PNWTIRC

Pacific Northwest Tree Improvement Research Cooperative

Annual Report 2005-2006

Oregon State University

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PNWTIRC

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About the PNWTIRC

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

Our Mission is to:

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

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

Director: Glenn Howe

Assistant Director: Marilyn Cherry

Graduate Student: Vikas Vikram

Contents

Highlights of 2005-06

- PNWTIRC personnel published one journal article and one PNWTIRC Report, and gave seven research presentations.

- We welcomed one new member, Cascade Timber Consulting, Inc. Bill Marshall will serve as their representative on the Policy/Technical Committee.

- In the *Wood Quality Study*, we supervised destructive sampling (thinning) of progeny tests, gathered field measurements, milled boards from our study logs, tested stiffness of the kiln-dried lumber, and conducted preliminary data analyses.

- We carried out crown management in the *Miniaturized Seed Orchard Study* at Plum Creek to control tree height and remove rootstock branches.

- We continued our pruning treatments at the Roseburg Forest Products Regeneration Center Vaughn miniaturized seed orchard, and counted flowers in the spring of 2006.

- In June of 2006, we held a demonstration of lumber stiffness testing in the OSU Department of Wood Science and Engineering laboratory. This demonstration was held in conjunction with the PNWTIRC 2005-06 Annual Meeting.

- The PNWTIRC is participating in the planning and implementation of a National Science Foundation Center for Advanced Forestry Systems (CAFS) with North Carolina State University, Virginia Tech, Purdue, and the Tree Biosafety and Genomics Research Cooperative at OSU.

- We co-hosted the 2005 Annual Meeting of the Western Forest Genetics Association in Corvallis, OR. Our co-hosts were the USFS Pacific Northwest Research Station and the Northwest Tree Improvement Cooperative.

Message from the Director

Wood quality is now the major focus of our activities. During the past year, we initiated a major collaborative project to understand the genetics of Douglas-fir wood stiffness, and to develop recommendations for incorporating wood stiffness into operational breeding programs. This research is partly driven by the concern that shorter rotations will lead to higher proportions of juvenile wood in the harvested trees, and thus lower quality wood in the future. This is because juvenile wood is less stiff than mature wood, and has other undesirable characteristics. There is a renewed interest in wood quality among geneticists and silviculturists in the Pacific Northwest, southeastern U.S., and southern hemisphere. This change seems to be driven by the availability of new tools to obtain reliable, indirect estimates of wood stiffness and other wood properties, and the increasing realization that selection for volume growth alone may adversely affect wood quality and value. These new technologies include acoustic tools that can be used to predict the stiffness of standing trees or logs, and near-infrared spectroscopy (NIR) which can be used to rapidly predict a large number of fundamental wood properties.

In addition to wood quality, we are continuing to study the design and management of miniaturized seed orchards (MSOs) for Douglas-fir. Our main MSO experiment at the Plum Creek Seed Orchard Complex is developing nicely, and will be the focus of much research once the orchard trees become older and large enough to begin testing operational crown management and flower stimulation treatments.

We were very pleased that a new Center for Advanced Forestry Systems (CAFS) received funding from the National Science Foundation to plan a new nationwide forestry research partnership linking industry and universities under the prestigious NSF Industry-University Cooperative Research Center (I/UCRC) program. This grant is being used to support CAFS planning meetings that will bring together forest geneticists and silviculturists from across the country. Our CAFS partners include the Tree Biosafety and Genomics Research Cooperative at OSU, Forest Nutrition Cooperative at North Carolina State University and Virginia Tech, Loblolly Pine Growth and Yield Research Cooperative at Virginia

Tech, and the Hardwood Tree Improvement and Regeneration Center at Purdue. We anticipate joining ranks with about 50 NSF-sponsored centers that represent the entire spectrum of technological fields across the nation. We are now in the planning phase, and will soon submit a full proposal to NSF to fund the Center. If we successfully negotiate this second hurdle, the PNWTIRC will receive NSF funds to conduct research on topics aimed at linking genes, genomes, and physiological processes to silvicultural performance and value in forest stands. Because participation in the Center may require us to increase membership dues slightly, and update our Memorandum of Agreement, these are two topics that we expect to discuss and vote on during the next year. More information on the NSF I/UCRC program can be found at http://www.nsf.gov/eng/iip/iucrc/.

Another important highlight of the past year is the addition of a new member: Cascade Timber Consulting. Although Howard Dew was instrumental in getting CTC to join the cooperative, Bill Marshall has become their PNWTIRC representative now that Howard has retired. We look forward to working with CTC and other new members in the coming years.

Glenn Howe

Research Overview

We currently have two major research projects: (1) genetics of Douglas-fir wood stiffness and strength and (2) miniaturized seed orchards (MSOs). The *Wood Quality Study* was our most active research project during the past year. Both Marilyn Cherry (PNWTIRC Assistant Director) and Vikas Vikram (graduate student) were heavily involved in the planning, fieldwork, and data analyses associated with our wood quality research. The *Miniaturized Seed Orchard Study* required relatively little attention during the past year, mostly routine maintenance at the Plum Creek MSO and the initiation of a pruning experiment at the Roseburg Forest Products Vaughn seed orchard. The pruning experiment will continue during 2006-07, and research at the Plum Creek MSO will begin once the trees become older and large enough to begin operational crown management treatments in the summer of 2007.

