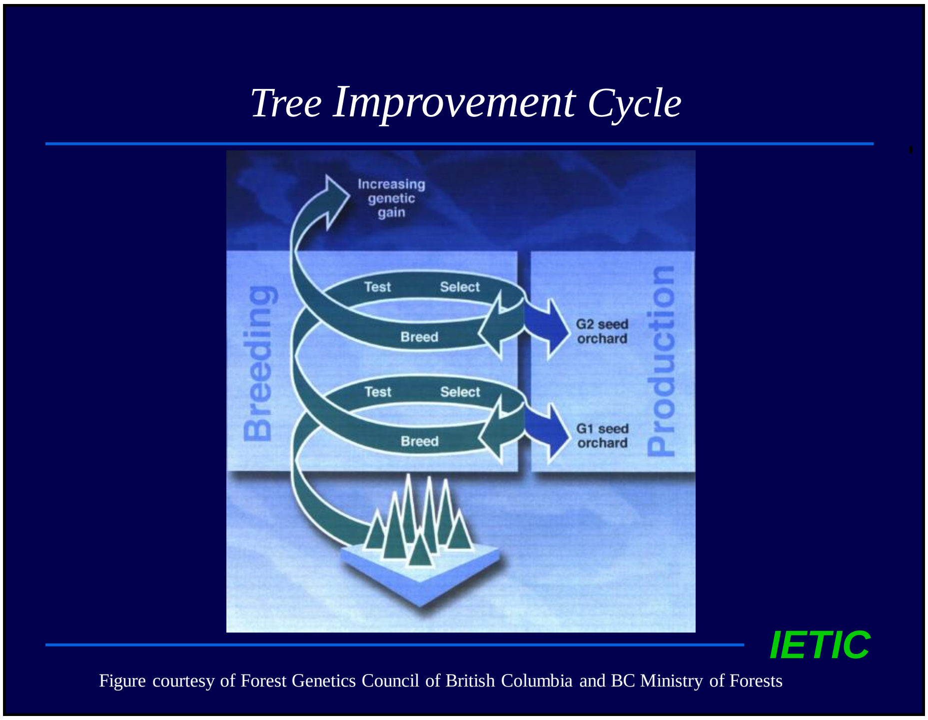
IETIC



In 1974, PPTIC became IETIC

- Ponderosa pine
- Western Larch
- Western White Pine
- Douglas-fir
- Lodgepole pine

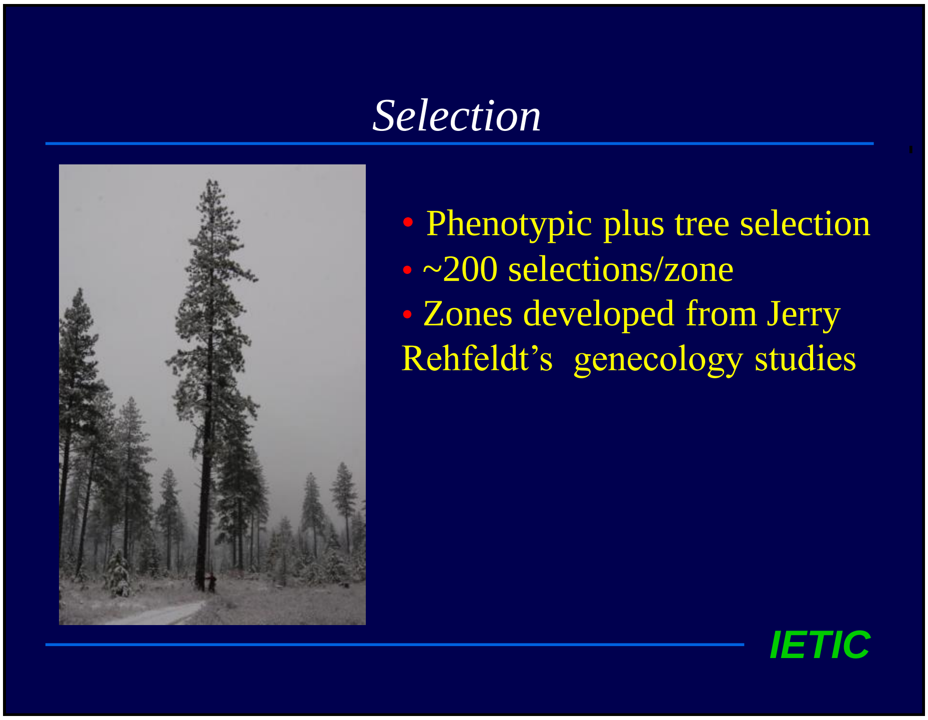
IETIC



Tree Improvement Cycle

IETIC

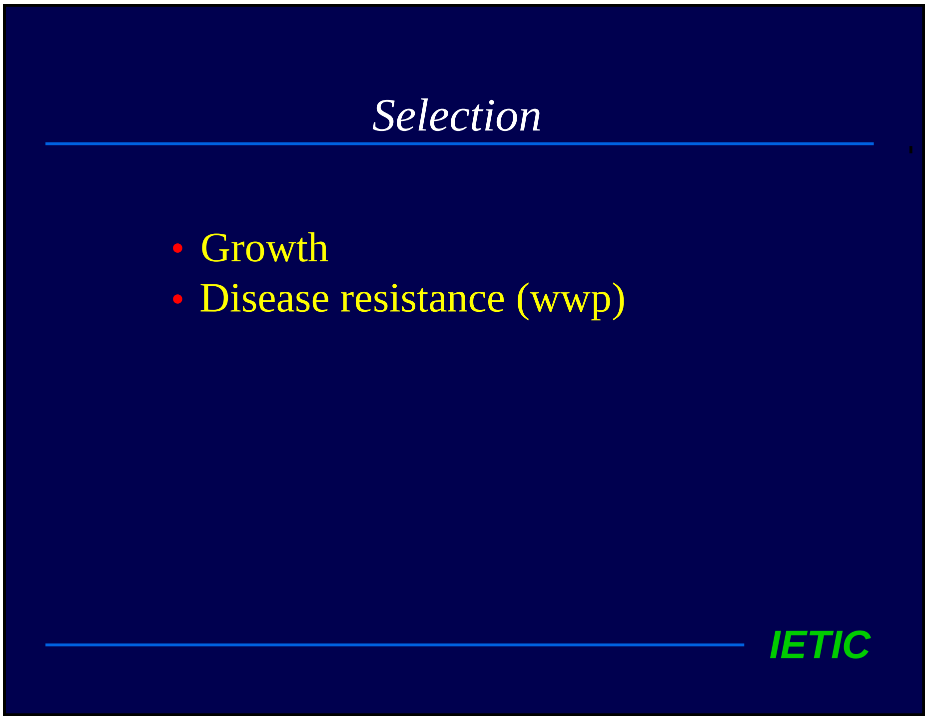
Figure courtesy of Forest Genetics Council of British Columbia and BC Ministry of Forests



Selection

- Phenotypic plus tree selection
- ~200 selections/zone
- Zones developed from Jerry Rehfeldt's genecology studies

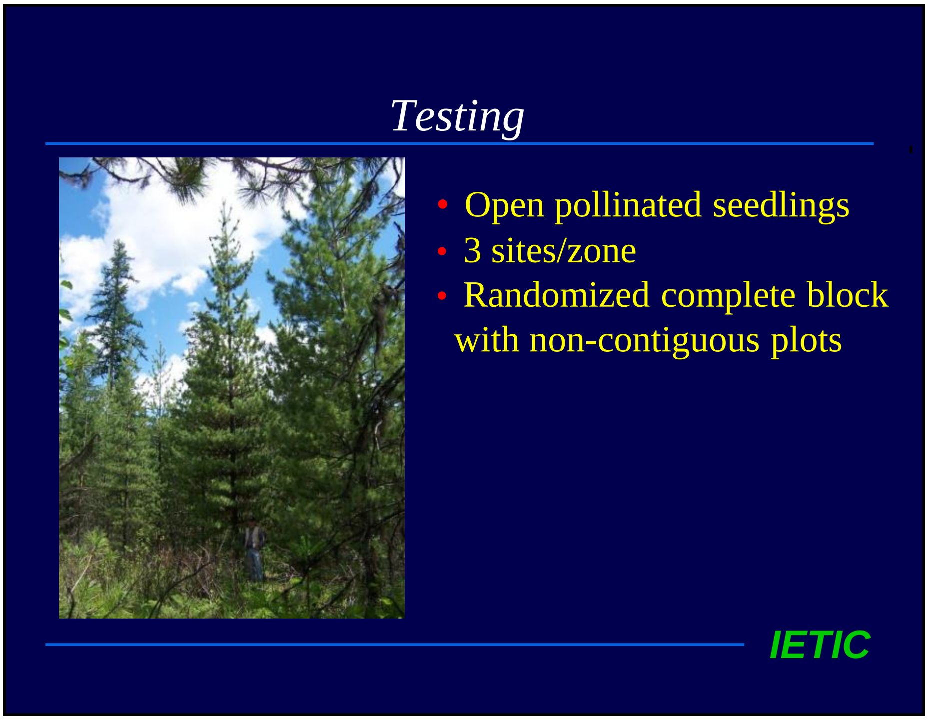
IETIC



Selection

- Growth
- Disease resistance (wwp)

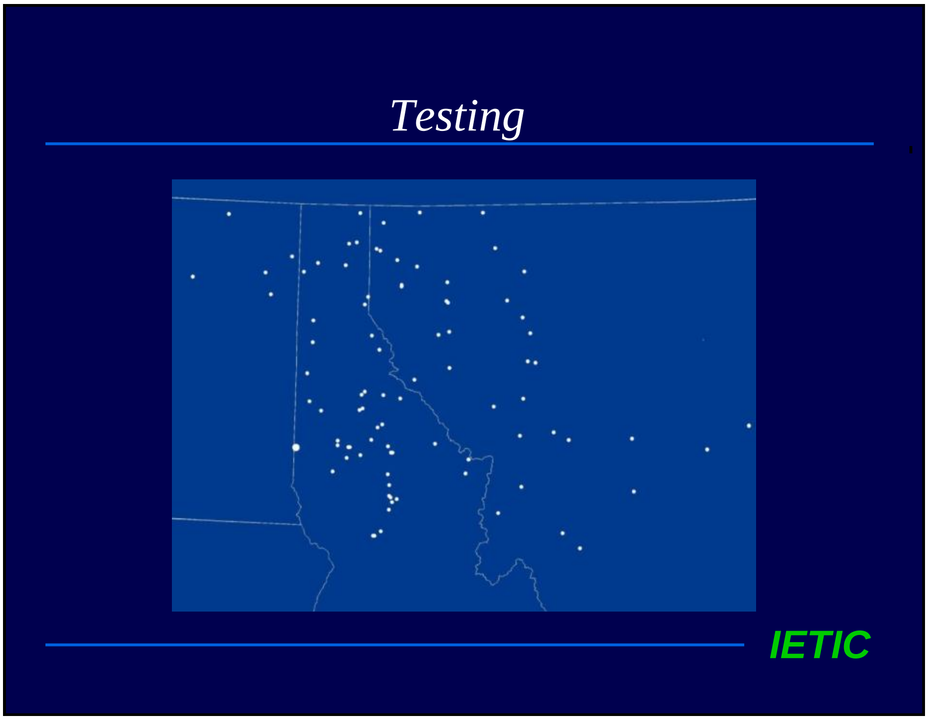
IETIC



Testing

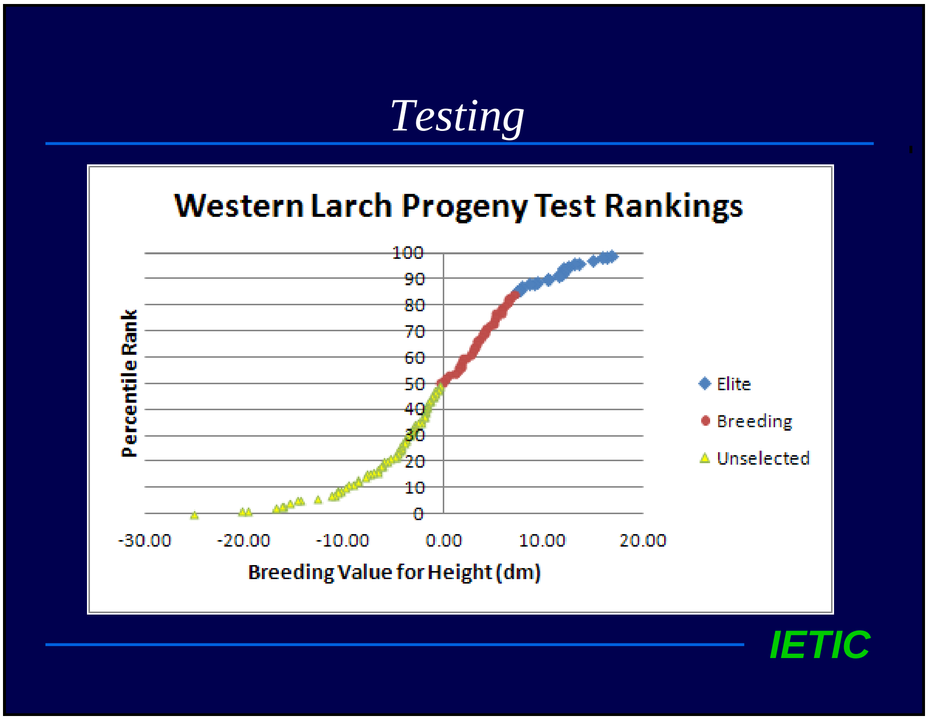
- Open pollinated seedlings
- 3 sites/zone
- Randomized complete block with non-contiguous plots

IETIC



Testing

IETIC



Testing

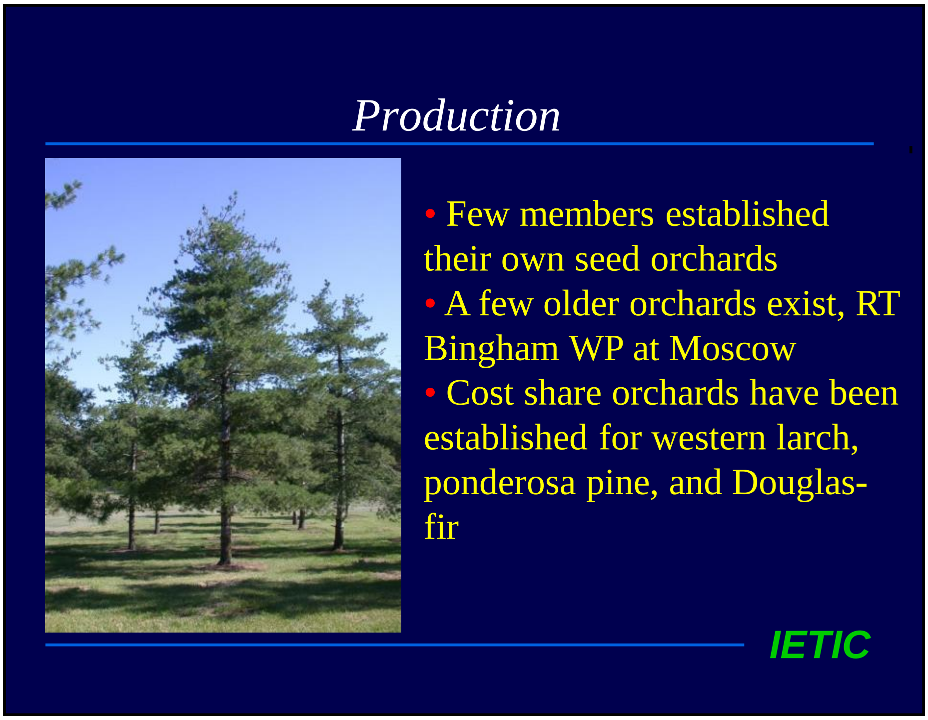
IETIC

Western White Pine Breeding

IETIC

Douglas-fir Breeding

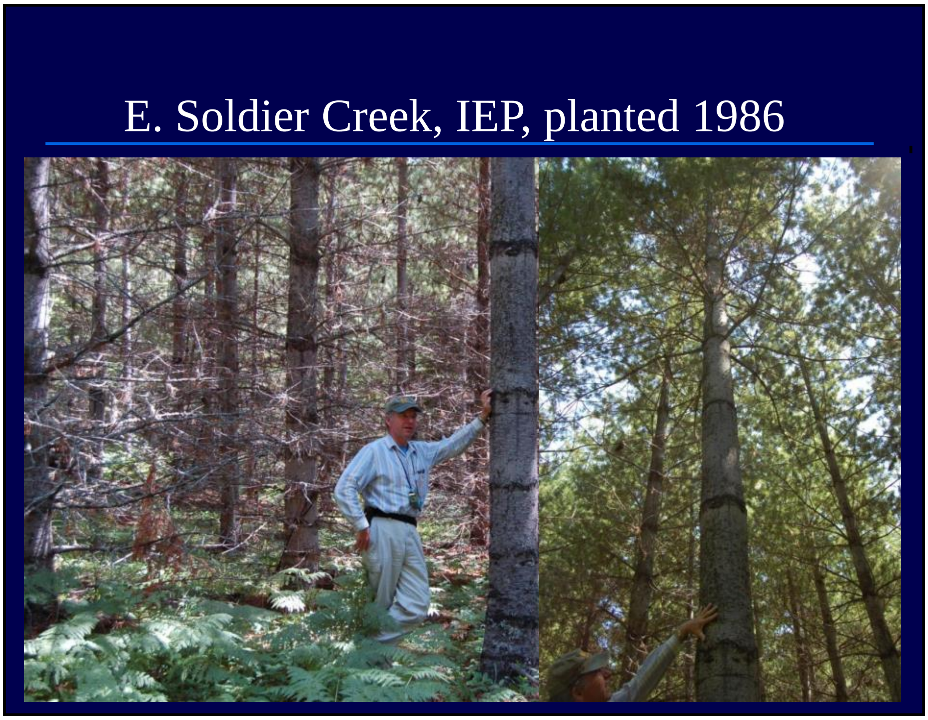
IETIC



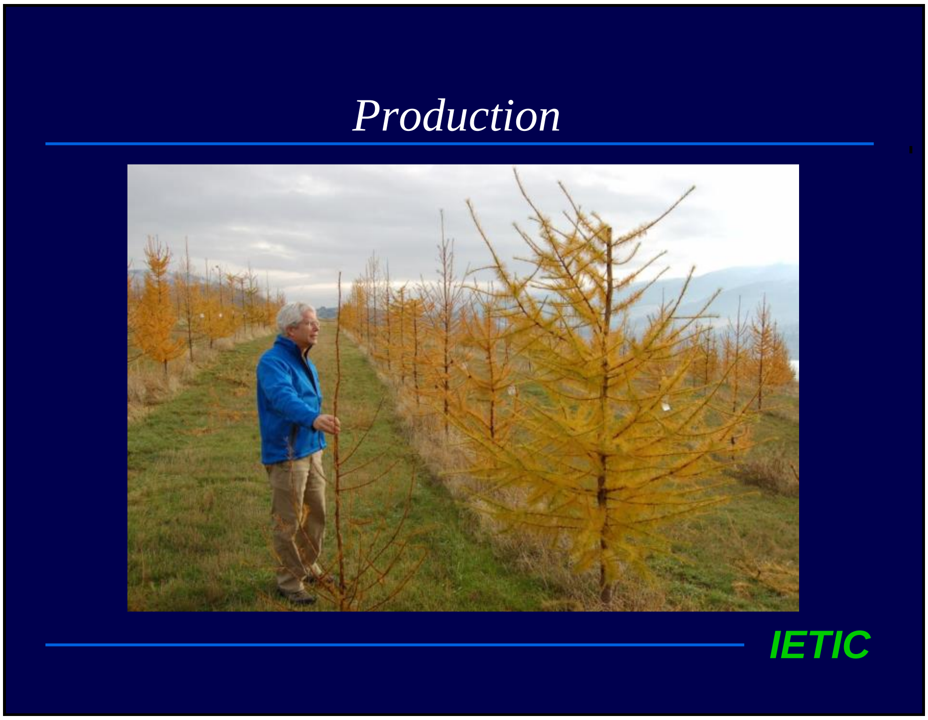
Production

- Few members established their own seed orchards
- A few older orchards exist, RT Bingham WP at Moscow
- Cost share orchards have been established for western larch, ponderosa pine, and Douglas-fir

IETIC



E. Soldier Creek, IEP, planted 1986



Production

IETIC

Studies

- Operational TI cooperative
- Studies aimed primarily at seed production/protection
- CAFS funding has provided additional opportunities for IETIC

IETIC

CAFS Studies

- Systemic insecticides for cone/seed insects
- Western white pine genomics
- WL genetics – compare op seed from seven orchard clones with orchard bulk
- Using endophytes to enhance blister rust resistance in improved western white pine

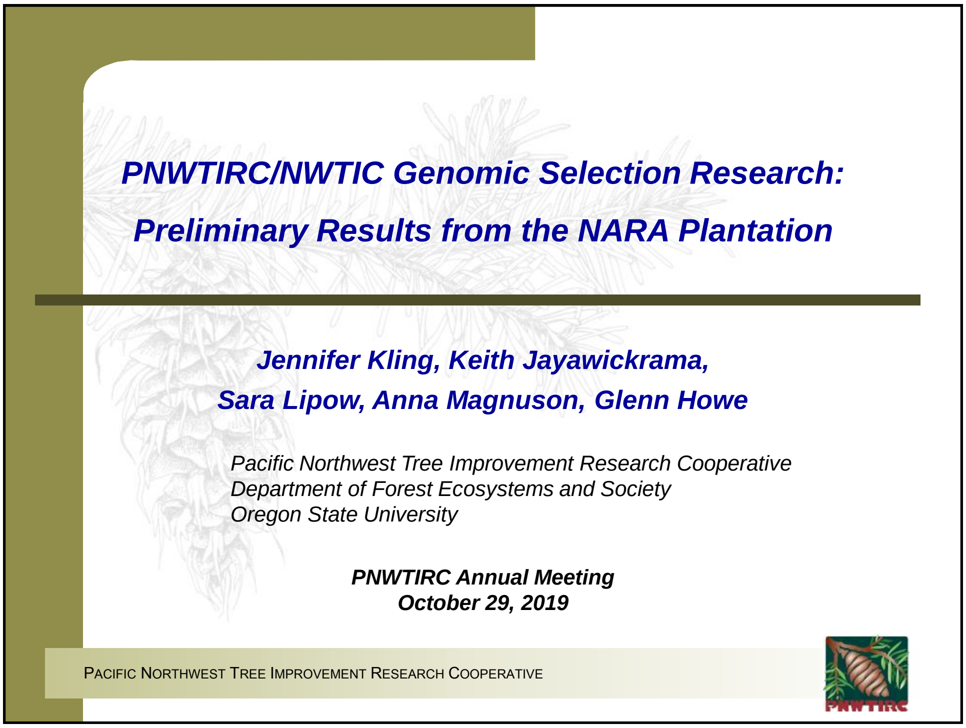
IETIC

IETIC

PNWTIRC/NWTIC genomic selection research: Preliminary results from the NARA plantation

Jennifer Kling, Keith Jayawickrama, Sara Lipow, Anna Magnuson, Glenn Howe

The development of a high-density Axiom array for genotyping in Douglas-fir has paved the way for applying genomic selection in breeding programs. Phenotypic data from a training population are used to develop an equation that predicts breeding values from molecular markers. Genomic selection can be applied to any trait. It is particularly beneficial for selection among progeny within families, because it can distinguish differences among genotypes that have the same pedigree. We conducted a pilot genomic selection study with funding from the PNWTIRC, Conifer Translational Genomics Network, and the Northwest Advanced Renewables Alliance (NARA). The pedigree for this project includes trees from three cycles of selection in populations from the South Central Coast and Coos Bay Low breeding populations that were genotyped using the Axiom array. Third cycle progeny from 25 full-sib families were planted on Roseburg Resources property near Elkton, Oregon in 2015. We collected data on 1,146 five-year-old trees at this site, including height in 2018 and 2019, diameter at breast height, second flushing, ramicorn branching, and sinuosity. In this annual report, we present results from our preliminary analyses. Family heritabilities were moderate for growth traits, but individual tree heritabilities were low, indicating a potential benefit from genomic selection. Second flushing, sinuosity, and ramicorn branching were positively correlated with growth (stem volume). Second flushing was positively correlated with ramicorn branching, but was not related to sinuosity. For the most part, results were consistent with our knowledge of the biology of these traits and with results from earlier studies. The NARA plantation should provide valuable data to further assess the potential gains from genomic selection.



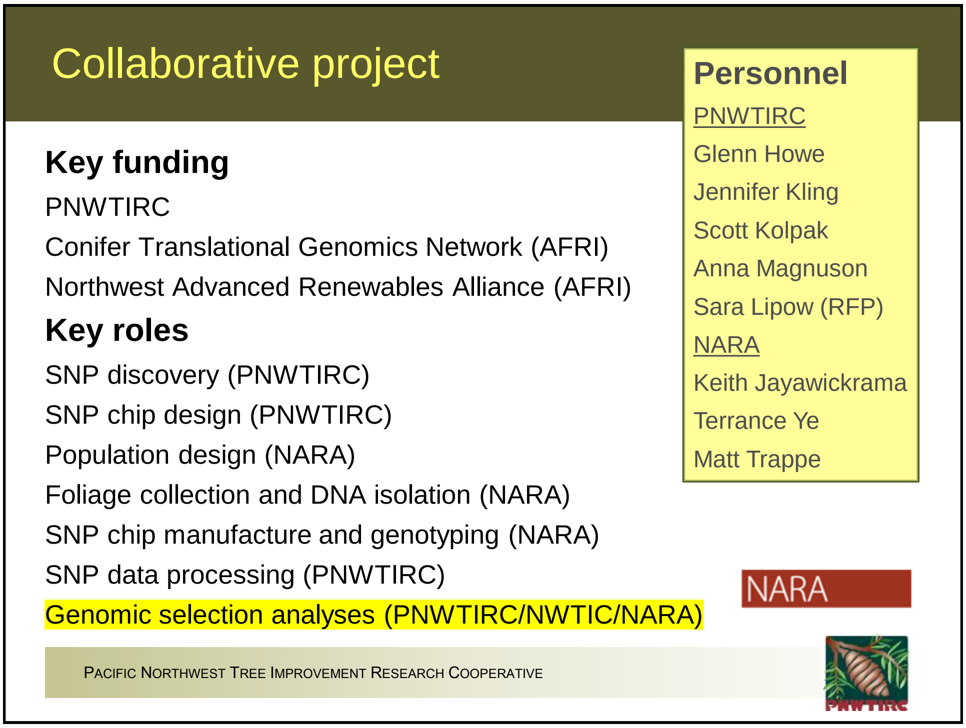
PNWTIRC/NWTIC Genomic Selection Research:
Preliminary Results from the NARA Plantation

Jennifer Kling, Keith Jayawickrama,
Sara Lipow, Anna Magnuson, Glenn Howe

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

PNWTIRC Annual Meeting
October 29, 2019

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Collaborative project

Key funding

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

Key roles

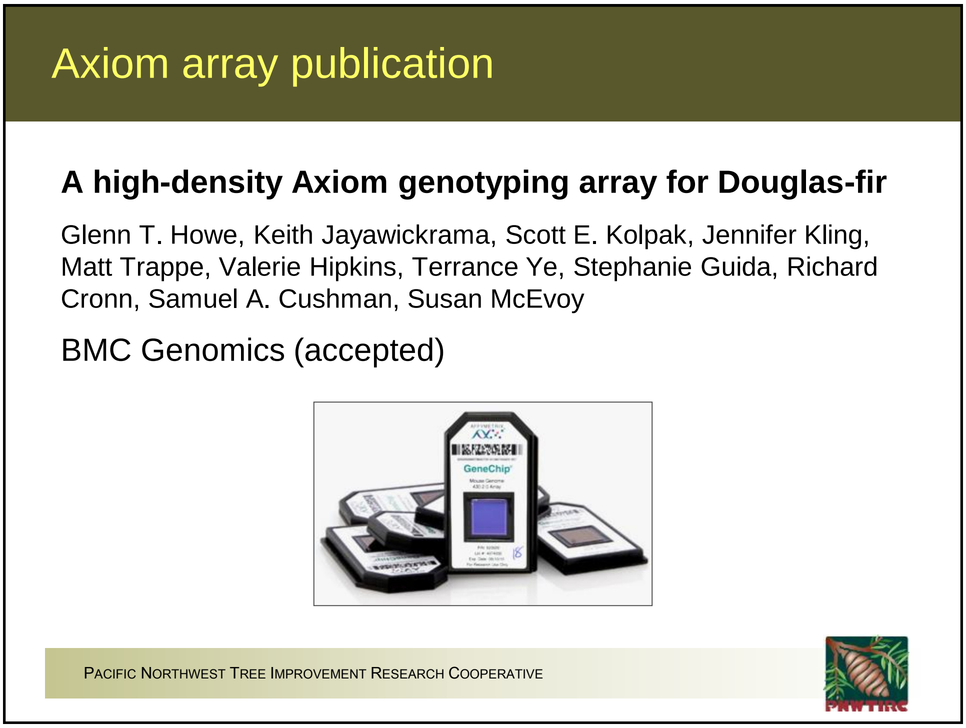
SNP discovery (PNWTIRC)
SNP chip design (PNWTIRC)
Population design (NARA)
Foliage collection and DNA isolation (NARA)
SNP chip manufacture and genotyping (NARA)
SNP data processing (PNWTIRC)
Genomic selection analyses (PNWTIRC/NWTIC/NARA)

Personnel

PNWTIRC
Glenn Howe
Jennifer Kling
Scott Kolpak
Anna Magnuson
Sara Lipow (RFP)
NARA
Keith Jayawickrama
Terrance Ye
Matt Trappe

NARA

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



Axiom array publication

A high-density Axiom genotyping array for Douglas-fir

Glenn T. Howe, Keith Jayawickrama, Scott E. Kolpak, Jennifer Kling, Matt Trappe, Valerie Hipkins, Terrance Ye, Stephanie Guida, Richard Cronn, Samuel A. Cushman, Susan McEvoy

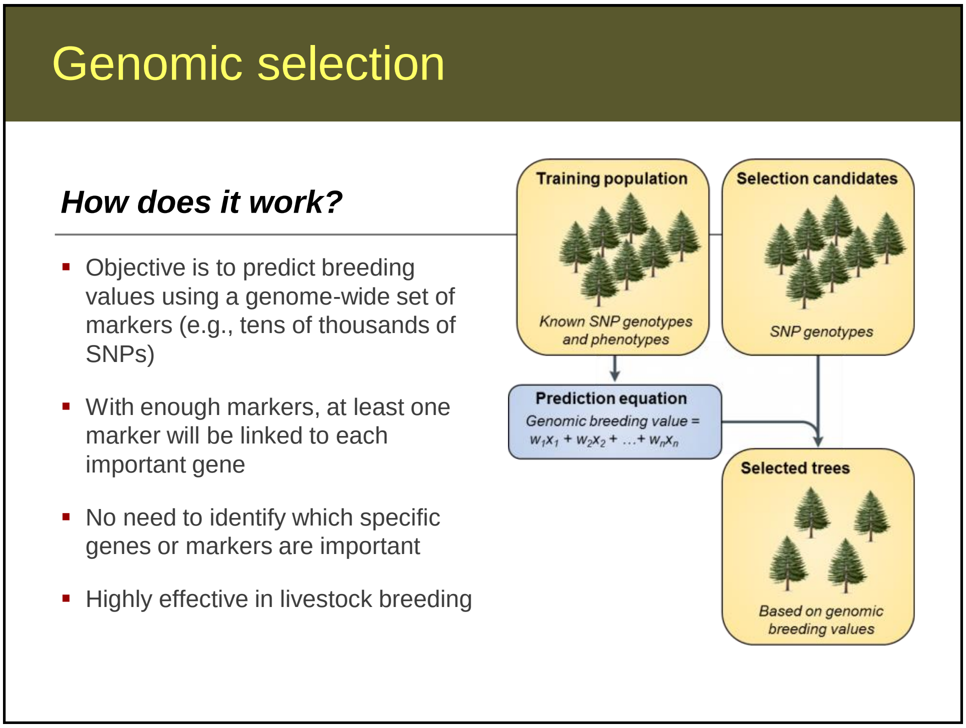
BMC Genomics (accepted)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Outline of presentation

- What is genomic selection?
- Description of the experiment
- Description of traits
- Preliminary results from the NARA site
  - *Heritability of traits*
  - *Correlations among traits*
- Next steps

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

Training population

Known SNP genotypes and phenotypes

Selection candidates

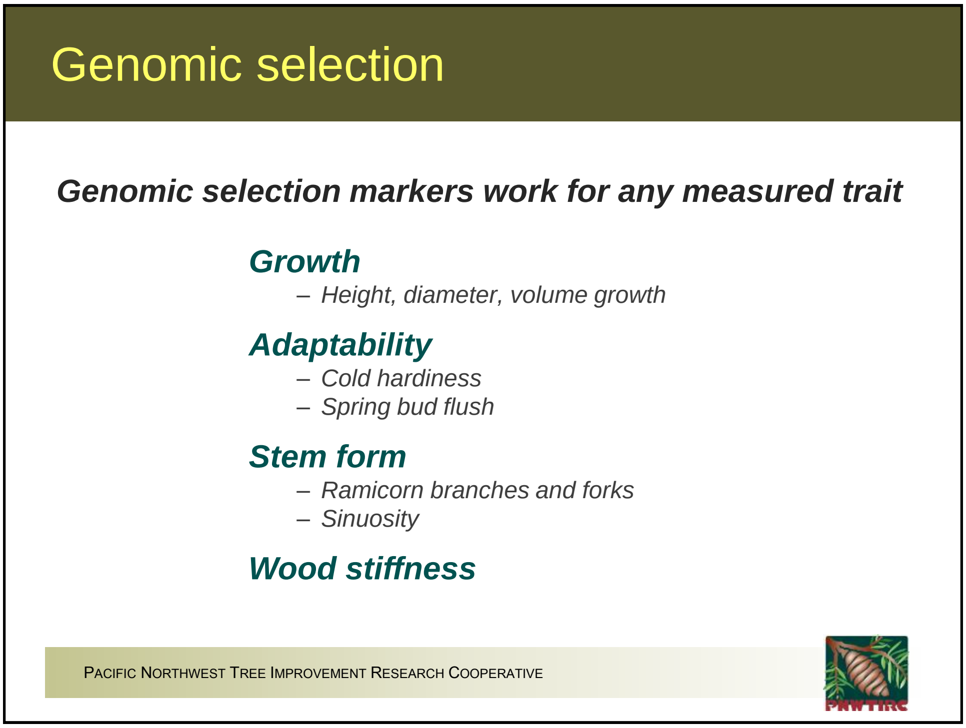
SNP genotypes

Prediction equation

Genomic breeding value = $w_1x_1 + w_2x_2 + \ldots + w_nx_n$

Selected trees

Based on genomic breeding values



Genomic selection

Genomic selection markers work for any measured trait

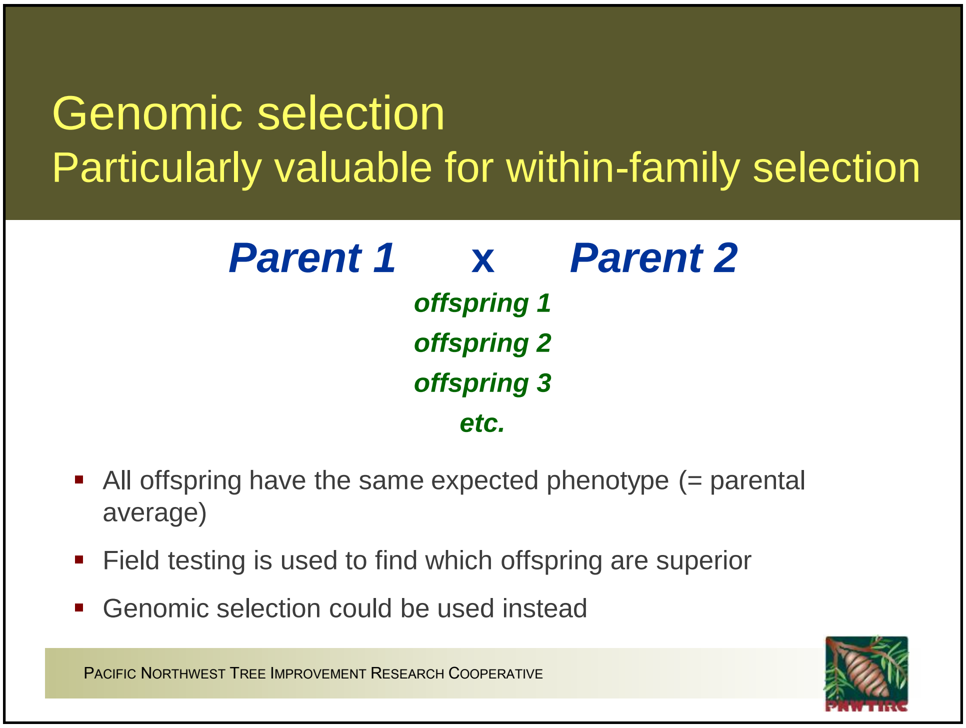
Growth
  - *Height, diameter, volume growth*

Adaptability
  - *Cold hardiness*
  - *Spring bud flush*

Stem form
  - *Ramicorn branches and forks*
  - *Sinuosity*

Wood stiffness

Pacific Northwest Tree Improvement Research Cooperative



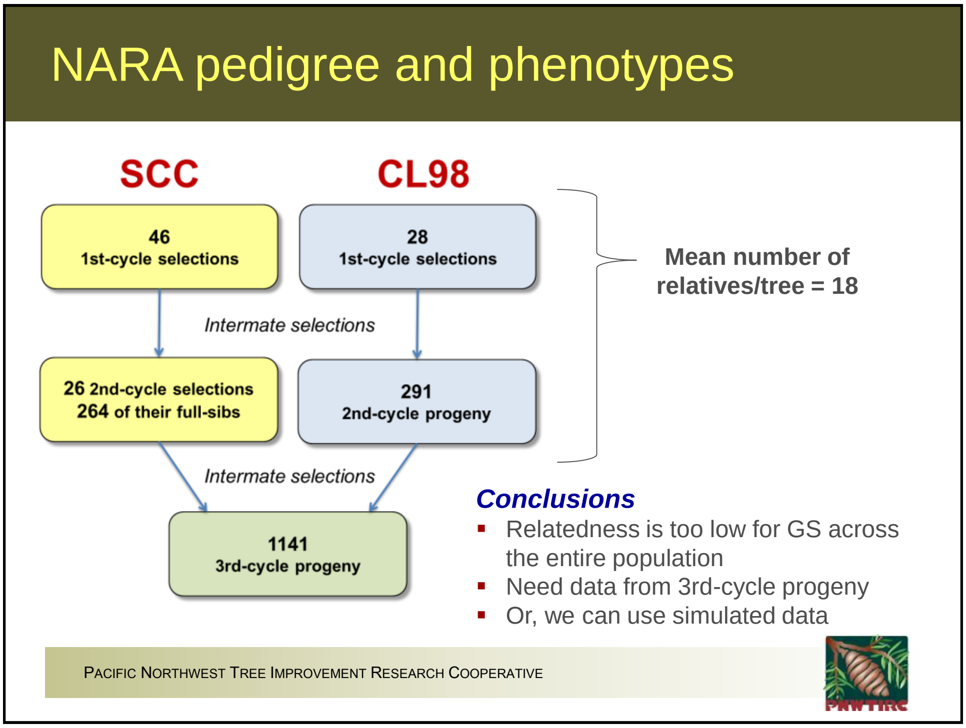
Genomic selection
Particularly valuable for within-family selection

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

Pacific Northwest Tree Improvement Research Cooperative



NARA pedigree and phenotypes

SCC

CL98

46
1st-cycle selections

28
1st-cycle selections

Intermate selections

26 2nd-cycle selections
264 of their full-sibs

291
2nd-cycle progeny

Intermate selections

1141
3rd-cycle progeny

Mean number of relatives/tree = 18

Conclusions

- Relatedness is too low for GS across the entire population
- Need data from 3rd-cycle progeny
- Or, we can use simulated data

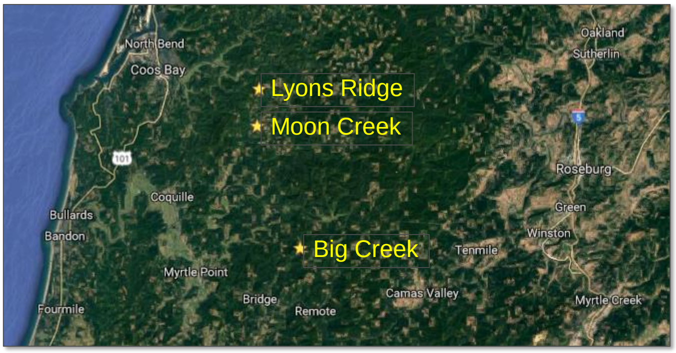
Pacific Northwest Tree Improvement Research Cooperative

Seed orchards

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Progeny test plantations



Plantation	Samples
Moon Creek	293
Lyons Ridge	208
Big Creek	55

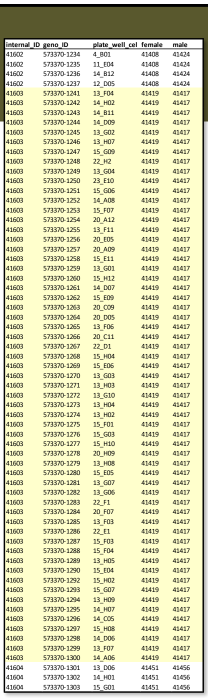
Greenhouse

- Plum Creek greenhouse
- 25 full-sib families
- 1,146 trees
- Planted on Roseburg Resources Property near Elkton, Oregon in March, 2015