Wood Quality Study

Our wood quality research combines a number of novel elements, including the (1) evaluation of new acoustic tools that can be used to obtain indirect estimates of wood stiffness on standing trees and logs, (2) comparisons of wood stiffness estimated indirectly from the acoustic tools with stiffness measured directly on lumber harvested from the same trees, (3) evaluation of wood stiffness of seed orchard parents and their progeny growing in genetic test plantations, and (4) discovery of genes associated with wood properties using genomic approaches. To accomplish these goals, we are collaborating with the Stand Management Cooperative (SMC) at the University of Washington (David Briggs, Director), scientists at the University of California at Davis (Dave Neale, professor), the Genetics Team at the USFS Pacific Northwest Research Station (Brad St. Clair, Team Leader), and Olympic Resource Management (Dan Cress, PNWTIRC representative). Some of the key questions we hope to answer are: "What are the potential genetic gains for wood stiffness?," "What are the most efficient approaches for improving wood stiffness in operational breeding programs?," "Can acoustic tools such as the Fibre-gen HM200 and ST300 be used to measure and select for wood stiffness in operational programs?," "Can we improve wood stiffness by simply measuring and selecting for increased wood specific gravity?," and "Will wood stiffness and specific gravity decline if we select and breed for volume growth alone?" We hope to have clear answers to these and other questions within the next one to two years. Our progress on the *Wood Quality Study* is described on page 11.

Miniaturized Seed Orchard Study

Miniaturized seed orchards (MSOs) are orchards in which the trees are planted at close spacings in clonal rows, and then maintained at a height of only 2 to 4 m (Sweet and Krugman 1977). We undertook three experiments which were designed to help us develop methods for establishing and managing miniaturized seed orchards of Douglas-fir. First, we tested flower stimulation techniques for very young grafts of Douglas-fir, resulting in a PNWTIRC Report entitled "Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*)" (Cherry et al 2006). This research demonstrated that male and female flowering can be stimulated on very young grafts using a combination of girdling and gibberellic acid (GA). Because the treated trees had higher mortality than the untreated trees, the best approach would be to delay flower stimulation until the grafts are 5 years old, i.e. just before their sixth growing season.

The second, largest experiment is a long-term test of alternative MSO designs that was established at the Plum Creek Seed Orchard Complex in western Oregon. We completed the grafting for this experiment in 2004, and the trees should be large enough to begin testing crown management treatments as early as the summer of 2007. Our progress on this experiment is described on page 19.

The third experiment is a pruning study that we initiated at the Roseburg Forest Products Vaughn Seed Orchard in the spring of 2005. This experiment is designed to determine the best time to prune the crowns of MSO trees. Pruning is needed to keep the trees small, but is also expected to reduce the number of cones (because flowers or cones may be removed in the process), thereby adversely affecting seed production. This study is based on the hypothesis that the timing (season and frequency) of crown pruning can be physiologically optimized to maximize seed production. This experiment is described on page 21. Eventually, we expect to test one or more of the best pruning treatments from this experiment at the Plum Creek MSO, in addition to other crown management treatments.

Technology Transfer

Workshops and Meetings

The PNWTIRC places a strong emphasis on transferring research results to tree improvement practitioners. Our technology transfer efforts include distribution of cooperative research reports, meetings with cooperators, annual meetings, and annual reports. In July 2005, the PNWTIRC co-organized a workshop on the Douglas-fir Genome Project with the University of California at Davis. We also co-hosted the 2006 Western Forest Genetics Association conference in Corvallis. This was the 50th anniversary of WFGA, and the theme of the meeting was Looking Back - Looking Ahead [see http://www.fsl.orst.edu/wfga/index_files/2005_Program.pdf].

Glenn Howe gave talks on the *Wood Quality Study* at both the Agenda 2020 Annual Meeting and at the Stand Management Cooperative Annual Meeting, and gave a presentation at the OSU Department of Horticulture entitled *Ecological genetics and breeding of Douglas-fir: merging traditional and genomic approaches in forest genetics*.

Demonstration of Wood Quality Testing

We held a laboratory demonstration in conjunction with the PNWTIRC 2006 Annual Meeting on June 20, 2006. We visited the OSU Department of Wood Science and Engineering laboratory, where Milo Clausen showed participants the drying kiln, and demonstrated some of the techniques we are using to test stiffness of dimensional lumber (Figure 1).

PNWTIRC Reports

We published a PNWTIRC Report entitled *Early flower stimulation in young miniaturized seed orchards*

Figure 1. Demonstration of lumber testing at the 2005-06 PNWTIRC Annual Meeting, OSU Dept. of Wood Science and Engineering. A force of 500 pounds is applied to the center of a supported beam, and lumber deflection is measured to estimate wood stiffness.

of Douglas-fir. Plant Breeding Reviews published a review paper entitled *Breeding Douglas-fir*. The citations for these papers can be found in Appendix 1.

National Science Foundation Center for Advanced Forestry Systems

The PNWTIRC is participating in the planning and implementation of a National Science Foundation Center for Advanced Forestry Systems (CAFS). CAFS received competitive funding from NSF to plan a new nationwide forestry research partnership linking industry and universities under their NSF Industry-University Cooperative Research Center (I/UCRC) program. The participating universities include OSU, North Carolina State University (NCSU), Virginia Tech, and Purdue. The participating research cooperatives include the PNWTIRC, Tree Biosafety and Genomics Research Cooperative at OSU, Forest Nutrition Cooperative at NCSU and Virginia Tech, Loblolly Pine Growth and Yield Research Cooperative at Virginia Tech, and the Hardwood Tree Improvement and Regeneration Center at Purdue. These NSF centers are designed to foster multi-university, interdisciplinary collaborations to solve industry-wide problems through multi-faceted approaches. A key focus of CAFS will be studies that link knowledge of genes, genomes, and physiological processes to silvicultural performance and value in forest stands.

Plans for 2006-07

We plan to host a workshop on Douglas-fir wood stiffness, in conjunction with Glen Murphy (OSU Department of Forest Engineering) and Mike Bondi (OSU Department of Forest Science).

Wood Quality Study

Introduction

Wood stiffness is the most important property of structural lumber, and has been identified as a high priority research topic by PNWTIRC members. Because juvenile wood is less stiff than mature wood, the quality of Douglas-fir lumber may decline as rotation lengths decrease and proportionally more of the wood is derived from the juvenile core. Furthermore, because wood traits of coniferous species are often highly heritable, and can be improved through selection and breeding, it may be valuable to incorporate wood stiffness into Douglas-fir breeding programs. Nonetheless, direct measures of wood stiffness are costly and require destructive sampling. Therefore, alternatives to destructive testing are needed to measure wood stiffness prior to harvest. For these reasons, we are carrying out extensive research on the measurement, quantitative genetics, and molecular genetics of Douglas-fir wood stiffness and strength.

Our research proposal for the *Wood Quality Study* was approved by the PNWTIRC members at the 2004-05 Annual Meeting. To accomplish this large research project, we are collaborating with the Stand Management Cooperative (SMC), the University of California at Davis (UC Davis), Olympic Resource Management (ORM), and the USDA Forest Service Pacific Northwest Research Station (PNWRS).

Objectives of the Wood Quality Study

Objective 1: To estimate potential genetic gains for direct measures of Douglas-fir wood stiffness (modulus of elasticity, MOE) and strength (modulus of rupture, MOR)

Objective 2: To determine which indirect measurements of MOE and MOR are useful for improving wood stiffness in operational tree improvement programs, and to estimate the relative gain efficiencies of the various indirect measures tested

Objective 3: To determine whether the wood properties of seed orchard parents can be used to predict the wood properties of their progeny

Objective 4: To identify molecular genetic markers that are associated with desirable wood properties

Wood disks were collected from the base of every log. Specific gravity (SG), annual ring counts, and bark thicknesses were measured in the field. Wood disks were later shipped to Corvallis, kiln-dried, and weighed again.

Accomplishments for 2005-06

Plant Materials

We are utilizing the Port Gamble first-generation progeny test series plus its associated seed orchard for our wood quality research. These materials are owned by Olympic Resource Management (ORM), and are located on the Kitsap Peninsula in Washington. Three progeny tests (Shine, Watershed, and Opsata) were established by ORM using a reps-in-sets design with non-interlocking blocks (Figure 2). Before it was rogued, the Hood Canal Seed Orchard contained grafted parents of the progeny test trees. Trees at the seed orchard and progeny tests were measured and harvested when they were 25 years old (e.g. Figure 3).

Shine

reps within sets	Sets			
	set 1 30 fam	set 2 30 fam	set 3 30 fam	set 4 40 fam
	1	1	1	1
	2	2	2	2
	3	3	3	3
	4	4	4	4
	5	5	5	5
	6	6	6	6
	7	7	7	7
	8	8	8	8

Opsata

reps within sets	Sets			
	set 1 30 fam	set 2 30 fam	set 3 30 fam	set 4 40 fam
	1	1	1	1
	2	2	2	2
	3	3	3	3
	4	4	4	4
	5	5	5	5
	6	6	6	6
	7	7	7	7
	8	8	8	8

Watershed

reps within sets	Sets			
	set 1 30 fam	set 2 30 fam	set 3 30 fam	set 4 40 fam
	1	1	1	1
	2	2	2	2
	3	3	3	3
	4	4	4	4
	5	5	5	5
	6	6	6	6
	7	7	7	7
	8	8	8	8

Figure 2. Study design of the Port Gamble series first-generation progeny tests. Each set contains between 28 to 40 families.

Data Collection

During the summer of 2005, we measured diameter at breast height (dbh_{25}), stem form, and branching traits on the 25-year-old trees in the three progeny tests (Table 1). These measurements were recorded on all 129 families at the tests. The acoustic modulus of elasticity (MOE_a) was measured on standing trees on a subset of 50 families (8 trees per family) at two of the tests (Shine and Opsata) using the Fibre-gen Director ST300™ (see Box on p. 13).

Table 1 .Approximate sample sizes per location for measured traits in the *Wood Quality Study.*

Trait	SO	Shine	Watershed	Opsata
Dbh, stem form		3,500	3,600	2,500
Height post-thinning		600	1,100	400
Specific gravity of wood disks	180	1,600		
HM200 MOEa	150	1,600	1,400	
ST300 MOEa	100	400		400
Trees milled for bending tests		400		
Foliage for DNA analysis	180			

SO = Hood Canal Seed Orchard, Port Gamble first-generation progeny test series
Shine, Watershed, and Opsata = Port Gamble first-generation progeny test sites