NARA site with full-sib progeny

Large full-sib families in the third cycle

Rigorous test of genomic selection



internal_ID	geno_ID	plate_well_cel	female	male
41602	573370-1234	4_B01	41408	41424
41602	573370-1235	11_E04	41408	41424
41602	573370-1236	14_B12	41408	41424
41602	573370-1237	12_D05	41408	41424
41603	573370-1241	13_F04	41419	41417
41603	573370-1242	14_H02	41419	41417
41603	573370-1243	14_B11	41419	41417
41603	573370-1244	14_D09	41419	41417
41603	573370-1245	13_G02	41419	41417
41603	573370-1246	13_H07	41419	41417
41603	573370-1247	15_G09	41419	41417
41603	573370-1248	22_H2	41419	41417
41603	573370-1249	13_G04	41419	41417
41603	573370-1250	23_E10	41419	41417
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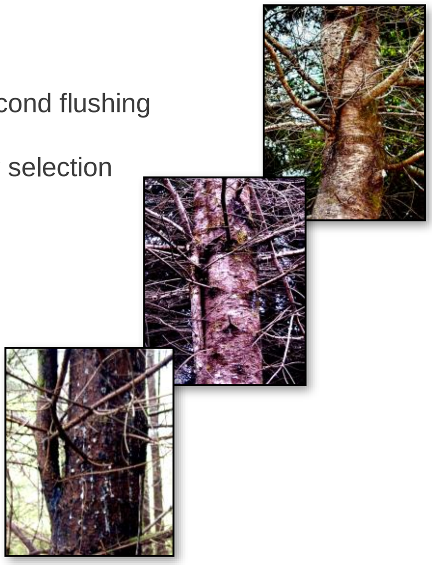
41603	573370-1256	20_E05	41419	41417
41603	573370-1257	20_A09	41419	41417
41603	573370-1258	15_E11	41419	41417
41603	573370-1259	13_G01	41419	41417
41603	573370-1260	15_H12	41419	41417
41603	573370-1261	14_D07	41419	41417
41603	573370-1262	15_E09	41419	41417
41603	573370-1263	20_C09	41419	41417
41603	573370-1264	20_D05	41419	41417
41603	573370-1265	13_F06	41419	41417
41603	573370-1266	20_C11	41419	41417
41603	573370-1267	22_D1	41419	41417
41603	573370-1268	15_H04	41419	41417
41603	573370-1269	15_E06	41419	41417
41603	573370-1270	13_G03	41419	41417
41603	573370-1271	13_H03	41419	41417
41603	573370-1272	13_G10	41419	41417
41603	573370-1273	13_H04	41419	41417
41603	573370-1274	13_H02	41419	41417
41603	573370-1275	15_F01	41419	41417
41603	573370-1276	15_G03	41419	41417
41603	573370-1277	15_H10	41419	41417
41603	573370-1278	20_H09	41419	41417
41603	573370-1279	13_H08	41419	41417
41603	573370-1280	15_E05	41419	41417
41603	573370-1281	13_G07	41419	41417
41603	573370-1282	13_G06	41419	41417
41603	573370-1283	22_F1	41419	41417
41603	573370-1284	20_F07	41419	41417
41603	573370-1285	13_F03	41419	41417
41603	573370-1286	22_E1	41419	41417
41603	573370-1287	15_F03	41419	41417
41603	573370-1288	15_F04	41419	41417
41603	573370-1289	13_H05	41419	41417
41603	573370-1290	15_E04	41419	41417
41603	573370-1292	15_H02	41419	41417
41603	573370-1293	15_G07	41419	41417
41603	573370-1294	13_H09	41419	41417
41603	573370-1295	14_H07	41419	41417
41603	573370-1296	14_C05	41419	41417
41603	573370-1297	15_H08	41419	41417
41603	573370-1298	14_D06	41419	41417
41603	573370-1299	13_F07	41419	41417
41603	573370-1300	14_A06	41419	41417
41604	573370-1301	13_D06	41451	41456
41604	573370-1302	14_H01	41451	41456
41604	573370-1303	15_G01	41451	41456

Traits measured

- Plant height (2018, 2019)
- Height growth (height in 2019 – height in 2018)
- Diameter at breast height (DBH)
- Volume (volume index)
- Forks (there weren't many)
- Sinuosity (index = frequency x displacement)
- Ramicorn branching (present or absent)
- Second flushing (present or absent)

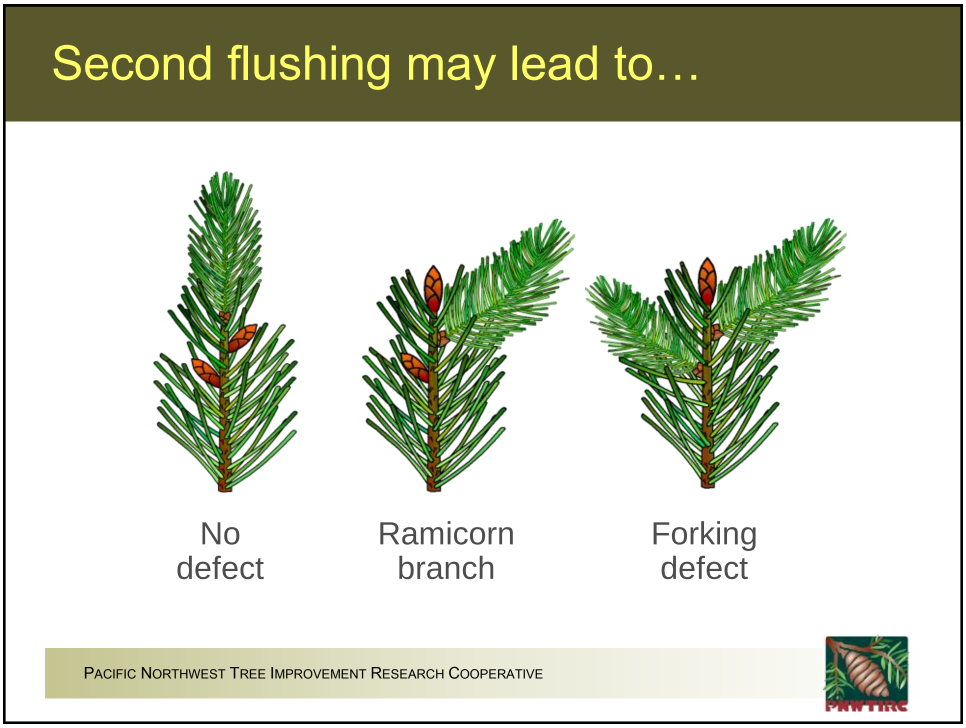
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Ramicorn branching and forking



Biology

- Most frequent cause is aberrant second flushing
- More frequent at early ages – Early selection should work well
- Low to moderate genetic control
- Large genetic gains possible
- Correlated with growth rate
- More frequent on productive sites



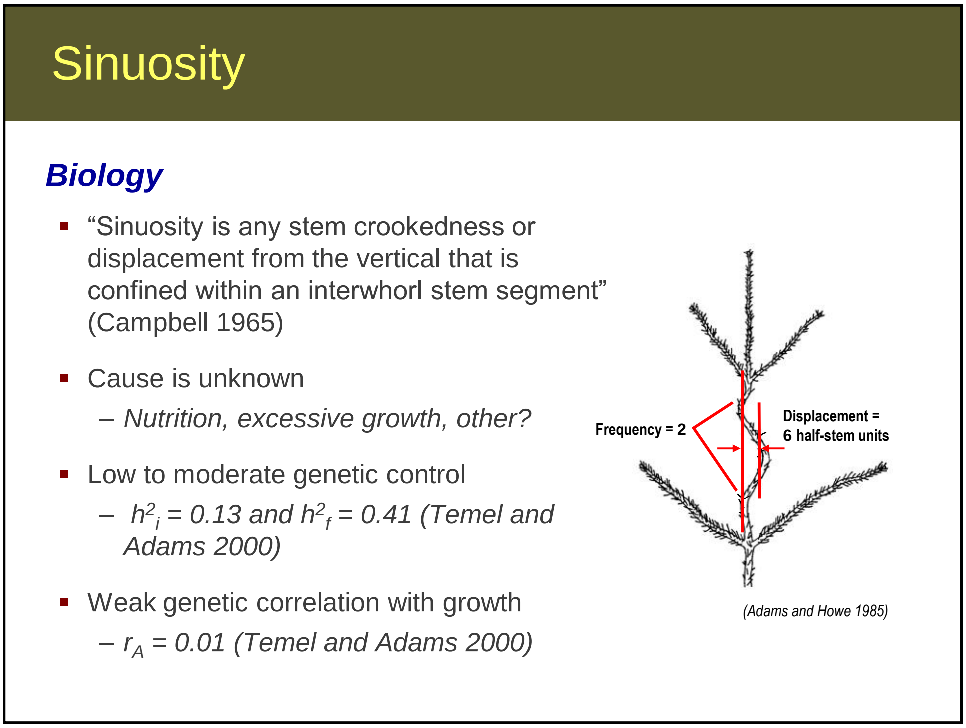
Second flushing may lead to…

No defect

Ramicorn branch

Forking defect

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

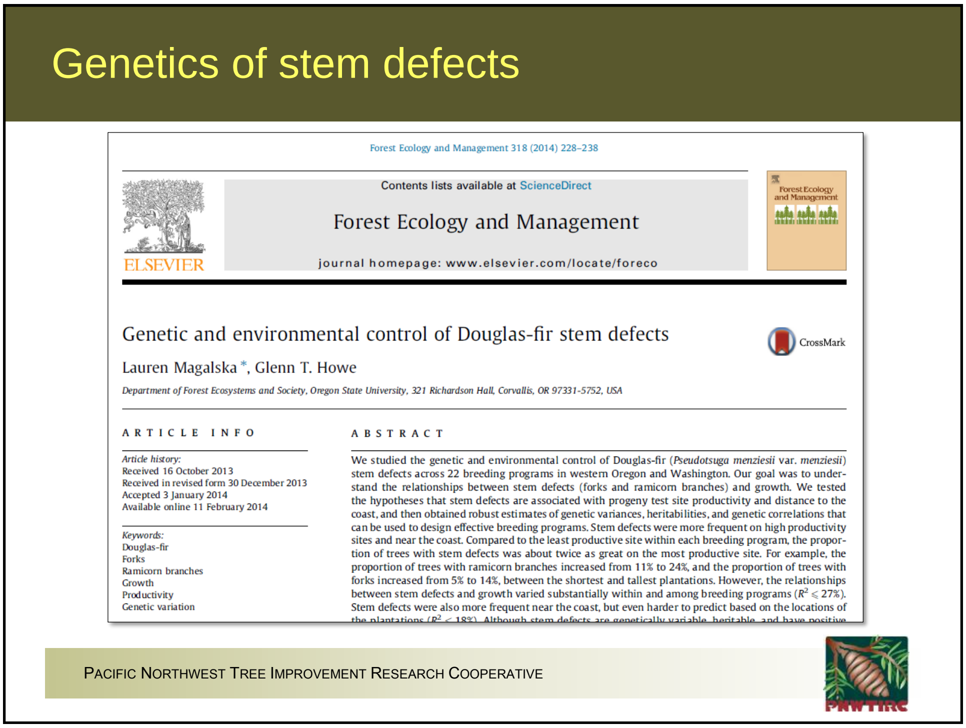


Sinuosity

Biology

- "Sinuosity is any stem crookedness or displacement from the vertical that is confined within an interwhorl stem segment" (Campbell 1965)
- Cause is unknown
  - *Nutrition, excessive growth, other?*
- Low to moderate genetic control
  - *$h^2_i = 0.13$ and $h^2_f = 0.41$ (Temel and Adams 2000)*
- Weak genetic correlation with growth
  - *$r_A = 0.01$ (Temel and Adams 2000)*

(Adams and Howe 1985)



Genetics of stem defects

Forest Ecology and Management 318 (2014) 228–238

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journal homepage: www.elsevier.com/locate/foreco

Genetic and environmental control of Douglas-fir stem defects

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ABSTRACT

We studied the genetic and environmental control of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) stem defects across 22 breeding programs in western Oregon and Washington. Our goal was to understand the relationships between stem defects (forks and ramicorn branches) and growth. We tested the hypotheses that stem defects are associated with progeny test site productivity and distance to the coast, and then obtained robust estimates of genetic variances, heritabilities, and genetic correlations that can be used to design effective breeding programs. Stem defects were more frequent on high productivity sites and near the coast. Compared to the least productive site within each breeding program, the proportion of trees with stem defects was about twice as great on the most productive site. For example, the proportion of trees with ramicorn branches increased from 11% to 24%, and the proportion of trees with forks increased from 5% to 14%, between the shortest and tallest plantations. However, the relationships between stem defects and growth varied substantially within and among breeding programs ($R^2 \leqslant 27\%$). Stem defects were also more frequent near the coast, but even harder to predict based on the locations of the plantations ($R^2 \leqslant 18\%$). Although stem defects are genetically variable, heritable, and have positive

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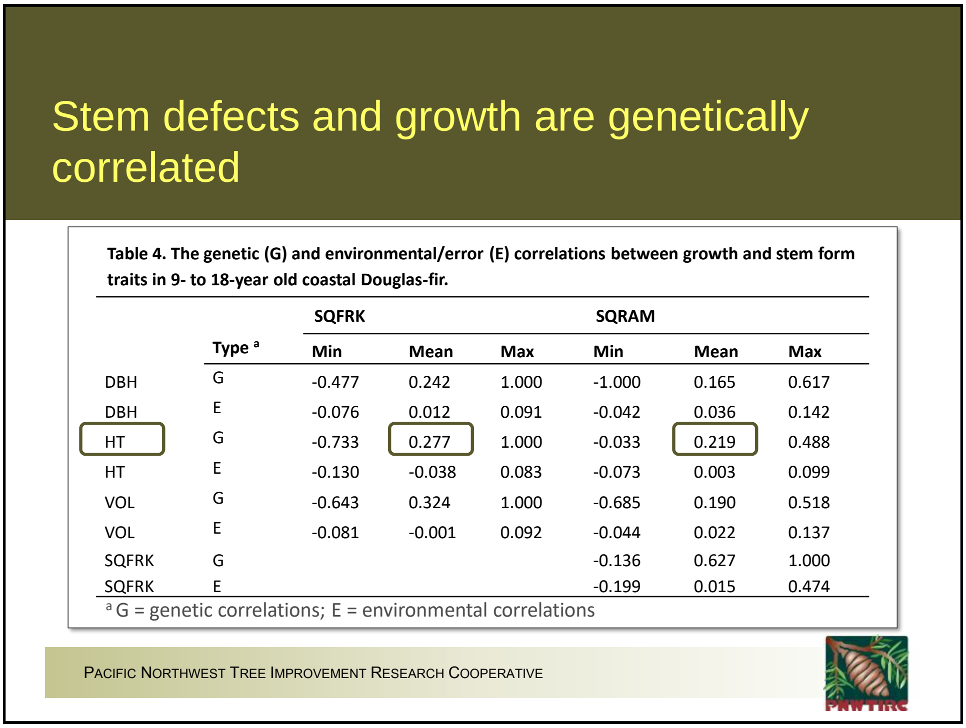


Stem defects and growth are genetically correlated

Table 4. The genetic (G) and environmental/error (E) correlations between growth and stem form traits in 9- to 18-year old coastal Douglas-fir.

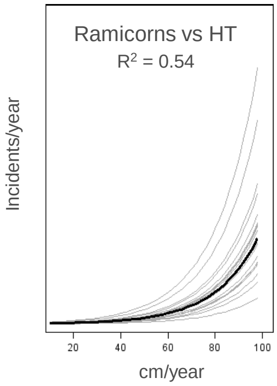
		SQFRK			**SQRAM**		
	Type [a]	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**
DBH	G	-0.477	0.242	1.000	-1.000	0.165	0.617
DBH	E	-0.076	0.012	0.091	-0.042	0.036	0.142
HT	G	-0.733	0.277	1.000	-0.033	0.219	0.488
HT	E	-0.130	-0.038	0.083	-0.073	0.003	0.099
VOL	G	-0.643	0.324	1.000	-0.685	0.190	0.518
VOL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

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

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Both stem forks and ramicorns increase with increasing height growth

- R^2 is greater than for other traits, which is consistent with biology
- For every additional cm/year
  - *Ramicorns/year increase 6%*