In the fall of 2005 and spring of 2006, the three progeny tests were thinned. At the Shine test, a large crew was assembled to harvest the trees and collect data in September 2005. Half of the trees at this site were felled by chainsaw, and the logs were skidded to a landing and processed by a Caterpillar® 325B (Figures 4 and 5). Wood disks were collected from the base of every log. Specific gravity (SG), annual ring counts, and bark thicknesses were measured in the field (Figure 6). Wood disks were later shipped to Corvallis, kiln-dried, and weighed again. After the logs were delimbed at the landing, acoustic velocities were measured with the Fibre-gen Director HM200™ (see Box on p. 13, Figure 7). The processed logs were spread one row thick to obtain clean HM200 readings. A subset of harvested trees from Shine (the same trees from which the ST300 measurements were taken) were cut to 9' butt logs and shipped to Corvallis

Tools used to obtain indirect estimates of wood stiffness

Acoustic tools assess wood stiffness by measuring the speed of soundwaves moving through wood. Wood stiffness is related to acoustic velocity by the following relationship:

= dynamic modulus of elasticity (Pa)

= acceleration due to gravity (9.8 m/s^2)

= acoustic wave velocity (m/s)

= density of the wood (kg/m^3)

where acoustic velocity is derived from the resonant frequency and distance that the soundwave travels.

Fibre-gen Director HM200™

This tool is designed to be used on recently harvested logs. A hammer strikes the end of the log, and the soundwave travels back and forth between the two ends of the log. A sensor in the HM200 detects the soundwave frequency and then calculates acoustic velocity based on the log length. This tool integrates the stiffness across the entire length and breadth of the log. Details can be found on the manufacturer's website:

http://www.fibre-gen.com/hm200.html

Fibre-gen Director ST300™

This tool is designed to be used on standing trees. The ST300 uses ultrasound technology to automatically measure the distance between two pins that are manually inserted into the bole about 1 meter apart. One probe (the transmitting probe) is hit with a hammer. The receiver probe monitors the time it takes for the soundwave to travel the distance between the two pins. Because the pins can only be inserted a small distance into the wood, the ST300 measures wood stiffness in the outer rings of the bole. Typically, repeated measures are taken, and averaged. It is desirable to insert the pins in at least two sides of the tree stem. Details can be found on the manufacturer's website:

http://www.fibre-gen.com/st300.html

The Watershed progeny test was thinned in March 2006 using a Timberjack harvester.

for milling into lumber. A portable WoodMizer sawmill was used to mill each log into 1.5" x 3.5" x 7' boards. These boards were then kiln-dried at OSU, and are now being used to obtain direct measurements of wood stiffness (Figure 8).

The Watershed progeny test was thinned in March 2006 using a Timberjack harvester. Trees were delimbed at the stump, and laid on the ground. HM200 measurements were subsequently taken by a small crew that followed behind the harvester at a safe distance. This harvesting system worked well for our data-gathering needs, and we found it to be much more efficient than the system used at Shine, where logs were skidded to a landing, processed, and then laid out for measurements.

Data Analysis

Heritabilities of dbh measured at age 25 served as a measure of test quality. Overall h^2_i for dbh_{25} was 0.12 across the 3 test sites, whereas h^2_f for dbh_{25} was 0.69. Heritabilities estimated per site (Table 2) were comparable to those estimated for dbh measured at age 13. Higher heritabilities were obtained for sets 1 and 3.

Table 3 lists the heritabilities for MOE_a measured in the field. The individual-tree heritabilities

Table 2. Heritabilities of dbh at age 25 at the Port Gamble progeny tests.

	Overall, 3 sites	Shine	Watershed	Opsata
h^2_i	0.12	0.18	0.16	0.13
h^2_f	0.69	0.60	0.61	0.46

$h^2_i = 3 * \sigma^2$ Family / σ^2 Phenotype

$h^2_f = 1 - (1/\text{family F-value})$

Based on these initial results, the HM200 appears to be a useful tool for breeders interested in improving wood quality through wood stiffness estimation during seed orchard roguing and progeny test thinning.

Table 3. Heritabilities of acoustic velocity (km/s) using the HM200 and ST300 tools at the Port Gamble progeny tests and Hood Canal Seed Orchard and wood specific gravity (SG) of wood disks from the base and top of the butt log of seed orchard trees.

	Progeny Tests			Seed Orchard			
	HM200	ST300		HM200	ST300	SG, basal	SG, top
h^2_i	0.30	0.17	H^2_i	0.74	0.65	0.31	0.42
h^2_f	0.66	0.59	H^2_c	0.99	0.99	0.41	0.55

h^2_i = 3 * σ^2 Family / σ^2 Phenotype
h^2_f = 1 - (1/Family F-value)
H^2_i = σ^2 Clone / σ^2 Phenotype
H^2_c = 1 - (1/Clone F-value)

for the HM200 measurements were moderate, whereas the heritabilities for the ST300 measurements were lower. At the two sites measured with the HM200, heritabilities were highest in sets 1 and 3, mirroring the dbh results. SG heritabilities estimated at the seed orchard were moderate, but lower than the heritabilities obtained for acoustic velocity of the HM200 and ST300 tools (Table 3). The genetic correlation of SG between the basal and top wood cookies (taken from either end of the basal log) was moderately strong (Figure 3).