Pacific Northwest Tree Improvement Research Cooperative

Genomic selection validation – NARA field test

Data collection - growth traits

Jennifer Kling
PNWTIRC/OSU

Anna Magnuson
PNWTIRC/OSU

Nancy Heater
Roseburg Forest Products

Data collection – Growth and stem defects

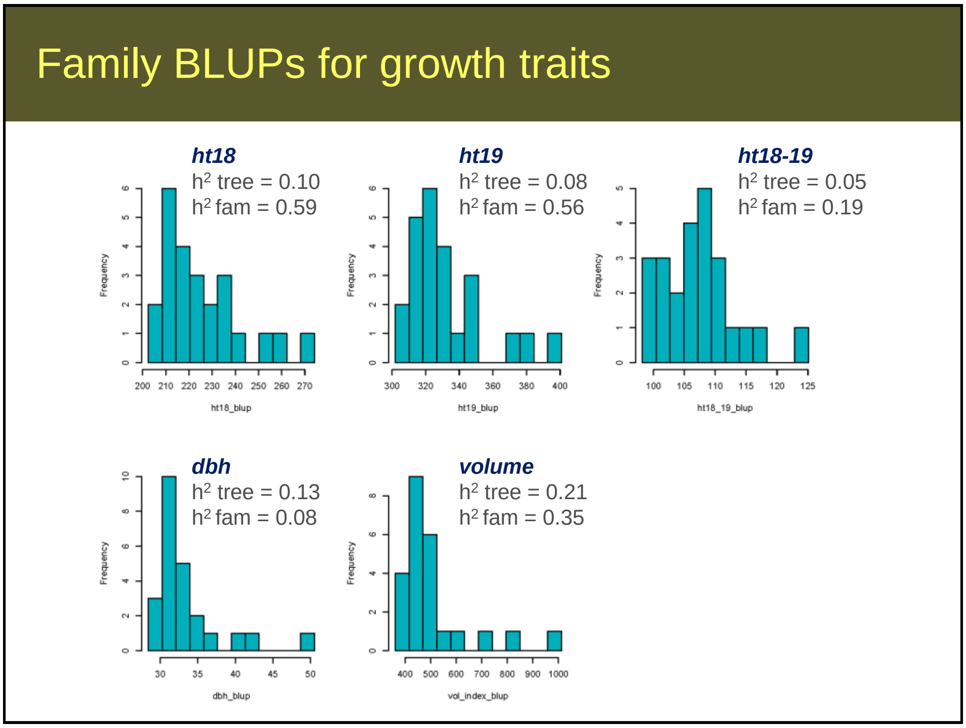
Second flushing

Height

Ramicorn branching

Sinuosity

DBH



Family BLUPs for growth traits

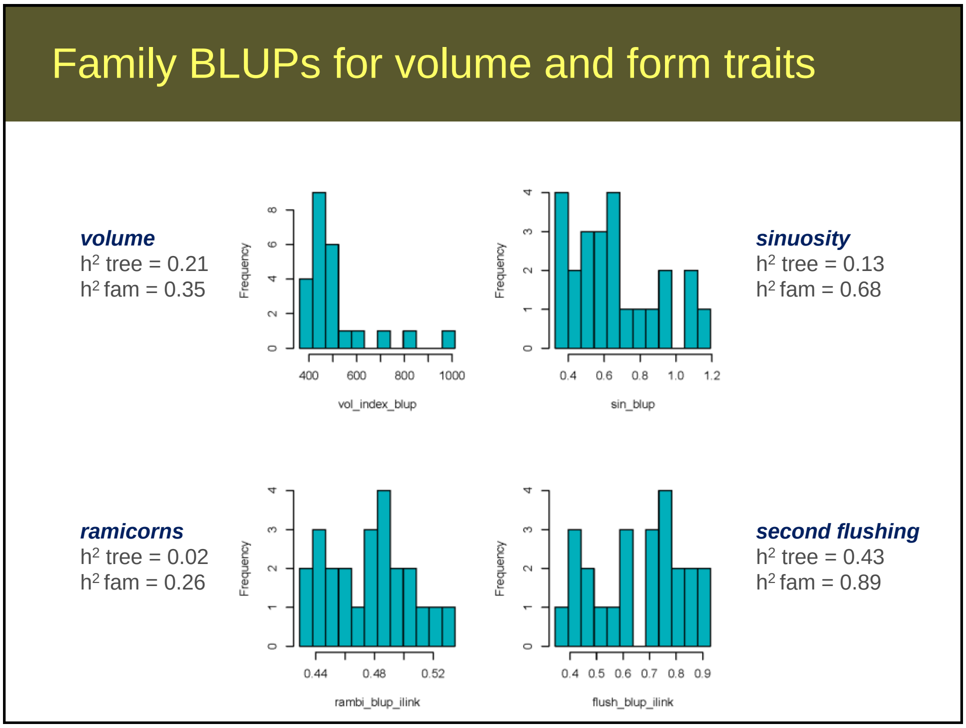
ht18
h^2 tree = 0.10
h^2 fam = 0.59

ht19
h^2 tree = 0.08
h^2 fam = 0.56

ht18-19
h^2 tree = 0.05
h^2 fam = 0.19

dbh
h^2 tree = 0.13
h^2 fam = 0.08

volume
h^2 tree = 0.21
h^2 fam = 0.35



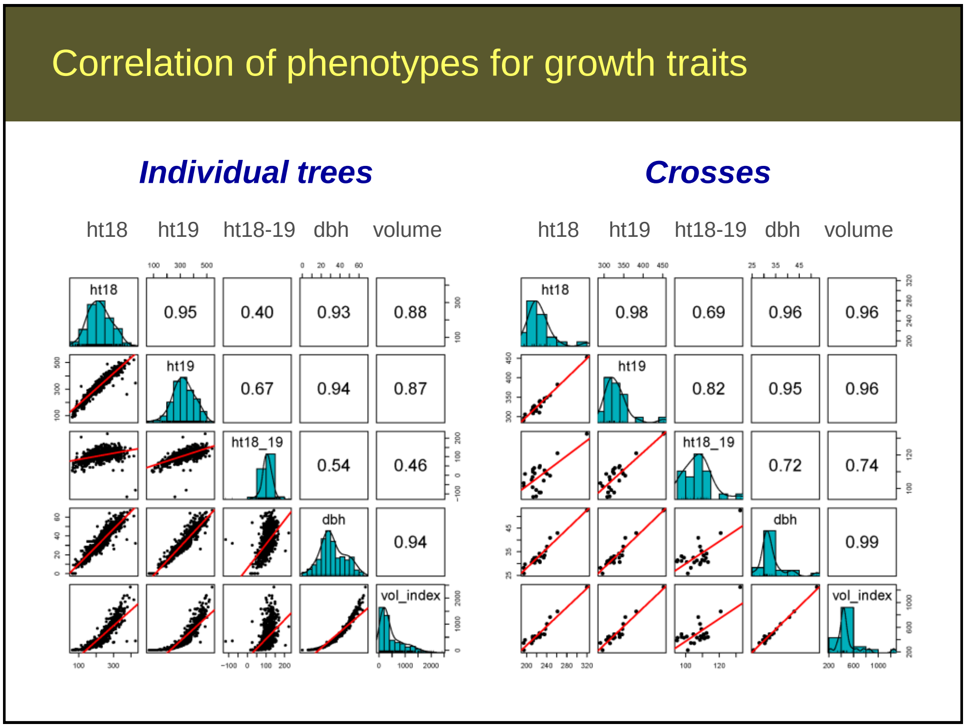
Family BLUPs for volume and form traits

volume
h^2 tree = 0.21
h^2 fam = 0.35

sinuosity
h^2 tree = 0.13
h^2 fam = 0.68

ramicorns
h^2 tree = 0.02
h^2 fam = 0.26

second flushing
h^2 tree = 0.43
h^2 fam = 0.89



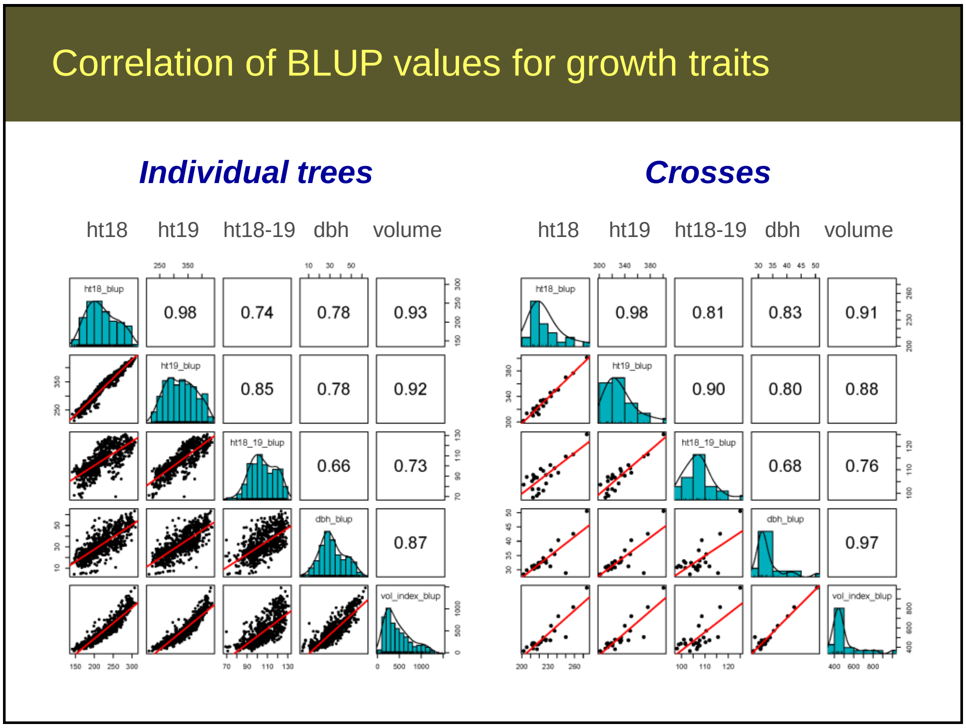
Correlation of phenotypes for growth traits

Individual trees

	ht18	ht19	ht18-19	dbh	volume
ht18	ht18	0.95	0.40	0.93	0.88
ht19		ht19	0.67	0.94	0.87
ht18_19			ht18_19	0.54	0.46
dbh				dbh	0.94
vol_index					vol_index

Crosses

	ht18	ht19	ht18-19	dbh	volume
ht18	ht18	0.98	0.69	0.96	0.96
ht19		ht19	0.82	0.95	0.96
ht18_19			ht18_19	0.72	0.74
dbh				dbh	0.99
vol_index					vol_index

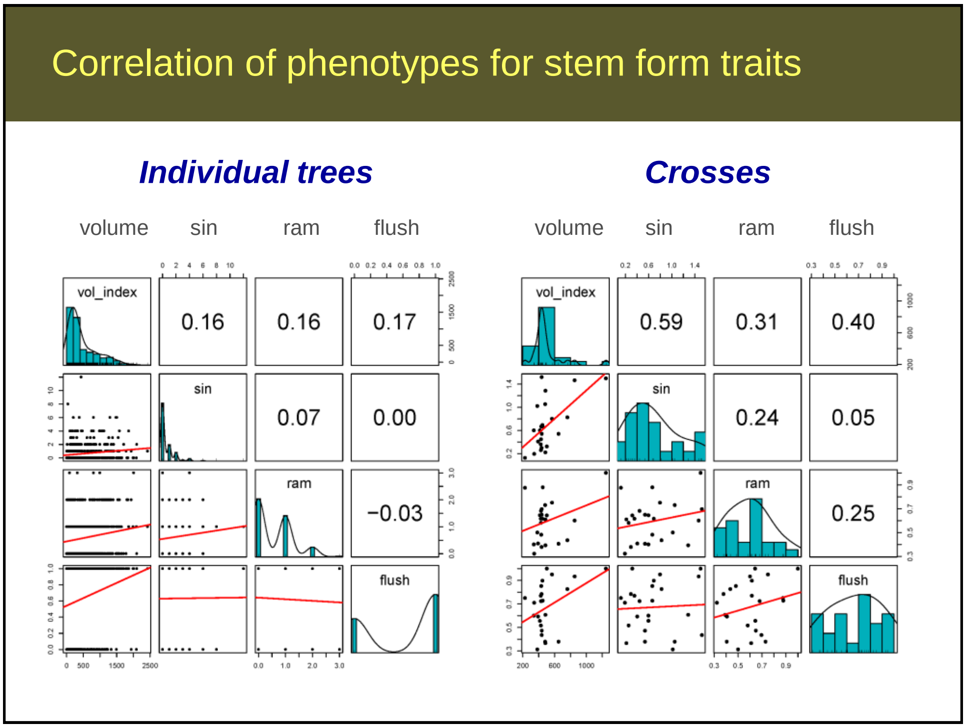


Correlation of BLUP values for growth traits

Individual trees ***Crosses***

ht18 ht19 ht18-19 dbh volume

ht18 ht19 ht18-19 dbh volume

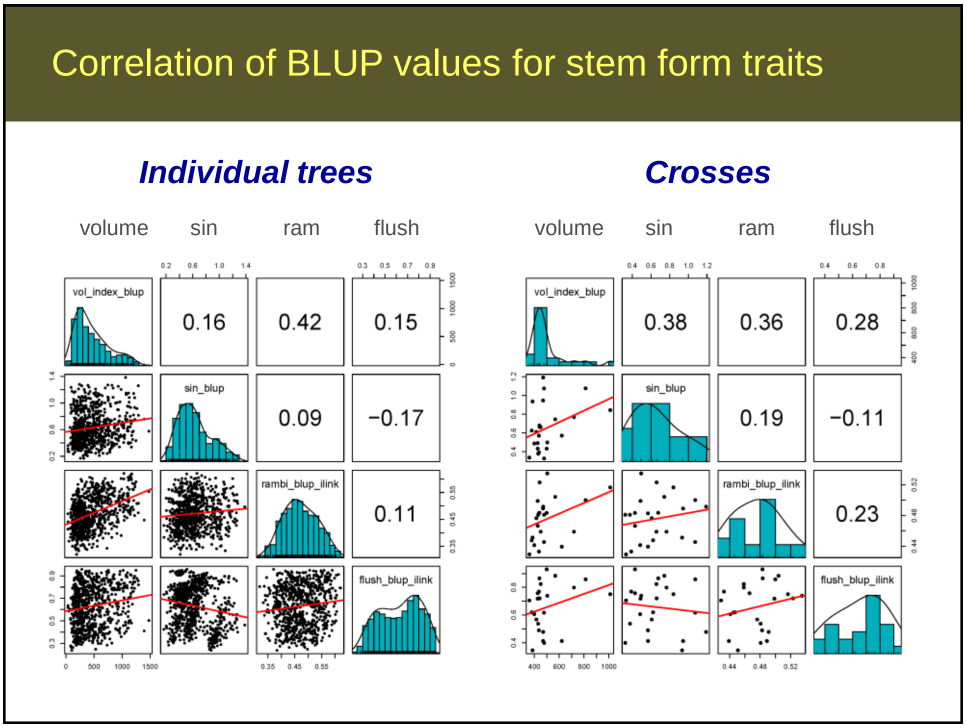


Correlation of phenotypes for stem form traits

Individual trees ***Crosses***

volume sin ram flush

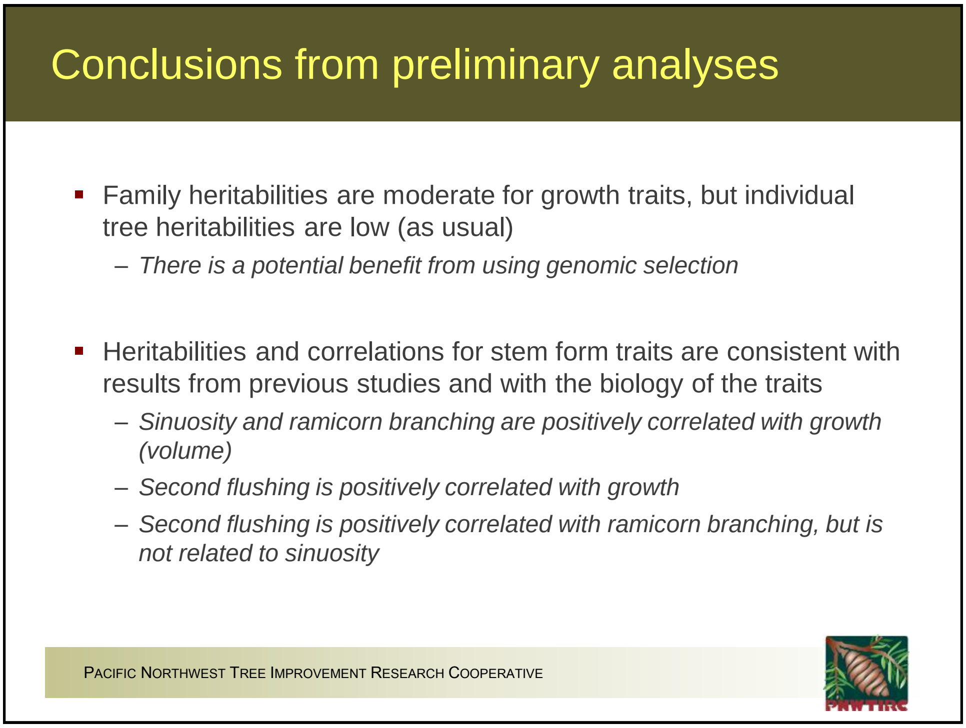
volume sin ram flush



Correlation of BLUP values for stem form traits

Individual trees

Crosses



Conclusions from preliminary analyses

- Family heritabilities are moderate for growth traits, but individual tree heritabilities are low (as usual)
  - *There is a potential benefit from using genomic selection*
- Heritabilities and correlations for stem form traits are consistent with results from previous studies and with the biology of the traits
  - *Sinuosity and ramicorn branching are positively correlated with growth (volume)*
  - *Second flushing is positively correlated with growth*
  - *Second flushing is positively correlated with ramicorn branching, but is not related to sinuosity*

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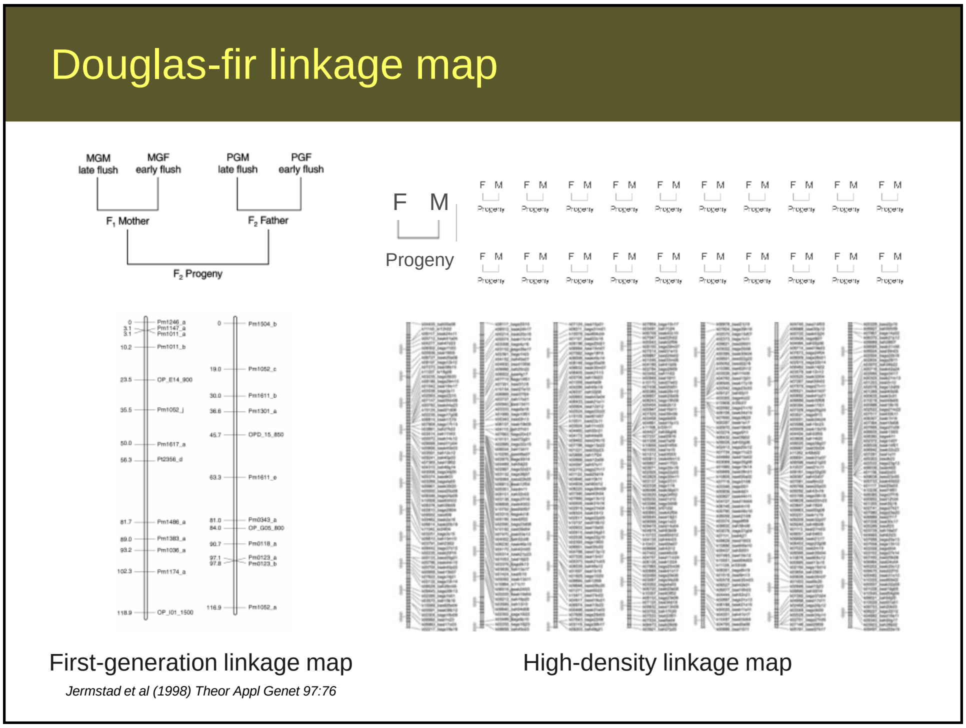
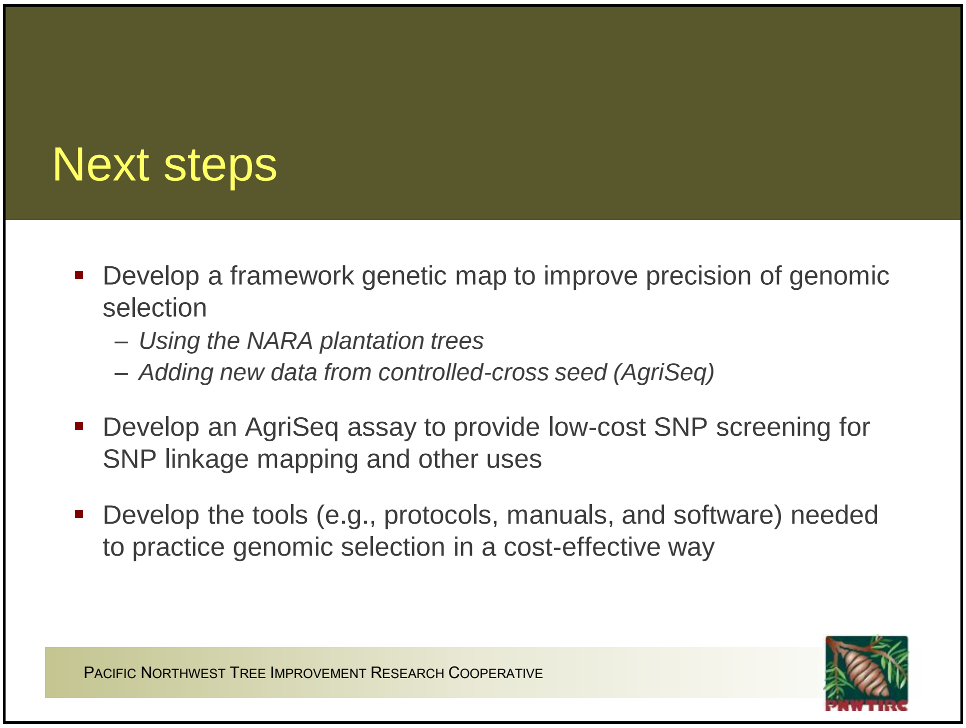


Douglas-fir linkage map

First-generation linkage map

Jermstad et al (1998) Theor Appl Genet 97:76

High-density linkage map



Next steps

- Develop a framework genetic map to improve precision of genomic selection
  - *Using the NARA plantation trees*
  - *Adding new data from controlled-cross seed (AgriSeq)*
- Develop an AgriSeq assay to provide low-cost SNP screening for SNP linkage mapping and other uses
- Develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Climate-based seed deployment zones for the Pacific Northwest

Meridith McClure and Glenn Howe

Native populations of forest trees are specifically adapted to the climates they inhabit. The farther trees are moved from their local climates (i.e., as the climatic transfer distance increases), the more growth and survival decrease. The transfer limit is the climatic transfer distance beyond which plantation performance is considered unacceptable. Thus, to ensure healthy and productive forests, the climatic adaptation of seedlots (i.e., seed from native stands or seed orchards) must be matched to the climate of the planting site. These concepts are important for current seed transfer and for practicing assisted migration. Currently, a fixed zone seed deployment system is used to match seedlots to potential planting sites in the Pacific Northwest. However, this method limits the deployment of seed, and makes it difficult to share seed among organizations that use different zone systems.

At the annual meeting, we presented a proposal to develop climate-based seed deployment zones for the Pacific Northwest. We propose to use a climate interpolation model called ClimateNA (Wang et al. 2016, PLoS One 11:e0156720) to match seed collection zones and seed deployment zones throughout the Pacific Northwest. This will expand seed deployment and pave the way to use assisted migration to help forests adapt to climate change. By determining climate matches between all seed deployment zones commonly-used in the Pacific Northwest, foresters will have a firmer basis for deploying alternative seedlots and for sharing seed among organizations and widely dispersed geographic areas.

Climate-based Seed Deployment Zones for the Pacific Northwest

October 29, 2019

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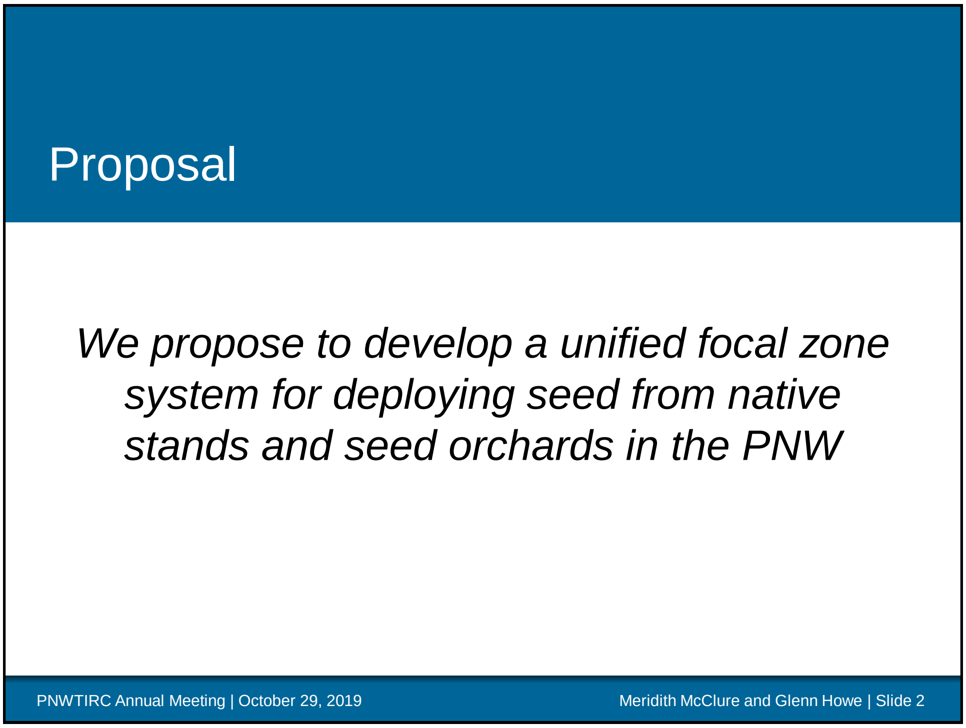
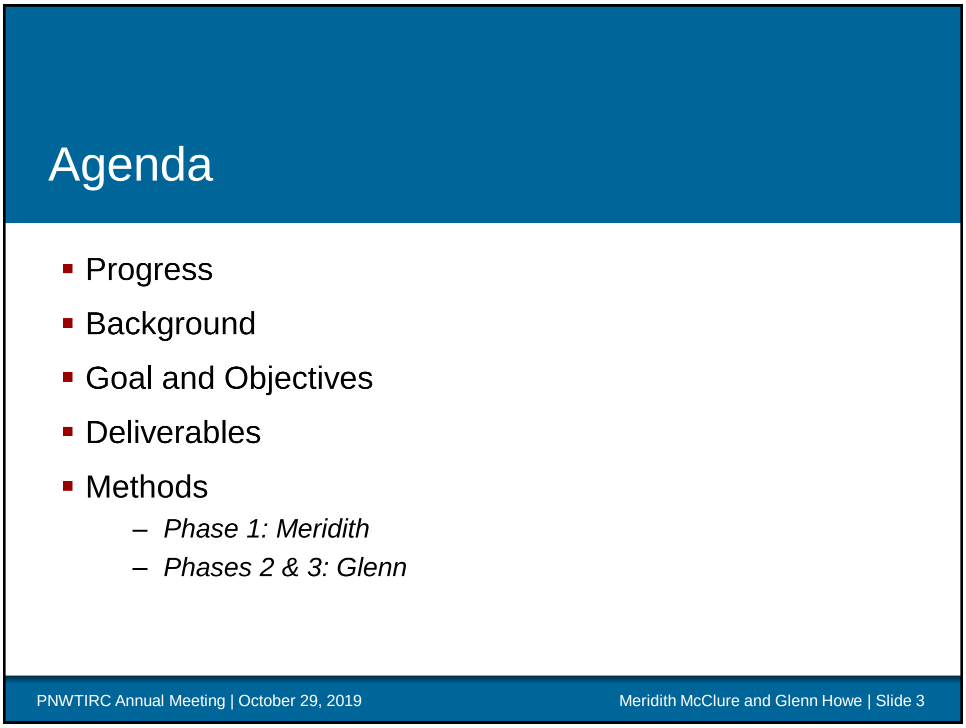


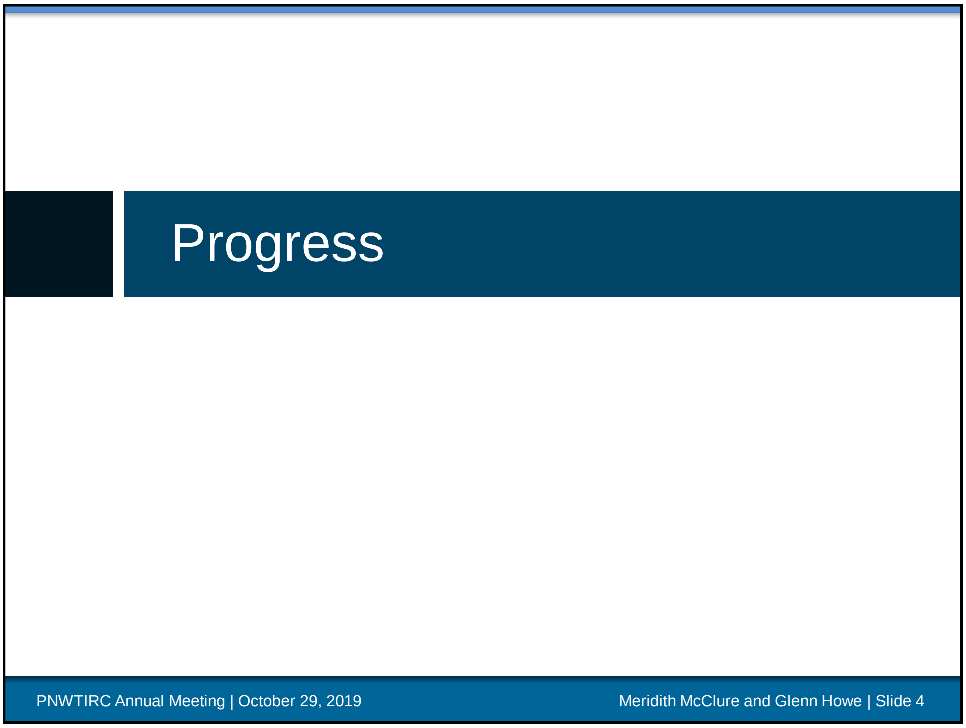
Proposal

We propose to develop a unified focal zone system for deploying seed from native stands and seed orchards in the PNW



Agenda

- Progress
- Background
- Goal and Objectives
- Deliverables
- Methods
  - *Phase 1: Meridith*
  - *Phases 2 & 3: Glenn*



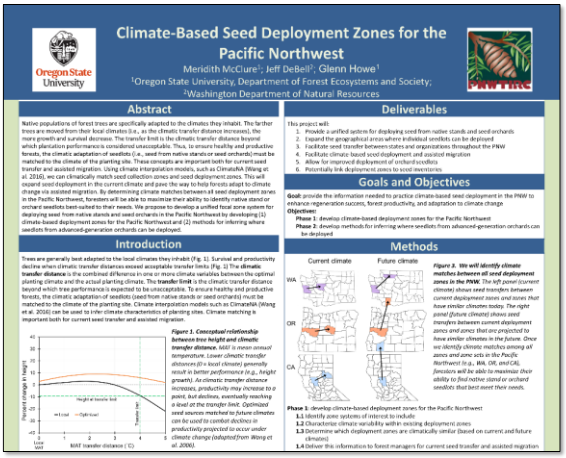
Progress

Stakeholder meeting (Olympia, WA)

- Met with Jeff DeBell, Lauren Magalska, Jeff deGraan, and Florian Deisenhofer for initial project feedback in May 2019
- Discussed project logistics, feasibility, and scope

NW Climate Conference (Portland, OR)

McClure, M., DeBell, J., & Howe, G. (2019). Climate-Based Seed Deployment Zones for the Pacific Northwest. Poster presented at: Northwest Climate Conference; 8 October, 2019; Portland, Oregon.



Climate-Based Seed Deployment Zones for the Pacific Northwest

Meridith McClure[1]; Jeff DeBell[2]; Glenn Howe[1]
[1]Oregon State University, Department of Forest Ecosystems and Society;
[2]Washington Department of Natural Resources

Abstract

Native populations of forest trees are specifically adapted to the climates they inhabit. The farther trees are moved from their local climates (i.e., as the climatic transfer distance increases), the more growth and survival decrease. The transfer limit is the climatic transfer distance beyond which plantation performance is considered unacceptable. Thus, to ensure healthy and productive forests, the climatic adaptation of seedlots (i.e., seed from native stands or seed orchards) must be matched to the climate of the planting site. These concepts are important both for current seed transfer and assisted migration. Using climate interpolation models, such as ClimateNA (Wang et al. 2016), we can climatically match seed collection zones and seed deployment zones. This will expand seed deployment in the current climate and pave the way to help forests adapt to climate change via assisted migration. By determining climate matches between all seed deployment zones in the Pacific Northwest, foresters will be able to maximize their ability to identify native stand or orchard seedlots best-suited to their needs. We propose to develop a unified focal zone system for deploying seed from native stands and seed orchards in the Pacific Northwest by developing (1) climate-based deployment zones for the Pacific Northwest and (2) methods for inferring where seedlots from advanced-generation orchards can be deployed.

Introduction

Trees are generally best adapted to the local climates they inhabit (Fig. 1). Survival and productivity decline when climatic transfer distances exceed acceptable transfer limits (Fig. 1) The **climatic transfer distance** is the combined difference in one or more climate variables between the optimal planting climate and the actual planting climate. The **transfer limit** is the climatic transfer distance beyond which tree performance is expected to be unacceptable. To ensure healthy and productive forests, the climatic adaptation of seedlots (seed from native stands or seed orchards) must be matched to the climate of the planting site. Climate interpolation models such as ClimateNA (Wang et al. 2016) can be used to infer climate characteristics of planting sites. Climate matching is important both for current seed transfer and assisted migration.

Figure 1. Conceptual relationship between tree height and climatic transfer distance. *MAT is mean annual temperature. Lower climatic transfer distances (0 = local climate) generally result in better performance (e.g., height growth). As climatic transfer distance increases, productivity may increase to a point, but declines, eventually reaching a level at the transfer limit. Optimized seed sources matched to future climates can be used to combat declines in productivity projected to occur under climate change (adapted from Wang et al. 2006).*

Deliverables

This project will:

1. Provide a unified system for deploying seed from native stands and seed orchards
2. Expand the geographical areas where individual seedlots can be deployed
3. Facilitate seed transfer between states and organizations throughout the PNW
4. Facilitate climate-based seed deployment and assisted migration
5. Allow for improved deployment of orchard seedlots
6. Potentially link deployment zones to seed inventories

Goals and Objectives

Goal: provide the information needed to practice climate-based seed deployment in the PNW to enhance regeneration success, forest productivity, and adaptation to climate change

Objectives:

Phase 1: develop climate-based deployment zones for the Pacific Northwest
Phase 2: develop methods for inferring where seedlots from advanced-generation orchards can be deployed

Methods

Figure 3. We will identify climate matches between all seed deployment zones in the PNW. *The left panel (current climate) shows seed transfers between current deployment zones and zones that have similar climates today. The right panel (future climate) shows seed transfers between current deployment zones and zones that are projected to have similar climates in the future. Once we identify climate matches among all zones and zone sets in the Pacific Northwest (e.g., WA, OR, and CA), foresters will be able to maximize their ability to find native stand or orchard seedlots that best meet their needs.*

Phase 1: develop climate-based deployment zones for the Pacific Northwest

- **1.1** Identify zone systems of interest to include
- **1.2** Characterize climate variability within existing deployment zones
- **1.3** Determine which deployment zones are climatically similar (based on current and future climates)
- **1.4** Deliver this information to forest managers for current seed transfer and assisted migration

Background

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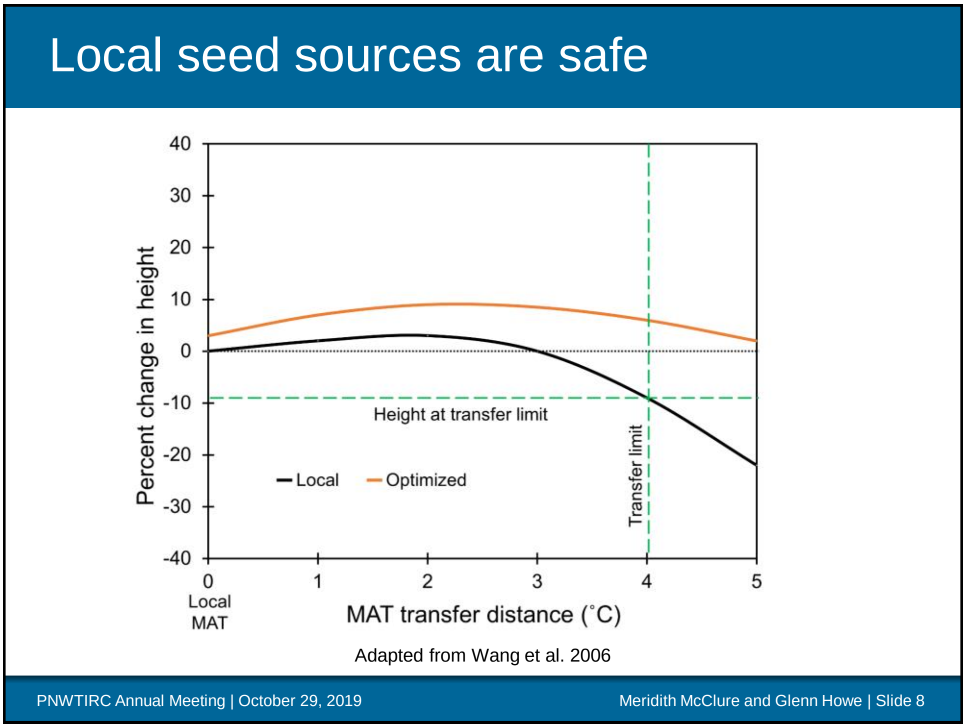

Local seed sources are safe

Adapted from Wang et al. 2006

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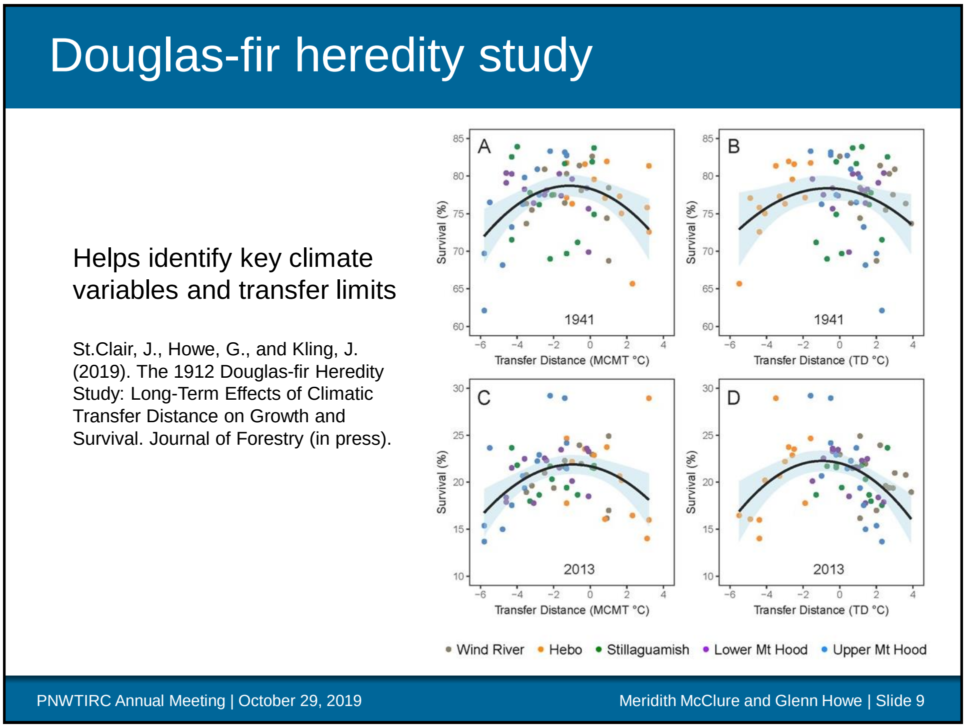

Douglas-fir heredity study

Helps identify key climate variables and transfer limits

St.Clair, J., Howe, G., and Kling, J. (2019). The 1912 Douglas-fir Heredity Study: Long-Term Effects of Climatic Transfer Distance on Growth and Survival. Journal of Forestry (in press).

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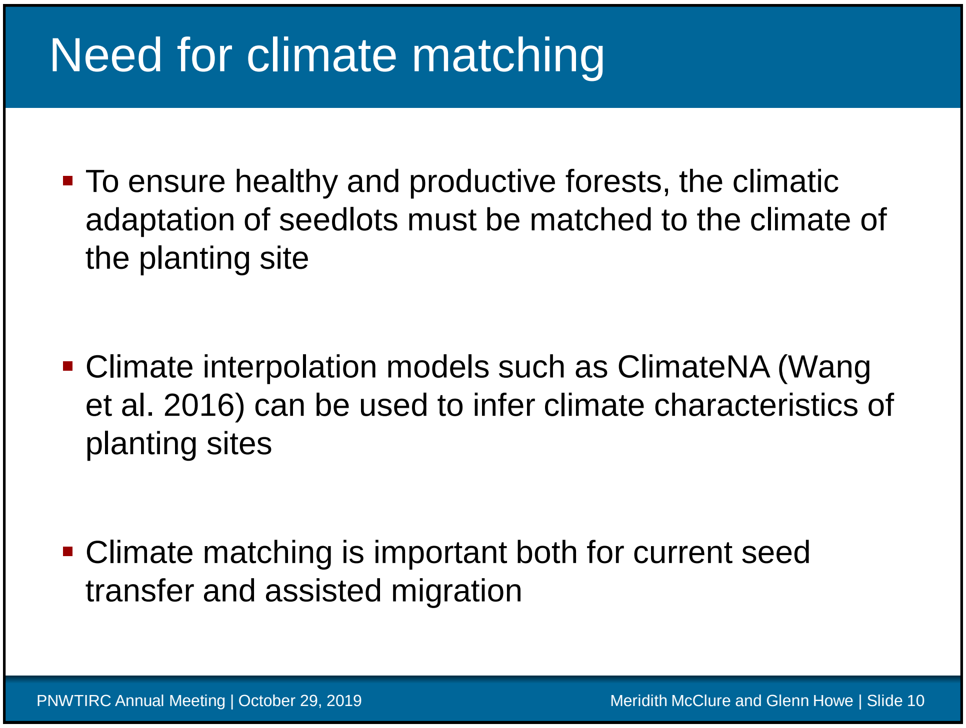

Need for climate matching

- To ensure healthy and productive forests, the climatic adaptation of seedlots must be matched to the climate of the planting site
- Climate interpolation models such as ClimateNA (Wang et al. 2016) can be used to infer climate characteristics of planting sites
- Climate matching is important both for current seed transfer and assisted migration

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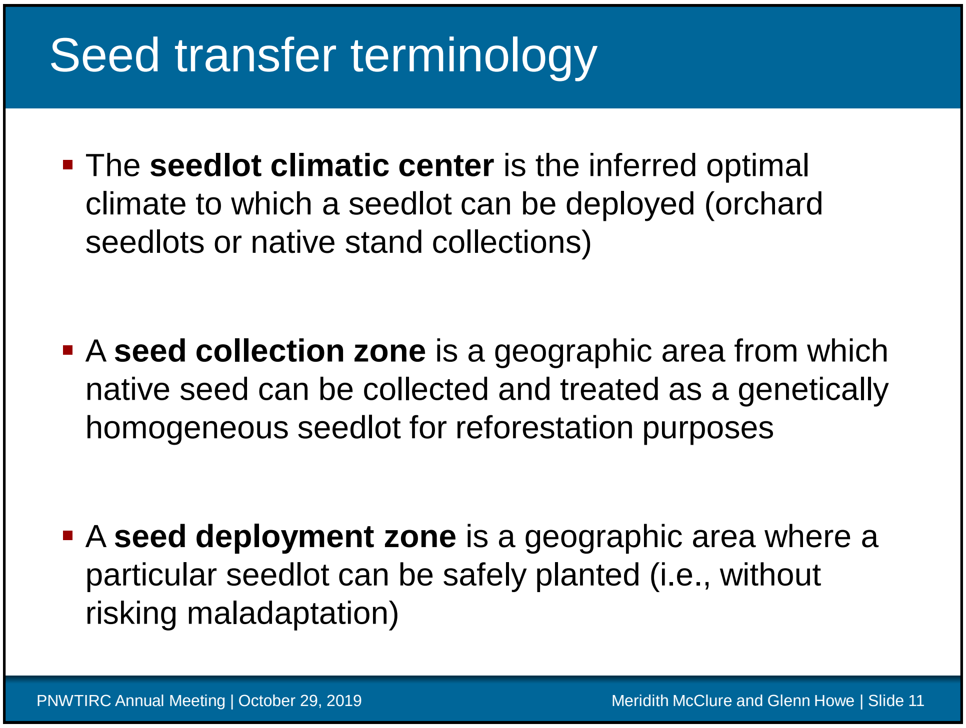
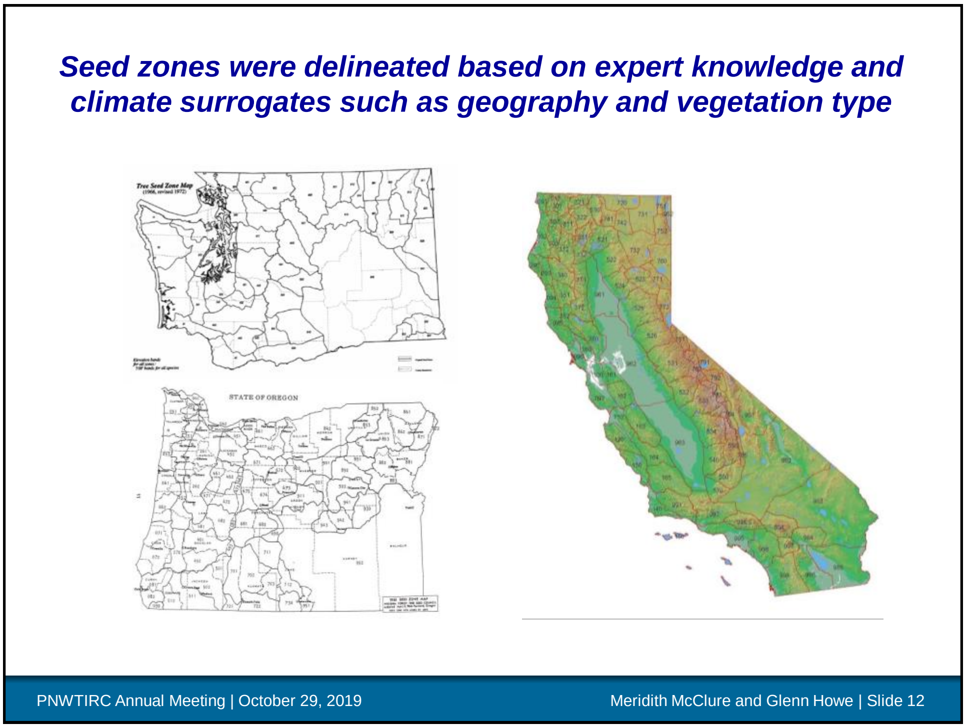
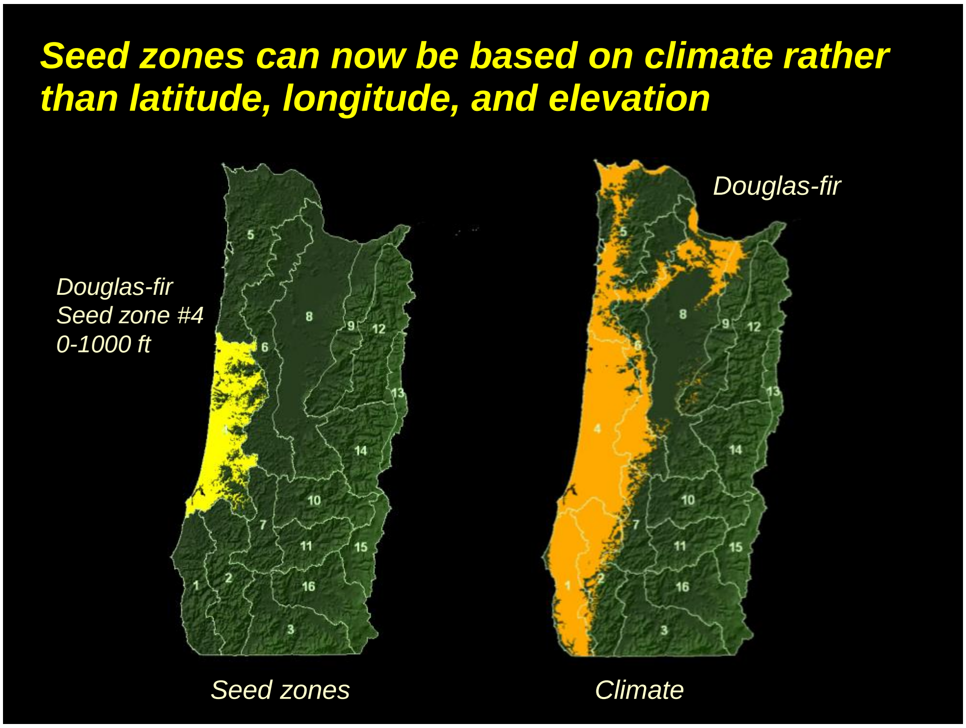