The genetic correlation (r_A) between SG and MOE_a for the HM200 was strong, but only a moderate r_A was estimated between SG and MOE_a based on the ST300 (Table 4). When we compared the genetic correlation of the two acoustic velocity measures (HM200 vs ST300), we observed a strong relationship for the Shine progeny test, but only a moderate relationship for the seed orchard (Table 5). We hypothesize that the lower sample size at the orchard may have affected these results. Furthermore, the orchard ramets were open-grown, and had larger branches and bigger knots in the lower bole. Because the ST300 measures acoustic velocity in the outer stem, these measurements may have been adversely affected by the large knots in the seed orchard trees. In contrast, the HM200 tool integrates the acoustic velocity across the entire log diameter, presumably resulting in more reliable estimates of wood stiffness.

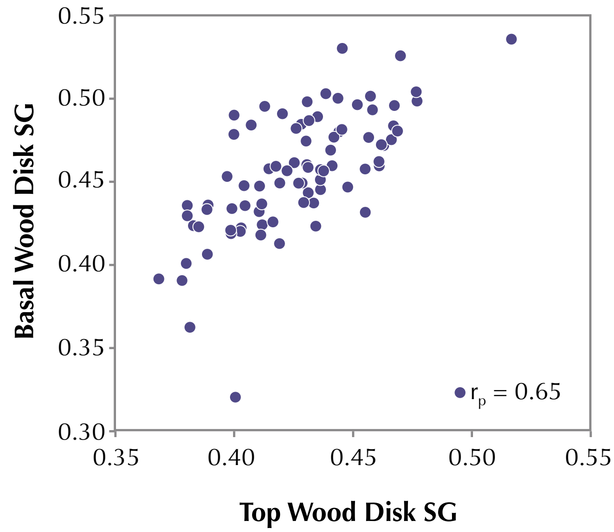


Figure 3. Phenotypic (r_P) family mean correlation of wood specific gravity (SG) between wood disks from the base and top of the butt log at the Hood Canal Seed Orchard. The genetic (r_A) correlation of these traits = 0.75.

Table 4. Genetic (r_A) correlations between wood specific gravity (SG) and MOE_a (GPa) for the HM200 and ST300 tools at the Hood Canal Seed orchard.

Wood specific gravity vs MOE_a at SO:	SG vs HM200	SG vs ST300
r_A, genetic correlation	0.97	0.54

Table 5. Genetic (r_A) correlations between acoustic velocity for the HM200 tool vs acoustic velocity (km/s) for the ST300 tool at the Shine progeny test and Hood Canal Seed Orchard (SO).

Acoustic velocity measured by HM200 vs ST300	Shine test	SO
r_A, genetic correlation	0.94	0.59

Figure 4. Thinning equipment at the progeny tests sites. Skidder and Caterpillar® 325B processor at the Shine progeny test site (a). Timberjack harvester at the Watershed progeny test site (b).

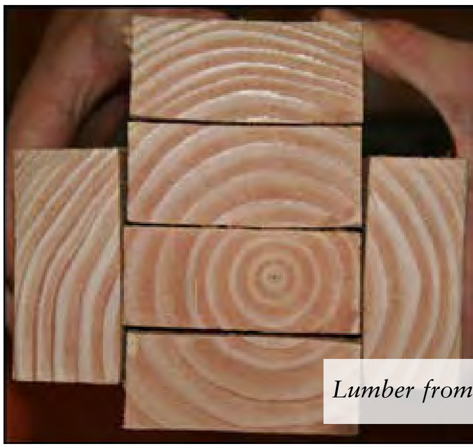
Figure 6. Wood disks at the Shine progeny test that were used to estimate wood specific gravity (SG) and obtain annual ring data.

Figure 5. Progeny test thinning (removing every second tree) at the Shine progeny test site.

Figure 7. Acoustic MOE measurements.HM200 log tool (a). ST300 standing tree tool (b).

Figure 8. Milling logs into lumber.

WoodMizer portable sawmill



Lumber from a single log

Labelling every board

Kiln-drying

…marker aided selection might be possible for improving wood properties in Douglas-fir.

Based on these initial results, the HM200 appears to be a useful tool for breeders interested in improving wood quality through wood stiffness estimation during seed orchard roguing and progeny test thinning. We are still working on estimating potential genetic gains compared to using direct measures of wood stiffness, whether stiffness can be estimated effectively using SG alone, and whether the ST300 is useful for making genetic selections as well.

Wood Quality Candidate Genes

The other important component of our *Wood Quality Study* is the discovery of wood quality candidate genes in Douglas-fir. Once these genes are identified, we will genotype the parents in the Hood Canal Seed Orchard and compare these genotypes with the wood quality phenotypes of (1) the parents in the seed orchard and (2) their progeny in the progeny test plantations. These *association genetic* studies will be used to test whether these genes are associated with genetic variation in important wood properties. Positive results would indicate that marker aided selection might be possible for improving wood properties in Douglas-fir.

During the past year, we isolated DNA from approximately 180 trees in the Hood Canal Seed Orchard, and began developing molecular genetic markers for 19 wood property candidate genes (Table 6). We are targeting these 19 genes because they play key roles in the formation of wood based on studies in loblolly pine. We are developing genetic markers called single nucleotide polymorphisms (SNPs), which are single-letter changes in the DNA code that occur between different alleles (copies) of the same gene. To date, we have identified 11 of our target genes in Douglas-fir, *During the past year, we isolated DNA from approximately 180 trees in the Hood Canal Seed Orchard, and began developing molecular genetic markers for 19 wood property candidate genes. We are targeting these 19 genes because they play key roles in the formation of wood based on studies in loblolly pine.*

and have begun developing SNP markers for these genes. During the next year, we will continue developing the SNP markers, and then begin genotyping the parents. This work is being conducted by Barnaly Pande and David Neale at UC-Davis, and is being supported by a USFS Agenda 2020 project involving the PNWTIRC, UC-Davis, and the USFS Pacific Northwest Research Station.