Seed transfer terminology

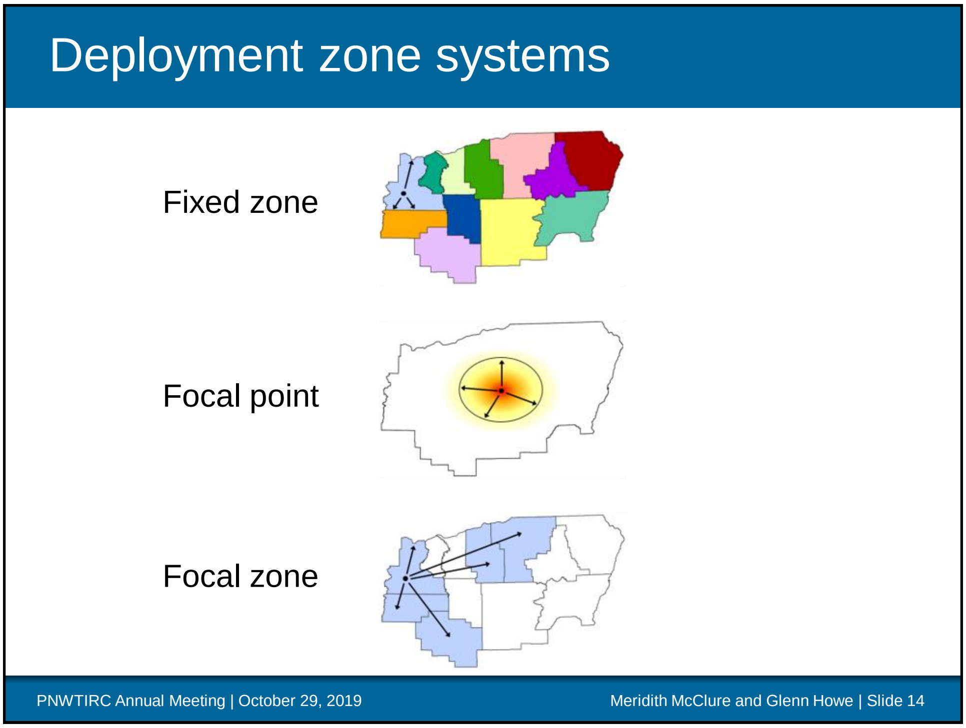
- The **seedlot climatic center** is the inferred optimal climate to which a seedlot can be deployed (orchard seedlots or native stand collections)
- A **seed collection zone** is a geographic area from which native seed can be collected and treated as a genetically homogeneous seedlot for reforestation purposes
- A **seed deployment zone** is a geographic area where a particular seedlot can be safely planted (i.e., without risking maladaptation)



Seed zones were delineated based on expert knowledge and climate surrogates such as geography and vegetation type



Seed zones can now be based on climate rather than latitude, longitude, and elevation



Deployment zone systems

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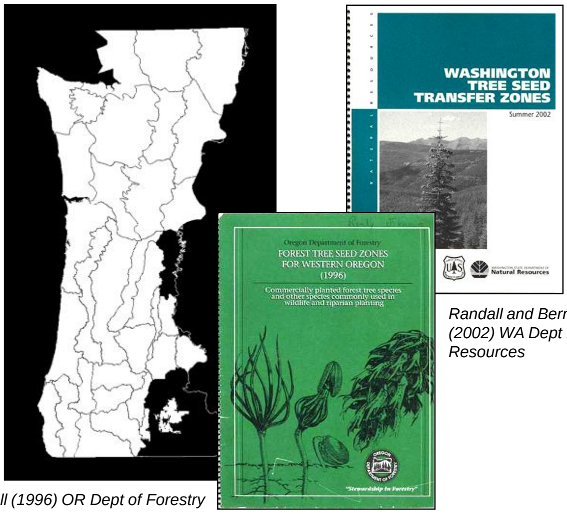
Fixed zone systems are common

Seed zones and breeding zones are largely delineated based on inferred climate



WASHINGTON TREE SEED TRANSFER ZONES

Summer 2002

Oregon Department of Forestry

FOREST TREE SEED ZONES FOR WESTERN OREGON (1996)

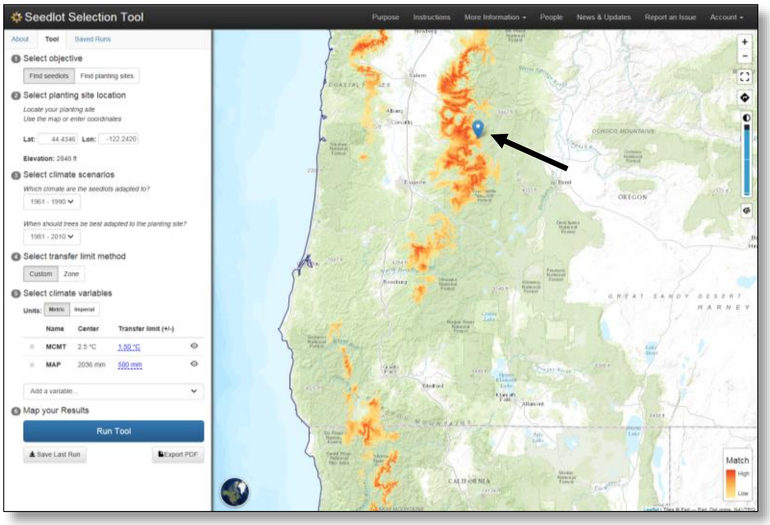
Commercially planted forest tree species and other species commonly used in wildlife and riparian planting

"Stewardship in Forestry"

Randall (1996) OR Dept of Forestry

Randall and Berrang (2002) WA Dept Nat Resources

SST uses a focal point approach



1 Select objective

Find seedlots | Find planting sites

2 Select planting site location

Locate your planting site

Use the map or enter coordinates

Lat: 44.4346 Lon: -122.2420

Elevation: 2848 ft

3 Select climate scenarios

Which climate are the seedlots adapted to?

1961 - 1990

When should trees be best adapted to the planting site?

1961 - 2010

4 Select transfer limit method

Custom | Zone

5 Select climate variables

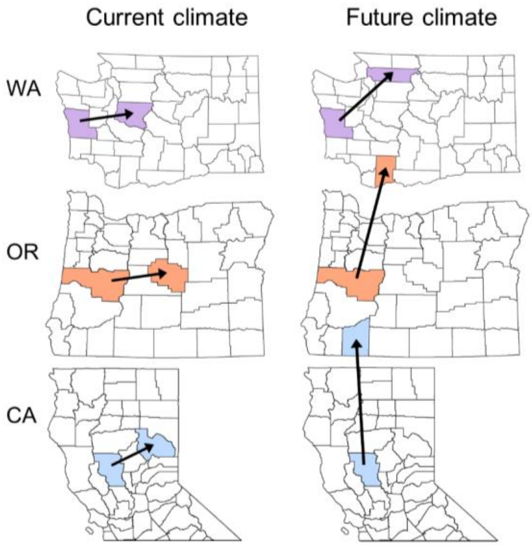
Units: Metric | Imperial

Name	Center	Transfer limit (+/-)
MCMT	2.5 °C	1.50 °C
MAP	2036 mm	500 mm

Add a variable...

6 Map your Results

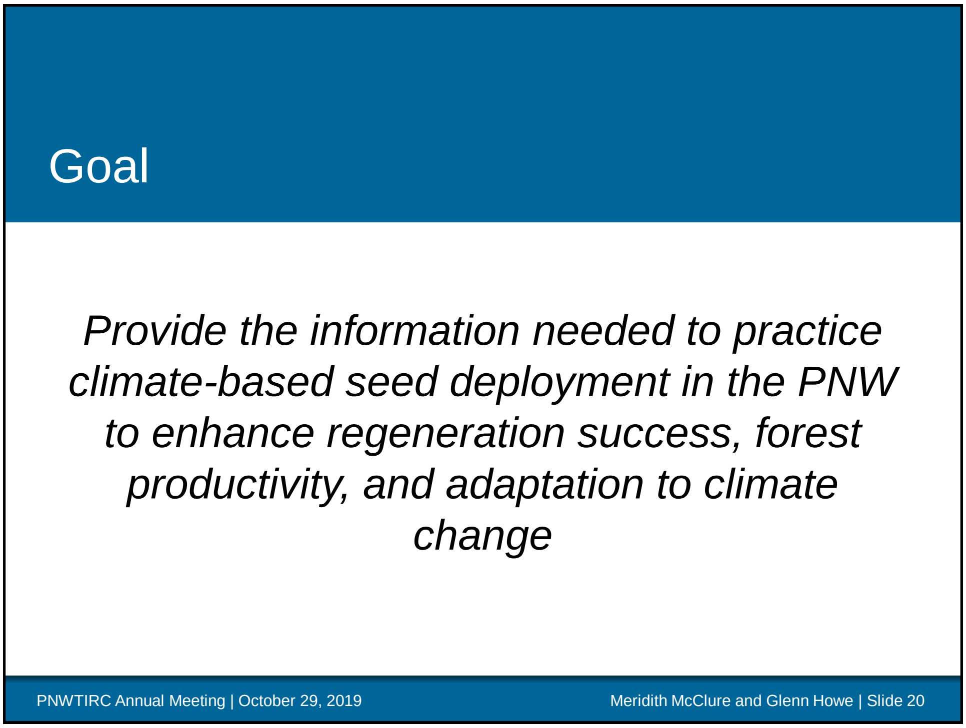
We propose a focal zone approach



Assisted migration

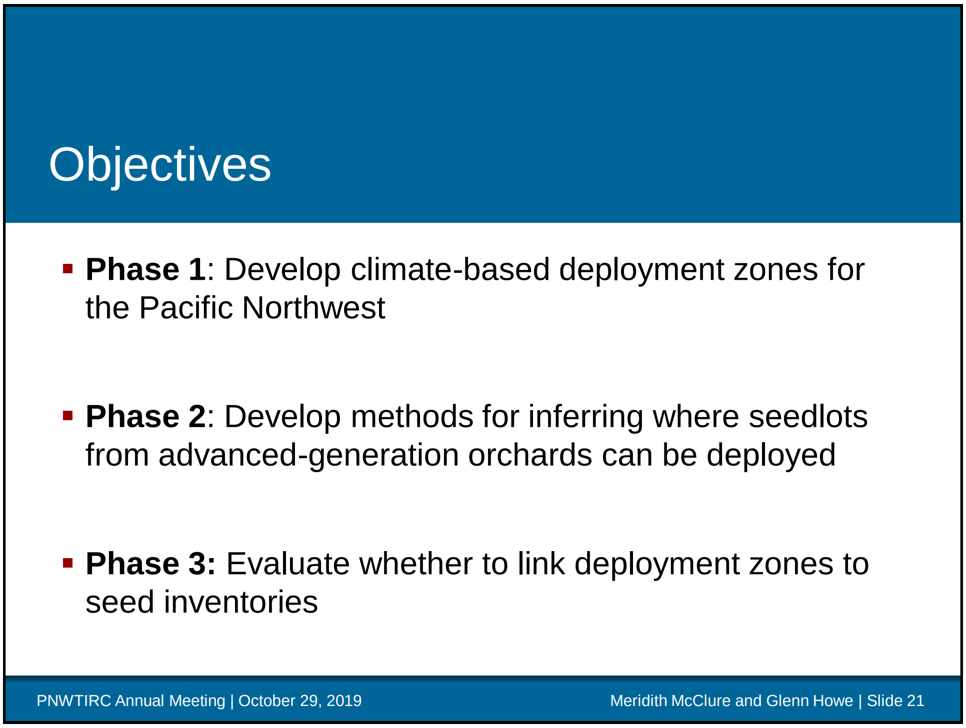
- Assisted migration will be necessary to maintain the health and productivity of forests
- **Assisted migration**: human facilitation of migration by planting tree seed or seedlings in areas where they would be unable to migrate naturally
- **Species-level assisted migration**: movement of a species outside its current range
- **Population-level assisted migration:** movement of a population within the species’ current range

Goal and Objectives



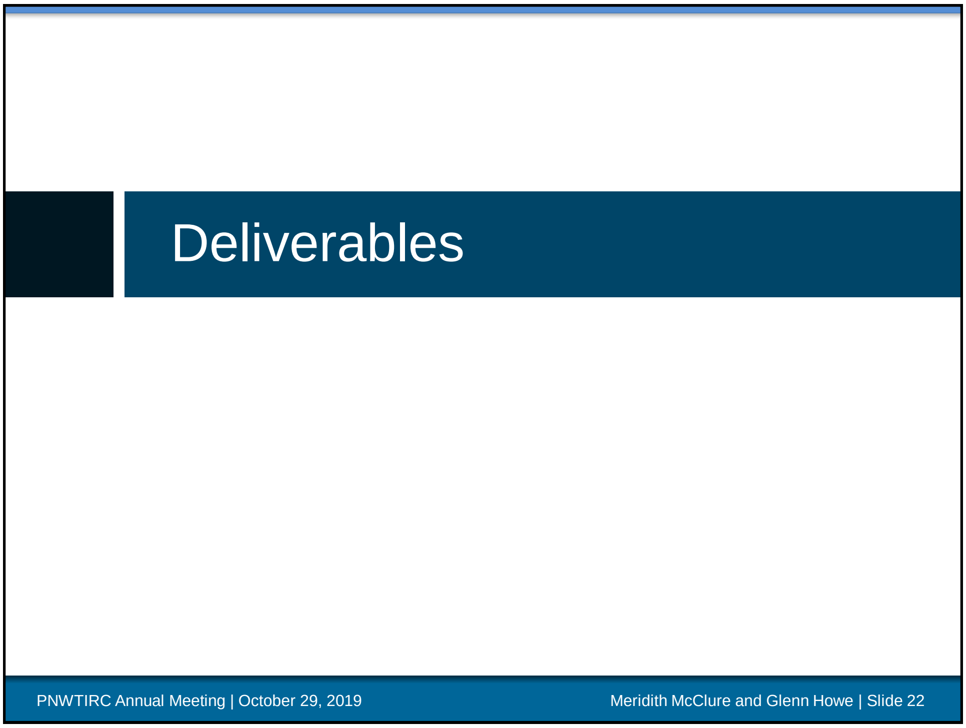
Goal

Provide the information needed to practice climate-based seed deployment in the PNW to enhance regeneration success, forest productivity, and adaptation to climate change

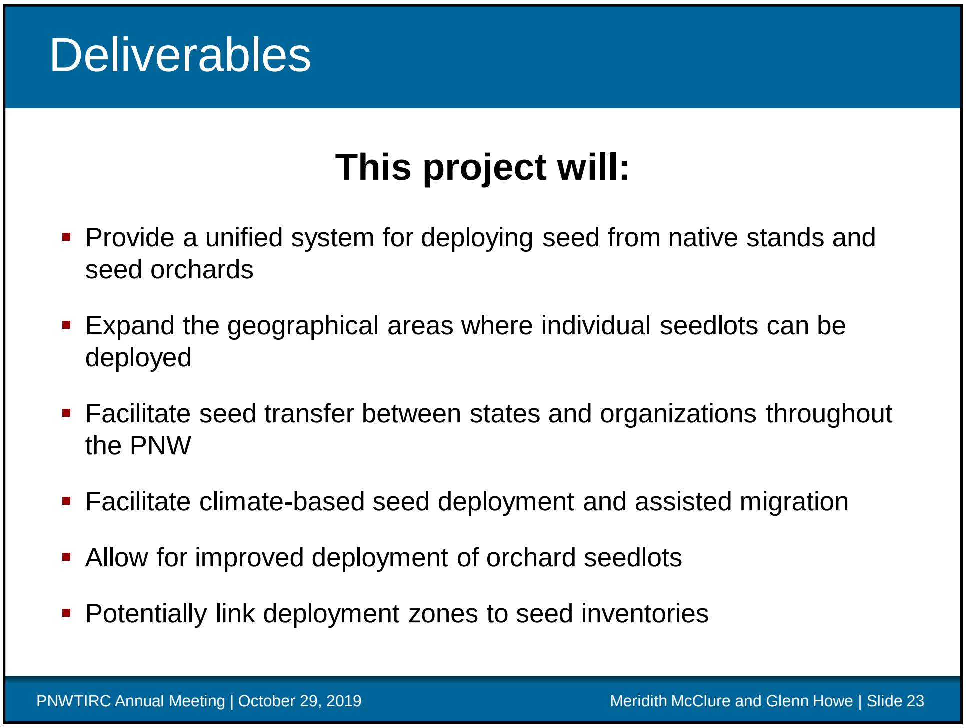


Objectives

- **Phase 1**: Develop climate-based deployment zones for the Pacific Northwest
- **Phase 2**: Develop methods for inferring where seedlots from advanced-generation orchards can be deployed
- **Phase 3:** Evaluate whether to link deployment zones to seed inventories



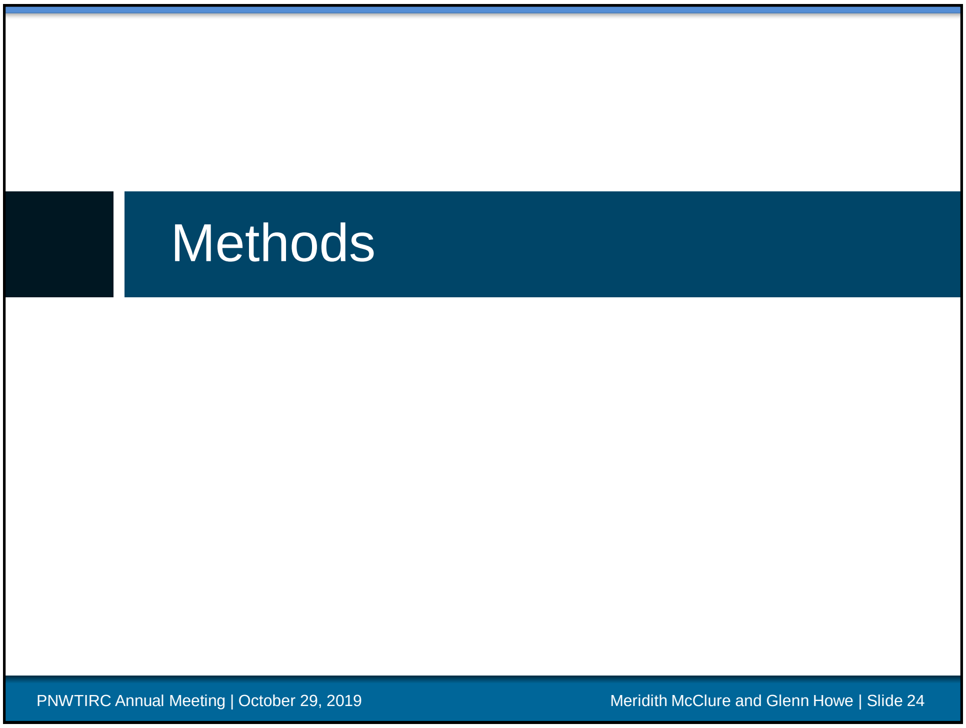
Deliverables



Deliverables

This project will:

- Provide a unified system for deploying seed from native stands and seed orchards
- Expand the geographical areas where individual seedlots can be deployed
- Facilitate seed transfer between states and organizations throughout the PNW
- Facilitate climate-based seed deployment and assisted migration
- Allow for improved deployment of orchard seedlots
- Potentially link deployment zones to seed inventories



Methods

Methods: Phase 1

Phase 1: *Develop climate-based deployment zones for the Pacific Northwest*

- Identify zone sets of interest to include
- Characterize climate variability within existing deployment zones
- Determine which deployment zones are climatically similar (based on current and future climates)
- Deliver this information to forest managers to be used for current seed transfer and assisted migration

Identify zone sets of interest to include

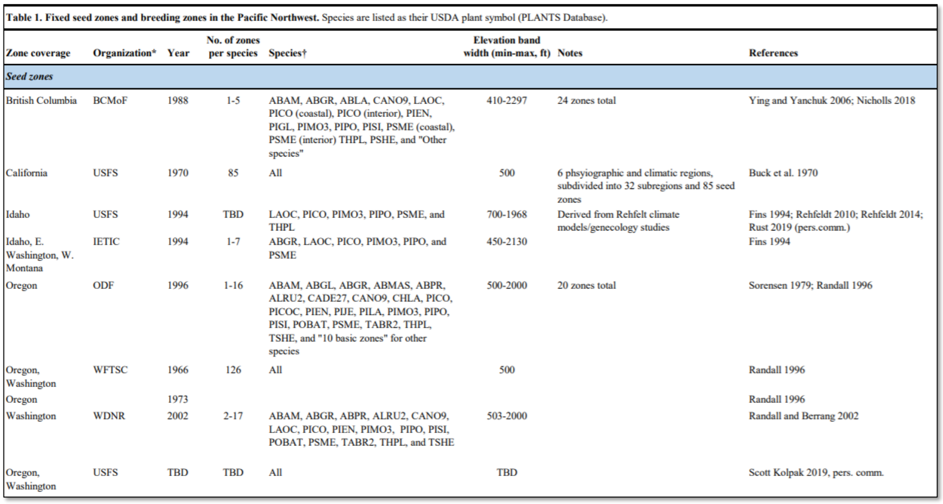
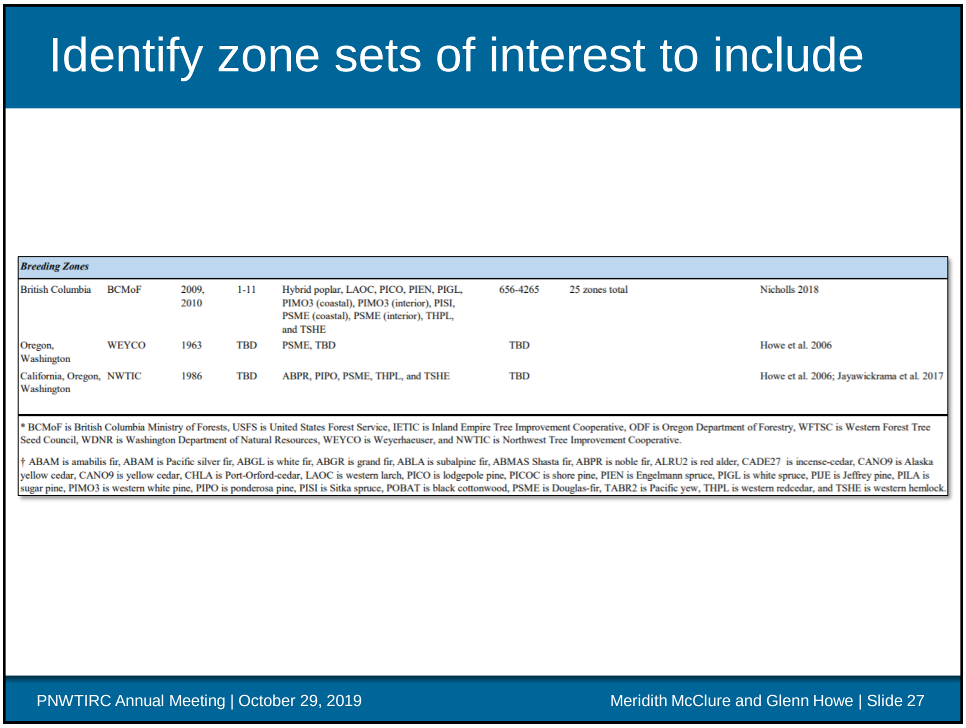


Table 1. Fixed seed zones and breeding zones in the Pacific Northwest. Species are listed as their USDA plant symbol (PLANTS Database).

Zone coverage	Organization*	Year	No. of zones per species	Species†	Elevation band width (min-max, ft)	Notes	References
Seed zones							
British Columbia	BCMoF	1988	1-5	ABAM, ABGR, ABLA, CANO9, LAOC, PICO (coastal), PICO (interior), PIEN, PIGL, PIMO3, PIPO, PISI, PSME (coastal), PSME (interior) THPL, PSHE, and "Other species"	410-2297	24 zones total	Ying and Yanchuk 2006; Nicholls 2018
California	USFS	1970	85	All	500	6 phsyiographic and climatic regions, subdivided into 32 subregions and 85 seed zones	Buck et al. 1970
Idaho	USFS	1994	TBD	LAOC, PICO, PIMO3, PIPO, PSME, and THPL	700-1968	Derived from Rehfelt climate models/genecology studies	Fins 1994; Rehfeldt 2010; Rehfeldt 2014; Rust 2019 (pers.comm.)
Idaho, E. Washington, W. Montana	IETIC	1994	1-7	ABGR, LAOC, PICO, PIMO3, PIPO, and PSME	450-2130		Fins 1994
Oregon	ODF	1996	1-16	ABAM, ABGL, ABGR, ABMAS, ABPR, ALRU2, CADE27, CANO9, CHLA, PICO, PICOC, PIEN, PIJE, PILA, PIMO3, PIPO, PISI, POBAT, PSME, TABR2, THPL, TSHE, and "10 basic zones" for other species	500-2000	20 zones total	Sorensen 1979; Randall 1996
Oregon, Washington	WFTSC	1966	126	All	500		Randall 1996
Oregon		1973					Randall 1996
Washington	WDNR	2002	2-17	ABAM, ABGR, ABPR, ALRU2, CANO9, LAOC, PICO, PIEN, PIMO3, PIPO, PISI, POBAT, PSME, TABR2, THPL, and TSHE	503-2000		Randall and Berrang 2002
Oregon, Washington	USFS	TBD	TBD	All	TBD		Scott Kolpak 2019, pers. comm.



Identify zone sets of interest to include

Breeding Zones							
British Columbia	BCMoF	2009, 2010	1-11	Hybrid poplar, LAOC, PICO, PIEN, PIGL, PIMO3 (coastal), PIMO3 (interior), PISI, PSME (coastal), PSME (interior), THPL, and TSHE	656-4265	25 zones total	Nicholls 2018
Oregon, Washington	WEYCO	1963	TBD	PSME, TBD	TBD		Howe et al. 2006
California, Oregon, Washington	NWTIC	1986	TBD	ABPR, PIPO, PSME, THPL, and TSHE	TBD		Howe et al. 2006; Jayawickrama et al. 2017

* BCMoF is British Columbia Ministry of Forests, USFS is United States Forest Service, IETIC is Inland Empire Tree Improvement Cooperative, ODF is Oregon Department of Forestry, WFTSC is Western Forest Tree Seed Council, WDNR is Washington Department of Natural Resources, WEYCO is Weyerhaeuser, and NWTIC is Northwest Tree Improvement Cooperative.

† ABAM is amabilis fir, ABAM is Pacific silver fir, ABGL is white fir, ABGR is grand fir, ABLA is subalpine fir, ABMAS Shasta fir, ABPR is noble fir, ALRU2 is red alder, CADE27 is incense-cedar, CANO9 is Alaska yellow cedar, CANO9 is yellow cedar, CHLA is Port-Orford-cedar, LAOC is western larch, PICO is lodgepole pine, PICOC is shore pine, PIEN is Engelmann spruce, PIGL is white spruce, PIJE is Jeffrey pine, PILA is sugar pine, PIMO3 is western white pine, PIPO is ponderosa pine, PISI is Sitka spruce, POBAT is black cottonwood, PSME is Douglas-fir, TABR2 is Pacific yew, THPL is western redcedar, and TSHE is western hemlock.

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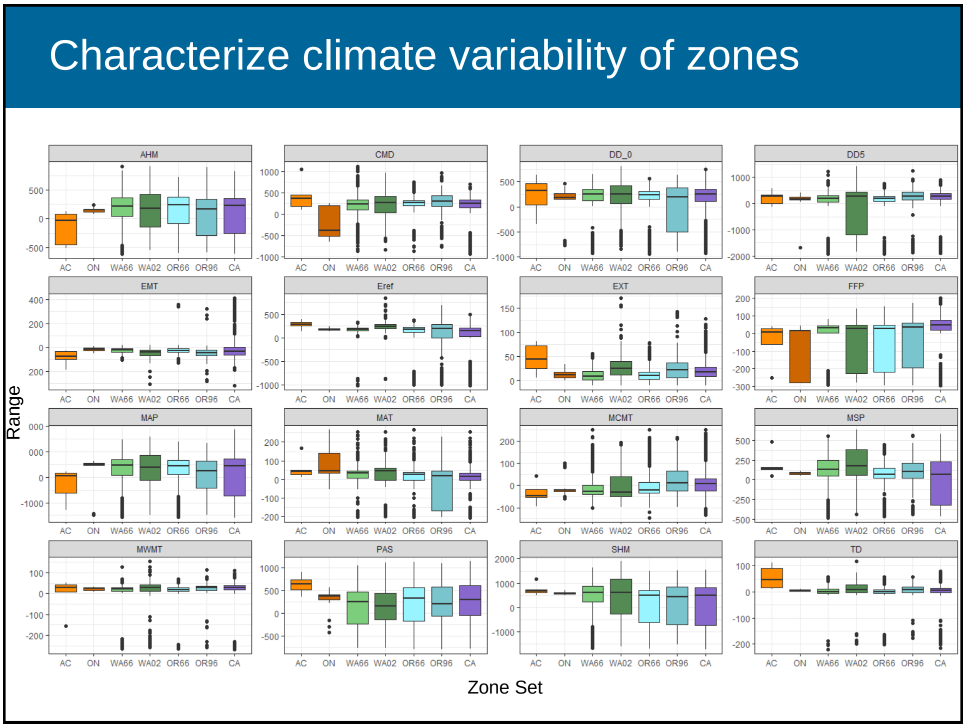


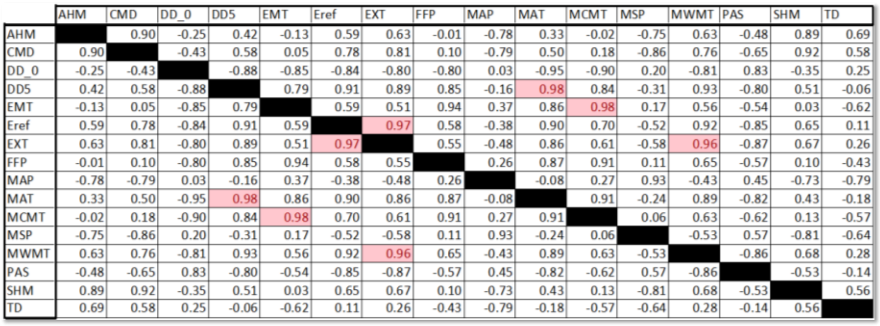
Characterize climate variability of zones

Characterize climate variability of deployment zones

Further analyses of these data will yield:

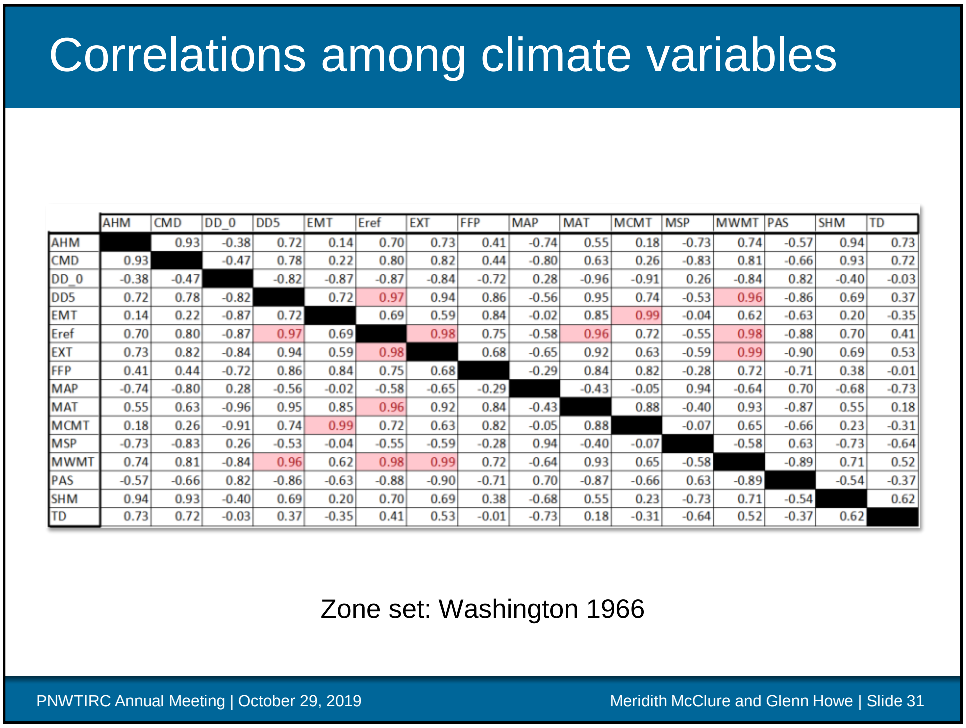
- Inferred transfer limits for each zone (16 climate variables)
- Transfer limit summaries by organization (e.g., zone set), region, species, and elevation
- Information useful for selecting focal climate variables
  - *Correlations among climate variables by zone*
  - *Partitioning of climate variation – among vs within zones*

Correlations among climate variables



	AHM	CMD	DD_0	DD5	EMT	Eref	EXT	FFP	MAP	MAT	MCMT	MSP	MWMT	PAS	SHM	TD
AHM	■	0.90	-0.25	0.42	-0.13	0.59	0.63	-0.01	-0.78	0.33	-0.02	-0.75	0.63	-0.48	0.89	0.69
CMD	0.90	■	-0.43	0.58	0.05	0.78	0.81	0.10	-0.79	0.50	0.18	-0.86	0.76	-0.65	0.92	0.58
DD_0	-0.25	-0.43	■	-0.88	-0.85	-0.84	-0.80	-0.80	0.03	-0.95	-0.90	0.20	-0.81	0.83	-0.35	0.25
DD5	0.42	0.58	-0.88	■	0.79	0.91	0.89	0.85	-0.16	0.98	0.84	-0.31	0.93	-0.80	0.51	-0.06
EMT	-0.13	0.05	-0.85	0.79	■	0.59	0.51	0.94	0.37	0.86	0.98	0.17	0.56	-0.54	0.03	-0.62
Eref	0.59	0.78	-0.84	0.91	0.59	■	0.97	0.58	-0.38	0.90	0.70	-0.52	0.92	-0.85	0.65	0.11
EXT	0.63	0.81	-0.80	0.89	0.51	0.97	■	0.55	-0.48	0.86	0.61	-0.58	0.96	-0.87	0.67	0.26
FFP	-0.01	0.10	-0.80	0.85	0.94	0.58	0.55	■	0.26	0.87	0.91	0.11	0.65	-0.57	0.10	-0.43
MAP	-0.78	-0.79	0.03	-0.16	0.37	-0.38	-0.48	0.26	■	-0.08	0.27	0.93	-0.43	0.45	-0.73	-0.79
MAT	0.33	0.50	-0.95	0.98	0.86	0.90	0.86	0.87	-0.08	■	0.91	-0.24	0.89	-0.82	0.43	-0.18
MCMT	-0.02	0.18	-0.90	0.84	0.98	0.70	0.61	0.91	0.27	0.91	■	0.06	0.63	-0.62	0.13	-0.57
MSP	-0.75	-0.86	0.20	-0.31	0.17	-0.52	-0.58	0.11	0.93	-0.24	0.06	■	-0.53	0.57	-0.81	-0.64
MWMT	0.63	0.76	-0.81	0.93	0.56	0.92	0.96	0.65	-0.43	0.89	0.63	-0.53	■	-0.86	0.68	0.28
PAS	-0.48	-0.65	0.83	-0.80	-0.54	-0.85	-0.87	-0.57	0.45	-0.82	-0.62	0.57	-0.86	■	-0.53	-0.14
SHM	0.89	0.92	-0.35	0.51	0.03	0.65	0.67	0.10	-0.73	0.43	0.13	-0.81	0.68	-0.53	■	0.56
TD	0.69	0.58	0.25	-0.06	-0.62	0.11	0.26	-0.43	-0.79	-0.18	-0.57	-0.64	0.28	-0.14	0.56	■

Zone set: Oregon 1966



Correlations among climate variables

	AHM	CMD	DD_0	DD5	EMT	Eref	EXT	FFP	MAP	MAT	MCMT	MSP	MWMT	PAS	SHM	TD
AHM		0.93	-0.38	0.72	0.14	0.70	0.73	0.41	-0.74	0.55	0.18	-0.73	0.74	-0.57	0.94	0.73
CMD	0.93		-0.47	0.78	0.22	0.80	0.82	0.44	-0.80	0.63	0.26	-0.83	0.81	-0.66	0.93	0.72
DD_0	-0.38	-0.47		-0.82	-0.87	-0.87	-0.84	-0.72	0.28	-0.96	-0.91	0.26	-0.84	0.82	-0.40	-0.03
DD5	0.72	0.78	-0.82		0.72	0.97	0.94	0.86	-0.56	0.95	0.74	-0.53	0.96	-0.86	0.69	0.37
EMT	0.14	0.22	-0.87	0.72		0.69	0.59	0.84	-0.02	0.85	0.99	-0.04	0.62	-0.63	0.20	-0.35
Eref	0.70	0.80	-0.87	0.97	0.69		0.98	0.75	-0.58	0.96	0.72	-0.55	0.98	-0.88	0.70	0.41
EXT	0.73	0.82	-0.84	0.94	0.59	0.98		0.68	-0.65	0.92	0.63	-0.59	0.99	-0.90	0.69	0.53
FFP	0.41	0.44	-0.72	0.86	0.84	0.75	0.68		-0.29	0.84	0.82	-0.28	0.72	-0.71	0.38	-0.01
MAP	-0.74	-0.80	0.28	-0.56	-0.02	-0.58	-0.65	-0.29		-0.43	-0.05	0.94	-0.64	0.70	-0.68	-0.73
MAT	0.55	0.63	-0.96	0.95	0.85	0.96	0.92	0.84	-0.43		0.88	-0.40	0.93	-0.87	0.55	0.18
MCMT	0.18	0.26	-0.91	0.74	0.99	0.72	0.63	0.82	-0.05	0.88		-0.07	0.65	-0.66	0.23	-0.31
MSP	-0.73	-0.83	0.26	-0.53	-0.04	-0.55	-0.59	-0.28	0.94	-0.40	-0.07		-0.58	0.63	-0.73	-0.64
MWMT	0.74	0.81	-0.84	0.96	0.62	0.98	0.99	0.72	-0.64	0.93	0.65	-0.58		-0.89	0.71	0.52
PAS	-0.57	-0.66	0.82	-0.86	-0.63	-0.88	-0.90	-0.71	0.70	-0.87	-0.66	0.63	-0.89		-0.54	-0.37
SHM	0.94	0.93	-0.40	0.69	0.20	0.70	0.69	0.38	-0.68	0.55	0.23	-0.73	0.71	-0.54		0.62
TD	0.73	0.72	-0.03	0.37	-0.35	0.41	0.53	-0.01	-0.73	0.18	-0.31	-0.64	0.52	-0.37	0.62	

Zone set: Washington 1966

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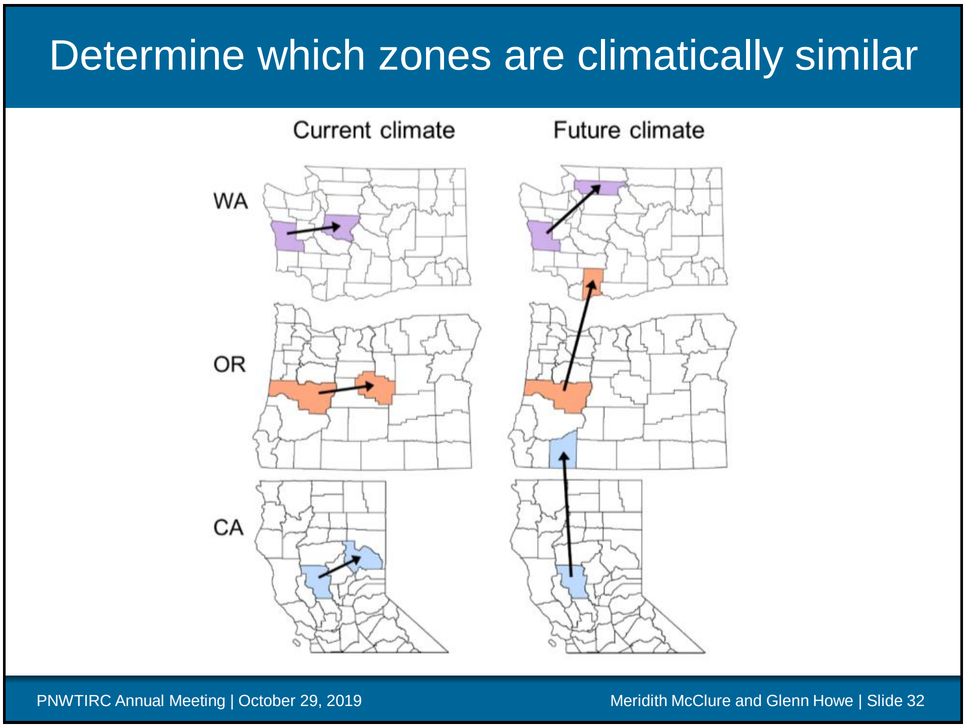