Table 6. Nineteen wood property candidate genes included in the *Wood Quality Study*. These genes were previously identified in loblolly pine, and are now being used to determine whether they are associated with wood quality in Douglas-fir.

	Length of the gene fragment being studied (base pairs)		Single nucleotide polymorphisms (SNPs) in the Douglas-fir genes	
Gene[1]	Loblolly pine	Douglas-fir [2]	Number of SNPs	Base-pairs per SNP
4cl	2,437	628	8	79
agp-4	367	-	-	-
agp-6	884	-	-	-
agp-like	886	-	-	-
alpha-tubulin	792	2,578	93	28
c3h	2,269	670	16	42
c4h-1	2,506	499	2	250
c4h-2	522	-	-	-
cad	440	197	4	49
ccoaomt	517	-	-	-
ccr-1	1,044	508	24	22
cesA3	1,023	-	-	-
comt-1	1,278	548	6	91
glyhmt	552	158	9	18
ptlim1	425	567	6	95
ptlim2	446	-	-	-
pal1	433	436	13	34
sam-1	739	602	3	201
sam-2	461	-	-	-

[1] *4cl* encodes 4-coumarate:CoA ligase; *agp-4*, *agp-6*, and *agp*-like encode arabinogalactan proteins; *alpha-tubulin* encodes a tubulin; *c3h* encodes coumarate 3-hydroxylase; *c4h-1* and *c4h-2* encode cinnamate 4-hydroxylases; *cad* encodes cinnamyl alcohol dehydrogenase; *ccoaomt* encodes caffeoyl CoA *O*-methyltransferase; *ccr-1* encodes cinnamoyl CoA reductase; *cesA3* encodes cellulose synthase; *comt-1* encodes caffeate *O*-methyltransferase; *glyhmt* encodes glycine hydroxymethyltransferase; *ptlim1* and *ptlim2* encode LIM transcription factors; *pa1* encodes phenylalanine ammonia-lyase; *sam-1* and *sam-2* encode s-adenosyl methionine synthetases.

[2] A dash indicates that this gene has not yet been identified in Douglas-fir.

Plans for 2006-07

Lumber stiffness testing will be carried out during the summer of 2006. Data analysis will continue, as will the candidate gene work. For example, genetic gains will be predicted, and we will further examine genetic correlations among traits. We will calculate relative gain efficiencies of incorporating green wood density into MOE_a estimates, and determine whether indirect and/or nondestructive methods of evaluating wood stiffness and strength will be useful in genetic tests. If genetic correlations and relative gain efficiencies are high, we will then be able to develop protocols and recommendations for using nondestructive test procedures in tree improvement programs.

Acknowledgements [1]

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Dan Cress	ORM
Glen Maxim	ORM
Dani Priest	ORM
David Briggs	SMC
Randol Collier	SMC
John Haukaas	SMC
Bob Gonyea	SMC
Bert Hasselberg	SMC
Gina Smith	SMC
Leon Burfiend	SMC
Angel Ratliff	SMC
Ahaaron Poorman	SMC
Brad St. Clair	PNWRS
Randy Johnson	PNWRS
Meg Sherwood	PNWRS
Jeff Riddle	PNWRS
Chris Poklemba	PNWRS
Randall Greggs	Green Diamond Resource Co.
Fred Pfund	Starker Forests
Thompson Timber Co.	Philomath, OR
Mike Doolittle	Logs to Lumber, Inc.
Jeff Kennedy	Sawyer
Dan Thompson	Sawyer
Calvin Lomsdalen	Tree processor operator
Fred Pleines	Tree processor operator
Richard Hughes	Skidder operator
Mike Ackerman	Skidder operator
Nick Augsburger	OSU work study student
Brooke Morris	OSU work study student
David Griffith	Work study student
Dana Howe	OSU Dept. of Forest Science
Milo Clauson	OSU Dept. of Wood Science and Engineering
Rakesh Gupta	OSU Dept. of Wood Science and Engineering
Northwest Projects	WA

[1] SMC, Stand Management Cooperative, Department of Forest Resources, University of Washington, Seattle; PNWRS, USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR; UC Davis, University of California at Davis; ORM, Olympic Resource Management, Port Gamble, WA.

Miniaturized Seed Orchard Study

Miniaturized Seed Orchard at the Plum Creek Seed Orchard Complex

Introduction

Miniaturized seed orchards (MSOs) are promising alternatives to conventional seed orchards. MSOs are designed for intensive management, efficiency, and cost-effectiveness. Seed crops are produced on numerous, closely-spaced, smaller trees instead of the fewer, wider-spaced, larger trees found in conventional orchards. MSOs have the potential to increase genetic gains through controlled pollination and supplemental mass pollination, thereby reducing pollen contamination and facilitating the production of elite crosses. Our goal is to compare management regimes for three alternative planting densities at an operational scale that will provide realistic estimates of management costs and seed yields for Douglas-fir (Anekonda and Adams 1999).

Grafts were established between 2002 and 2004. Our study compares 3 tree spacings and uses 24 clones. Ramets were grafted into 5- or 6-tree row plots per clone per replication. Replications within each tree spacing were grouped into block-pairs. Eight clones from backward selections were replicated in all 8 blocks per spacing, while 16 clones from forward selections were replicated in 4 of 8 blocks (2 block-pairs of 2 replications each) per spacing (see Box on this page).