Determine which zones are climatically similar

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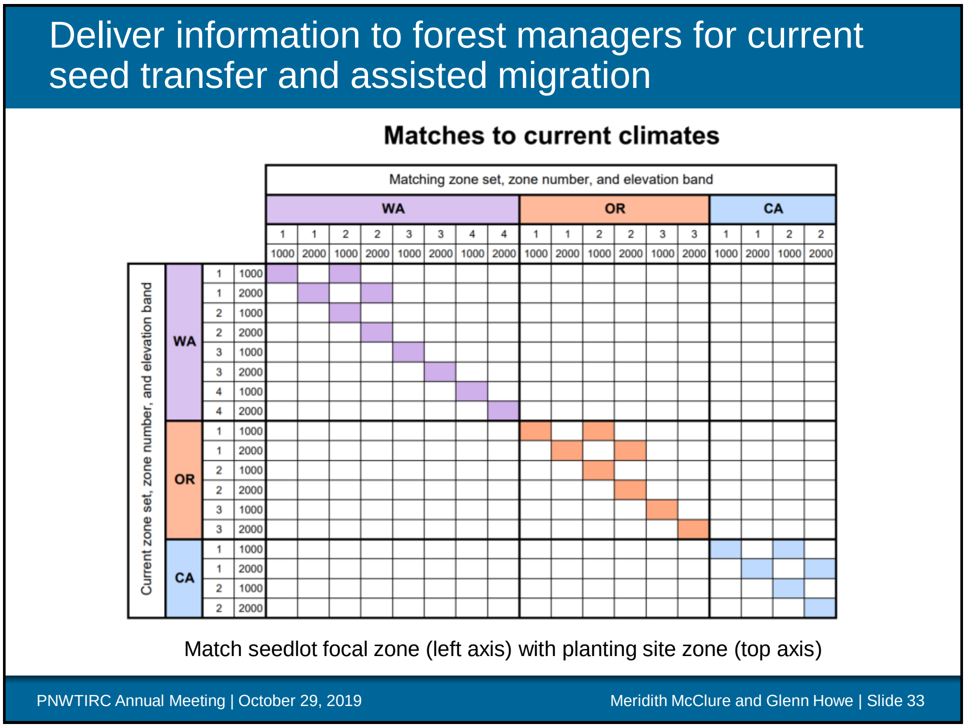

Deliver information to forest managers for current seed transfer and assisted migration

Matches to current climates

Current zone set, zone number, and elevation band			Matching zone set, zone number, and elevation band																	
			WA								OR						CA			
			1	1	2	2	3	3	4	4	1	1	2	2	3	3	1	1	2	2
			1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000
WA	1	1000	■		■															
	1	2000		■		■														
	2	1000			■															
	2	2000				■														
	3	1000					■													
	3	2000						■												
	4	1000							■											
	4	2000								■										
OR	1	1000									■		■							
	1	2000										■		■						
	2	1000											■							
	2	2000												■						
	3	1000													■					
	3	2000														■				
CA	1	1000															■		■	
	1	2000																■		■
	2	1000																	■	
	2	2000																		■

Match seedlot focal zone (left axis) with planting site zone (top axis)

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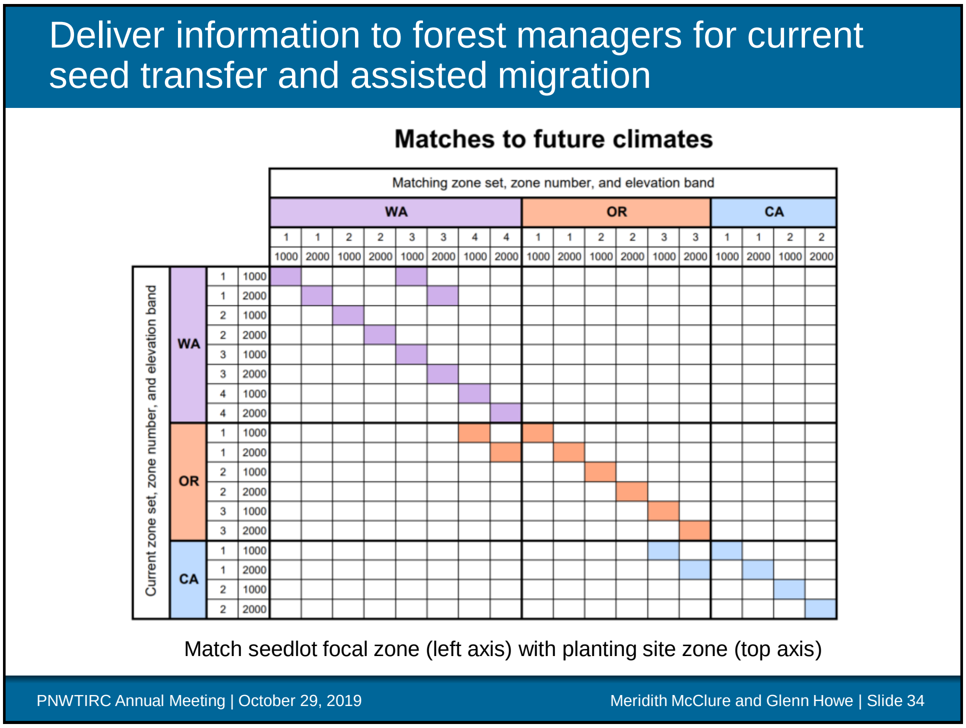

Deliver information to forest managers for current seed transfer and assisted migration

Matches to future climates

Current zone set, zone number, and elevation band			Matching zone set, zone number, and elevation band																	
			WA								OR						CA			
			1	1	2	2	3	3	4	4	1	1	2	2	3	3	1	1	2	2
			1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000	1000	2000
WA	1	1000	■				■													
	1	2000		■				■												
	2	1000			■															
	2	2000				■														
	3	1000					■													
	3	2000						■												
	4	1000							■											
	4	2000								■										
OR	1	1000							■		■									
	1	2000								■		■								
	2	1000											■							
	2	2000												■						
	3	1000													■					
	3	2000														■				
CA	1	1000													■		■			
	1	2000														■		■		
	2	1000																	■	
	2	2000																		■

Match seedlot focal zone (left axis) with planting site zone (top axis)

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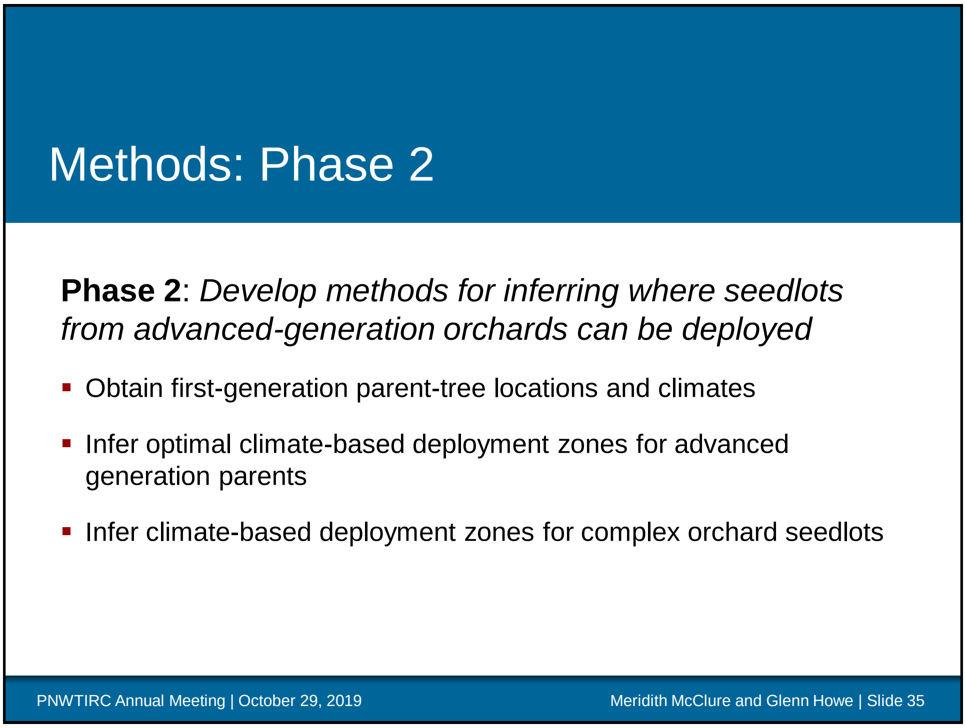

Methods: Phase 2

Phase 2: *Develop methods for inferring where seedlots from advanced-generation orchards can be deployed*

- Obtain first-generation parent-tree locations and climates
- Infer optimal climate-based deployment zones for advanced generation parents
- Infer climate-based deployment zones for complex orchard seedlots

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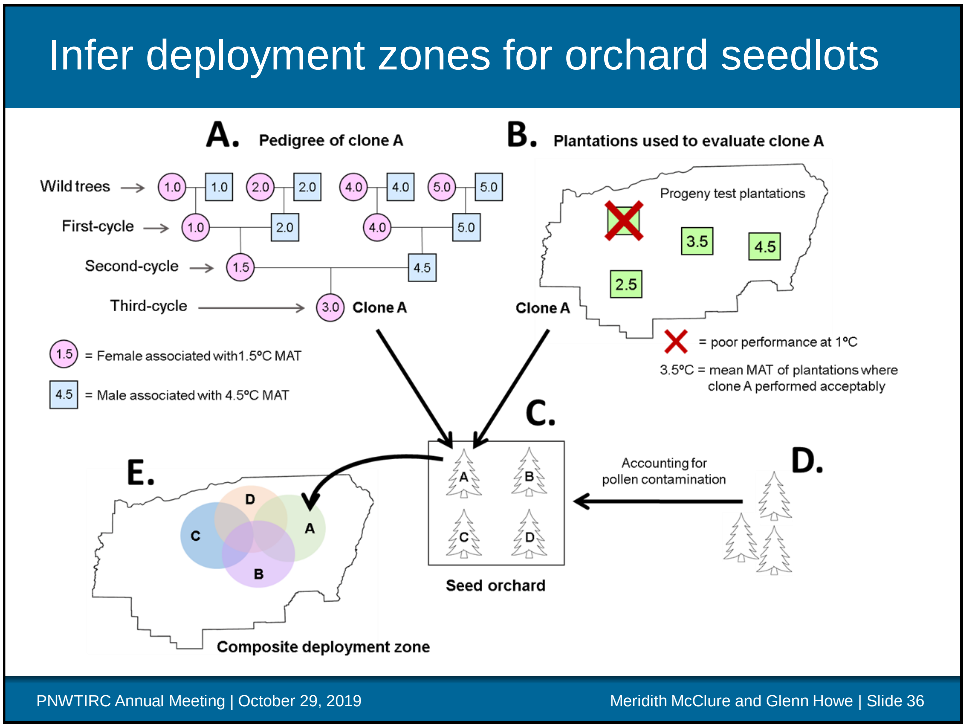

Infer deployment zones for orchard seedlots

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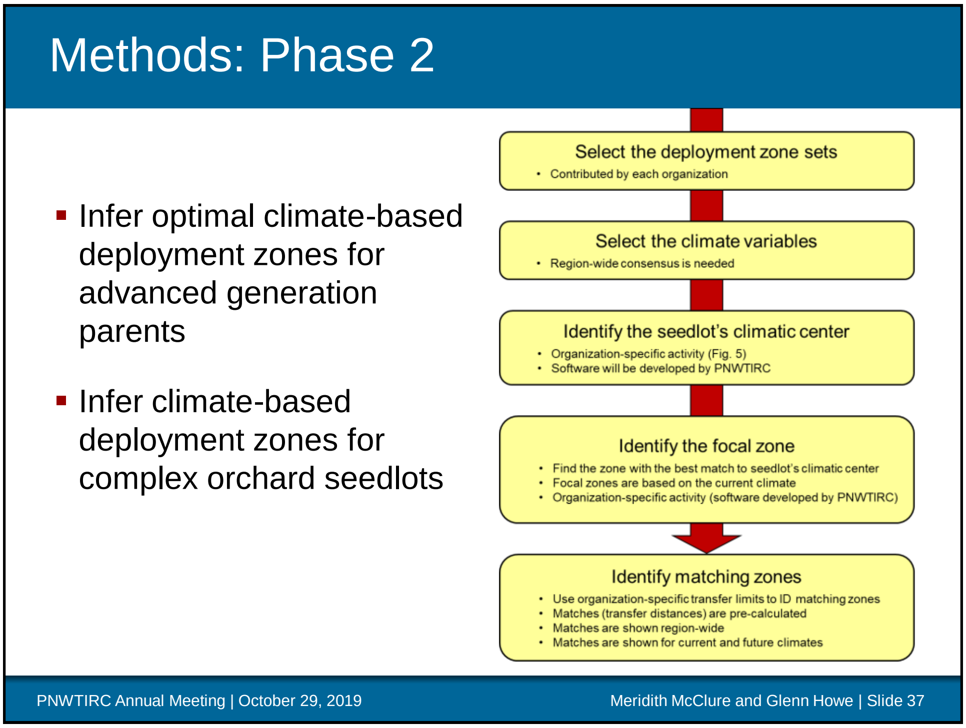


Methods: Phase 2

- Infer optimal climate-based deployment zones for advanced generation parents
- Infer climate-based deployment zones for complex orchard seedlots

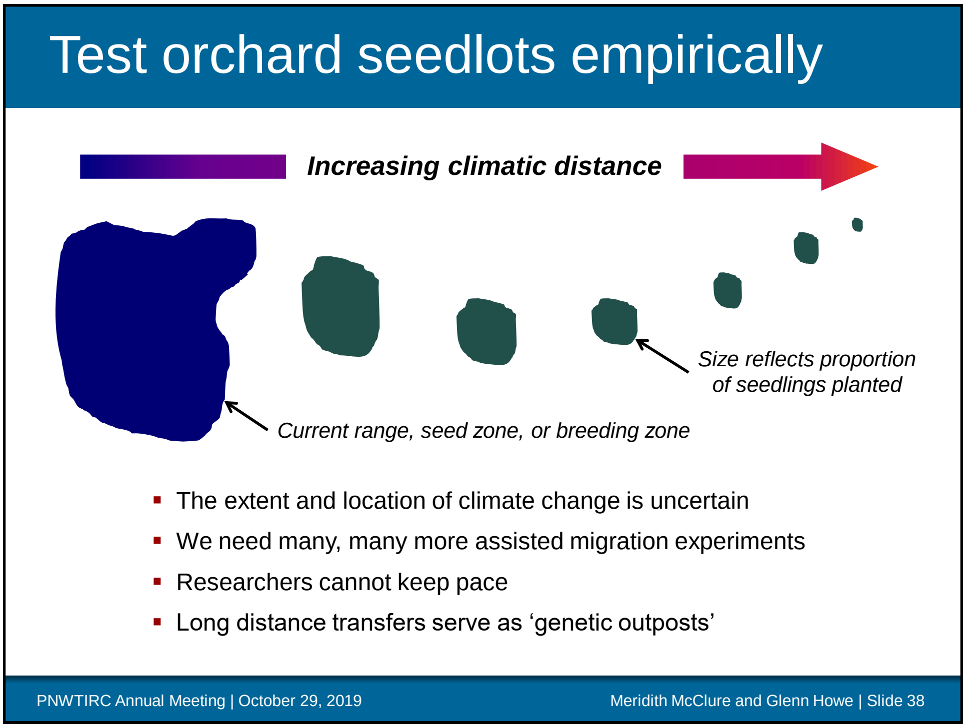
Select the deployment zone sets
- Contributed by each organization

Select the climate variables
- Region-wide consensus is needed

Identify the seedlot's climatic center
- Organization-specific activity (Fig. 5)
- Software will be developed by PNWTIRC

Identify the focal zone
- Find the zone with the best match to seedlot's climatic center
- Focal zones are based on the current climate
- Organization-specific activity (software developed by PNWTIRC)

Identify matching zones
- Use organization-specific transfer limits to ID matching zones
- Matches (transfer distances) are pre-calculated
- Matches are shown region-wide
- Matches are shown for current and future climates



Test orchard seedlots empirically

Increasing climatic distance

Size reflects proportion of seedlings planted

Current range, seed zone, or breeding zone

- The extent and location of climate change is uncertain
- We need many, many more assisted migration experiments
- Researchers cannot keep pace
- Long distance transfers serve as 'genetic outposts'

Methods: Phase 3

Phase 3: *Evaluate whether to link deployment zones to seed inventories*

- Work with stakeholders to determine whether we should link deployment zones to seed inventories
- If desired, determine how we would link deployment zones to seed inventories

Linking deployment zones to seed inventories

Forest Seedling Network is a marketplace that connects independent buyers and sellers.

Find the correct seedlings for your planting site from postings made by Growers and Other landowners.

Find vendor assistance services and contractors from the Vendor Service Directory.
Vendors create your own listing here

Growers and Landowners post your seedlings for sale to other landowners.

Find forest management related advice, events, maps and other resources.

APPENDIX I

PNWTIRC Activities 2018-2019

Publications and posters

Howe, G.T., Jayawickrama, K.J., Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S. 2019. An Axiom SNP genotyping array for Douglas-fir. *BMC Genomics, **21**:9*.

St.Clair, J.B., Howe, G.T., Kling, J.G. 2019. The 1912 Douglas-fir heredity study: Long-term effects of climatic transfer distance on growth and survival. *Journal of Forestry*, **118**(1): 1-13.

McClure, M., DeBell, J., and Howe, G.T. 2019. Climate-based seed deployment zones for the Pacific Northwest. Poster presented at: Northwest Climate Conference, 8 October, 2019, Portland, Oregon.

Collaborations and grants

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

PNWTIRC Annual Meeting Minutes

Oct 29, 2019

Clackamas Fire Station #3, Oak Grove, OR

PNWTIRC attendees
Michael Crawford – Bureau of Land Management
Darian Domes – Cascade Timber Consulting
Florian Deisenhofer – Hancock Forest Management
Keith Jayawickrama – NWTIC, OSU
Dan Cress – Olympic Resource Management
Glenn Howe – PNWTIRC, OSU
Jennifer Kling – PNWTIRC, OSU
Anna Magnuson – PNWTIRC, OSU
Sara Lipow – Roseburg Forest Products
Margaret Banks – Stimson Lumber Co.
Jeff DeBell – Washington State DNR
Brian Baltunis – Weyerhaeuser
Amy Jamruszka – Weyerhaeuser
David Barker – Rayonier Forest Resources
David Stafford – Olympic Resource Management
Doug Maguire – CIPS, OSU
Lauren Magalska – Port Blakely Tree Farms
Liam Gilson – CIPS, OSU
Marc Rust – Inland Emptire Tree Improvement Cooperative
Meridith McClure – PNWTIRC, OSU
Stu Olshevski – Washington State DNR
Brianna McTeague – Weyerhaeuser

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

II. PNWTIRC highlights for 2018-2019. Glenn Howe presented an overview of major accomplishments for 2018-2019

1. ***Administration and members***
   - Director - Glenn Howe
   - Research Coordinator - Anna Magnuson
   - Research Scientist - Jennifer Kling
   - Graduate students - Oguz Urhan
   - Policy/Technical Committee Chair - Lauren Magalska

2. ***Publications and posters by PNWTIRC personnel during 2018-2019***
   - Howe, G.T., Jayawickrama, K.J., Kolpak, S.E., Kling, J.G., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S. 2019. A high-density Axiom genotyping array for Douglas-fir. BMC Genomics (submitted).

- St.Clair, J.B., Howe, G.T., Kling, J.G. 2019. The 1912 Douglas-fir heredity study: Long-term effects of climatic transfer distance on growth and survival. Journal of Forestry, **118**(1): 1-13.
- McClure, M., DeBell, J., and Howe, G.T. 2019. Climate-based seed deployment zones for the Pacific Northwest. Poster presented at: Northwest Climate Conference, 8 October, 2019, Portland, Oregon.

3. ***Collaborations and grants during 2018-2019***
    - **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.

- Genomic selection workplan: A joint project between the PNWTIRC and NWTIC. Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak
- Low-density SNP genotyping in Douglas-fir: A joint project between the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and the Center for Intensive Plantation Silviculture (CIPS). Glenn Howe, Jennifer Kling, Doug Maguire.
- Climate-based seed deployment zones for the Pacific Northwest. Glenn Howe and Meridith McClure, PNWTIRC.

IV. PNWTIRC research presentations

1. ***Update: Why does Douglas-fir grow much better in New Zealand?*** *Liam Gilson, Doug Maguire*
2. ***Routine marker genotyping in Douglas-fir: From SSRs to SNPs.*** *Anna Magnuson, Glenn Howe*
3. ***Inland Emptire Tree Improvement Cooperative.*** *Marc Rust*
4. ***PNWTIRC/NWTIC genomic selection research, preliminary results from the NARA plantation.*** *Jennifer Kling*
5. ***Climate-based seed deployment for the PNW.*** *Meridith McClure, Glenn Howe*

V. Budget. Glenn Howe presented the budget for FY 2018-2019. The proposed budget for FY 2019-2020 was also presented. A motion to approve the budgets was offered by Brian Baltunis, seconded by Margaret Banks, and approved by unanimous voice vote.

VI. PNWTIRC Policy/Technical Committee Chair. Margaret Banks was nominated as new Policy/Technical Committee Chair by Lauren Magalska. The nomination was seconded and approved by unanimous voice vote.

VII. PNWTIRC annual meeting. Next year's meeting will be held ***Tuesday, October 13th, 2020.***

VIII. Meeting adjourned. The meeting adjourned about 3:30 pm.

APPENDIX III

Financial Statement 2018-2019

PNWTIRC Financial Support for Fiscal Year 2018-2019

Regular members[1]	110,000
Associate members[1]	5,000
Contracts	2,500
Forest Research Laboratory, Oregon State University[2]	128,192
Total	245,692

[1] Each Regular Member contributed $10,000 and each Associate Member contributed $5,000 excluding in-kind contributions of labor, supplies, etc.

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