Description of three orchard types in the MSO study

Orchard type	Spacing (m)	Trees per ha	# trees	Target final ht (m)
Macro	6 x 4	416	640	4
Mini	4 x 2	1,250	640	2
Micro	3 x 1	3,333	768	2

Clones in each block-pair (two replications) and spacing in the MSO study

	Forward selections[1]		Backward selections[2]	
Block-pair	clones	ortet age, 2004	clones	ortet age, 2004
A	I-P	8-31	Q-X	58-101
B	A-D, M-P	8	Q-X	58-101
C	E-L	8-31	Q-X	58-101
D	A-H	8	Q-X	58-101

1 Forward selection = graft scion selected from progeny of original parent

2 Backward selection = graft scion selected from original parent or parental clone

After viewing the Washington State Department of Natural Resources' sickle-bar pruning equipment in action, we are optimistic that it can be used operationally to efficiently control crown size in miniaturized seed orchards.

More details on the objectives, potential advantages, and design of the MSO project are included in previous PNWTIRC Annual Reports (Howe et al. 2002, 2003; Cherry et al. 2004).

Figure 9. Crown management at the Plum Creek Miniaturized Seed Orchard. Pruning and applying Tree Seal to cut branch tips (a). Pruned clonal rows (b).

Accomplishments for 2005-06

The trees are being managed and maintained until they are large enough to begin experimental treatments. The tallest orchard trees were topped and the lateral branches in the upper crown were pruned during the summer of 2006 to control crown size (Figure 9). This pruning protocol was designed to control tree height and to keep the trees below their intended final size. We continued to remove branches from the rootstocks of the grafted trees, and this process is now almost complete. The trees will soon be large enough to begin the pruning treatments designed to enhance cone production. The protocol for orchard pruning in 2006 was similar to previous prunings (Cherry and Howe 2005), except that Tree Seal was painted onto every cut branch and stem to prevent *Dioryctria* infestations. Site maintenance by Plum Creek is ongoing, including weed control, irrigation, and fertilization.

Plans for 2006-07

Rootstock branch removal will be completed in the summer of 2007, and operational crown management trials will be designed and implemented. After viewing the Washington State Department of Natural Resources' sickle-bar pruning equipment in action, we are optimistic that it can be used operationally to efficiently control crown size in miniaturized seed orchards. We plan to transport this equipment to the MSO next summer for crown shaping experiments. Based on results from our *Early Flowering Study*, we expect the trees to have reached sufficient size to initiate flower stimulating treatments by the spring of 2008.

This study is examining whether timing (season and frequency) of crown pruning can be physiologically optimized to maximize seed production.

Pruning Study at Roseburg Forest Products Regeneration Center

Introduction

The pruning study at Roseburg Forest Products' Vaughn Seed Orchard is designed to test the effects of pruning timing and leader retention on crown form and cone production in order to learn about physiological responses to pruning prior to applying similar treatments at the Plum Creek MSO. Pruning is used to manage crown size, but adversely affects seed production if flowers or cones are pruned off prior to cone harvest. This study is examining whether timing (season and frequency) of crown pruning can be physiologically optimized to maximize seed production.

Accomplishments for 2005-06

Eighteen clones were selected for the study. For each clone, we selected 5 to 9 previously untopped ramets per clone to be included in each treatment. The study involves six pruning treatments in a generalized randomized block design, with clones considered to be the random blocking variables (see Cherry and Howe 2005; Box on this page). Pruning will be carried out every 2 years. The Vaughn Seed Orchard contains slightly older, larger trees than the Plum Creek MSO. Initial experiments focus on the physiology of pruning and cone production, whereas later experiments will integrate operational concerns.

Flower stimulating treatments using a combination of stem girdling and gibberellic acid (GA) stem

Pruning treatments at the Roseburg Forest Products Vaughn Seed Orchard.

Treat	Description	Rationale
1	Control = no pruning	
Treatments in the year of flower stimulation (Spring-Summer '05)		
2	Top prune and prune branches before bud flush	Maximize growth of lateral branches by removing the main leader. Advantage: Laterals are pruned before flower buds form. Disadvantage: Growth of laterals may inhibit flower bud formation.
3	Prune branches before bud flush; top prune in summer, after bud set	Minimize growth of lateral branches by retaining the main leader. Advantage: May lead to more flower buds as compared to Treat. 2. Disadvantage: Two prunings are needed
4	Top prune and prune branches in summer, after bud set	Compare results with Treatments 2 and 3. Disadvantage: Pruning will remove flower buds.
Treatments in the year of cone production (Summer-Fall '06)		
5	Top prune and prune branches in summer, after bud set	Maximize bud growth following pruning. Advantage: May be able to avoid removing developing cones.
6	Top prune and prune branches in fall, after cone harvest	Minimize bud growth following pruning (similar to Treatment 2) Advantage: Don't have to worry about removing cones.

Further pruning treatments will be applied using the WDNR's sickle-bar pruning equipment. The results from this study will be used to design pruning treatments for the Plum Creek MSO

injections were applied in late spring 2005. The top and branch pruning protocols are listed in Box below. Treatment 4 was topped and lateral-pruned in July 2005 (Figure 10). In the spring of 2006, we measured crown diameter and branch length. Male and female flowers were also counted.

Figure 10. Pruned tree at the Roseburg Forest Products Miniaturized Seed Orchard.

Plans for 2006-07

The last remaining treatments (#5 and #6) will be carried out in 2006, one year after flower stimulation. Crown types will be assigned to qualitatively distinguish tight-branching, narrow crowns from lanky-limbed, sprawling crowns, etc. Further pruning treatments will be applied using the WDNR's sickle-bar pruning equipment (see *Miniaturized Seed Orchard at the Plum Creek Seed Orchard Complex*, p. 19). The results from this study will be used to design pruning treatments for the Plum Creek MSO.

Pruning protocols at the Vaughn Seed Orchard

Top pruning	The main stem was cut at about 2 m and the terminal buds were removed from all but one branch located near the top of the pruned tree. The branch that was left unpruned was chosen to form the new leader for the tree. Typically a smaller branch was chosen, to reduce tree height growth.
Branch pruning	Large branches – terminal buds were pruned from the leader of the main branch and distal second-order branches.
	Smaller branches – terminal buds were pruned from the leader of the main branch only, and the distal second-order branches were not pruned unless they appeared to be very vigorous.

Literature Cited

Anekonda, T.S. and Adams, W.T. 1999. Seed orchard research in coastal Douglas-fir: comparison of macro, micro and mini orchards. PNWTIRC Study Plan, July 26, 1999, 25 pp.

Cherry, M.L., Anekonda, T.S., Albrecht, M.J., and Howe, G.T. 2006. Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). Pacific Northwest Tree Improvement Research Cooperative Report #25, Oregon State University. 23 pp.

Cherry, M.L. and Howe, G.T. 2005. PNWTIRC Annual Report 2004-2005, 23 pp.

Cherry, M.L., Howe, G.T., and Slavov, G. 2004. PNWTIRC Annual Report 2003-2004, 27 pp.

Howe, G.T., Cherry, M.L., and Slavov, G. 2003. PNWTIRC Annual Report 2002-2003, 45 pp.

Howe, G.T., Anekonda, T., and Slavov, G. 2002. PNW-TIRC Annual Report 2001-2002, 29 pp.

Sweet, G.B. and Krugman, S.L. 1977. Flowering and seed production problems and a new concept of seed orchards. Proc. Third World Consultation on Forest Tree Breeding Vol. 2, N.Z. For. Serv., 749-759.

Appendix 1

Publications by PNWTIRC Personnel: 2005-06

Cherry, M.L., Anekonda, T.S., Albrecht, M.J., and Howe, G.T. 2006. Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). Pacific Northwest Tree Improvement Research Cooperative Report #25, Oregon State University. 23 pp.

Howe, G.T., Jayawickrama, K.J., Cherry, M.L., Johnson, G.R., and Wheeler, N.C. 2006. Breeding Douglas-fir. Pp. 245-353. *In* J. Janick (ed): Plant Breeding Reviews. 27, John Wiley and Sons, Inc.

Appendix 2

Workshops, Presentations, and Abstracts by PNWTIRC Personnel: 2005-06

Cherry, M.L., Howe, G.T., Briggs, D., Neale, D.B., St. Clair, J.B., and Cress, D. 2006. Genetics of wood stiffness. Stand Management Cooperative Annual Meeting. April 26, 2006, Hood River, OR.

Howe, D.K., Brunner, A.M., Cherry, M.L., Krutovsky, K.V., Neale, D.B., and Howe, G.T. 2005. Identifying candidate genes associated with cold hardiness in coastal Douglas-fir using DNA microarrays. *Abstract in*: Proceedings Western Forest Genetics Association 50th Anniversary Meeting: Looking Back—Looking Ahead, July 19-21, 2005, Corvallis, OR, p. 15.

Howe, G.T. 2006. Ecological genetics and breeding of Douglas-fir: merging traditional and genomic approaches in forest genetics. Department of Horticulture, Oregon State University, May 16, 2006, Corvallis.

Howe, G.T. 2006. Ecological genomics: a vision for OSU, the College of Forestry, and the H.J. Andrews. Meeting of the Long-term Ecological Research (LTER) Program, May 5, 2006, Corvallis, OR.

Howe, G.T. 2006. Genomics of Douglas-fir: implications for tree improvement, gene conservation, tree physiology, and beyond. Department of Forest Science, Oregon State University, March 2, 2006, Corvallis.

Neale, D.B., Howe, G.T., St. Clair, J.B., and Cherry, M.L. 2006. Discovery of genes controlling wood property traits in Douglas-fir. USDA-Forest Service Agenda 2020 Annual Research Meeting, April 25, 2006, Hood River, OR.

Pande, B., Howe, G.T., St. Clair, J.B., Wheeler, N., Krutovsky K.V., and Neale, D.B. 2005. Mapping adaptive traits in Douglas-fir using association genetics. *Abstract in*: Proceedings Western Forest Genetics Association 50th Anniversary Meeting: Looking Back—Looking Ahead, July 19-21, 2005, Corvallis, OR, p. 23.

Douglas-fir Genome Project Meeting, July 18, 2005, Corvallis, OR.

Western Forest Genetics Association Meeting, July 19-21, 2005, Corvallis, OR.

Appendix 3

PNWTIRC Financial Support for Fiscal Year 2005-06

Regular members[1]	$108,000
Associate members[1]	4,000
Contracts	2,000
Forest Research Laboratory, Oregon State University[2]	122,134
Total	**236,134**

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

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

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