Pacific Northwest Tree Improvement Research Cooperative

Annual Report
2006-2007

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Longview Fibre Company

Menasha Forest Products Corporation

Olympic Resource Management

Oregon Department of Forestry

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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

Policy/Technical Committee Chair: Dan Cress

Graduate Student: Vikas Vikram

Highlights of 2006-2007

- Field and laboratory work continued in the Wood Quality Study. Tree heights were measured on a subset of the unthinned trees at all three progeny test sites in the summer of 2006. Lumber testing continued during the fall of 2006, and wood disks were measured in the spring of 2007. Data analysis and candidate gene analyses are in progress.

- We continued the pruning treatments and collected flowering data in the spring of 2007 at Roseburg Resources' Vaughn seed orchard in Lebanon. The timing of pruning seems to affect the number of flowers per tree. Graft maintenance continued at the other orchards.

- We published the first PNWTIRC Report on the Wood Quality Study entitled *Genetic variation in wood quality in a clonal Douglas-fir seed orchard.* We studied the genetics of wood stiffness (modulus of elasticity, MOE) in a clonal seed orchard of Douglas-fir. Indirect estimates of MOE were obtained using two tools that measure acoustic velocity in wood. The HM200 can be used to measure acoustic velocity and estimate dynamic MOE of logs, whereas the ST300 can be used to measure comparable traits on standing trees. We observed low to moderate individual-tree broad-sense heritabilities for HM200 velocity (0.27), HM200 MOE (0.36), and basic wood density (0.48), and our projections suggest that very high clonal mean heritabilities can be obtained by measuring relatively few ramets per clone. In contrast, clonal variation was nonsignificant for the ST300 traits. These results suggest that seed orchard trees can be used to measure wood properties and select genotypes with superior wood stiffness. Overall, it appears that the HM200 can be used to obtain rapid, inexpensive assessments of wood quality in breeding programs, and we recommend that additional comparisons of the HM200 and ST300 (or other standing tree tools) be conducted when trees are harvested in other seed orchards.

- PNWTIRC personnel completed four journal articles and reports (i.e., published or in press) and gave 10 presentations (see Appendices 1-2).

- The Early Flowering Study was completed with the publication of the results in the Canadian Journal of Forest Research [Cherry, M.L., Anekonda, T.S., Albrecht, M.J., and Howe, G.T. 2007. Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). Can. J. For. Res. 37:1-10].

- Glenn Howe co-organized the International Conference on Conifer Genomics, Cold Spring Harbor, NY, March 18-21, 2007 (with Dave Neale, Jeff Dean, and Mike Greenwood).

Message from the Director

The most noticeable trend over the past year was the participation of the PNWTIRC as an important collaborator, rather than as a sole participant, in a number of new research projects. For example, PNWTIRC members approved the initiation of a climate change "subproject"—which is a research project that is financed and involves the participation of only a subset of PNWTIRC members. In addition to the extra contributions from a subset of the members, this project involves a substantial contribution from the USFS Pacific Northwest Research Station. The goal of this project, "Forest Management and Climate Change: A Synthesis of Genetic and Silvicultural Options for the Western U.S.," is to provide public and private forest landowners with science-based management options suitable for meeting diverse management objectives under alternative climate change scenarios.

Another new project in which the PNWTIRC will play an important role is the Center for Advanced Forestry Systems (CAFS). Forestry cooperatives at Oregon State University (Howe, Strauss, Jayawickrama), North Carolina State University (Goldfarb, Allen), Virginia Tech (Burkhart, Fox), and Purdue (Michler, Meilan) recently submitted a proposal to the National Science Foundation to fund a project designed to link cooperative research among universities and forestry disciplines, including genetics and silviculture.

Other collaborative projects do not involve direct participation by the PNWTIRC, but are highly relevant to PNWTIRC members, and were at least partly facilitated by the existence of the PNWTIRC and the large Douglas-fir breeding programs in the Pacific Northwest. A large genomics project was recently funded by the U.S. Department of Energy Joint Genome Initiative Community Sequencing Program. The goal of this project is to obtain many more gene sequences for Douglas-fir and other conifers. This project, "An expanded EST resource for pines and other conifers," is a joint effort of the University of Georgia (Dean), Oregon State University (Howe), University of California at Davis (Neale, Rogers), and the USFS Pacific Southwest Research Station (Jermstad).

A proposal for a second, large genomics project is pending with the USDA National Research Initiative Competitive Grants Program. The goal of this project, which is entitled the "Conifer Translational Genomics Network," is to bring marker-aided breeding knowledge and tools to breeders of Douglas-fir, loblolly pine, and slash pine. Again, this is a multi-university collaborative project involving the University of California at Davis, (Neale, Lee, Wegrzyn), Oregon State University (Howe, Harry, Wheeler), North Carolina State University (McKeand, Isik, Whetten), Texas A&M University (Byram), University of Florida (Huber), University of Georgia (Dean), and the USFS (Nelson, St.Clair).

I expect that these large multi-organizational research projects will become even more common in the future, leading to a more complex, but more effective and valuable PNWTIRC research portfolio in the future.

Glenn Howe

Introduction

Currently, the PNWTIRC has two main areas of research: wood quality and miniaturized seed orchards (MSOs). The aim of the Wood Quality Study is to understand the genetics of wood stiffness and the potential for using new acoustic tools to improve wood stiffness in Douglas-fir. The impetus for this study is the availability of new tools that can measure the speed of acoustic (or stress) waves in logs and standing trees. Because acoustic velocity is positively correlated with stiffness, the standing-tree tools in particular may enable us to readily measure and select for wood stiffness in progeny tests. Furthermore, the log tool may be useful when progeny tests or seed orchards are thinned or rogued. In this annual report, we focus on our experience with these tools in the Hood Canal Seed Orchard.

Our Miniaturized Seed Orchard Study is a long-term experiment designed to explore whether MSOs can be used to facilitate controlled mass pollination, reduce pollen contamination, increase genetic gains, and reduce costs of cone harvest and pest control. In contrast to conventional orchards, MSOs are orchards in which the trees are planted at close spacings, and then maintained at a height of only 2 to 4 m. In our experiment, we are testing three alternative MSO spacings, and the trees are planted in clonal rows.

New Research Directions

NSF 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.

Technology Transfer

Our technology transfer efforts include distribution of cooperative research reports, meetings with cooperators, annual meetings, annual reports, and workshops.

Publications

We published a PNWTIRC Report entitled *Genetic Variation in Wood Quality in a Clonal Douglas-fir Seed Orchard*, and the Canadian Journal of Forest Research published the results of the Early Flowering Study in a paper entitled *Flower stimulation in young miniaturized seed orchards of Douglas-fir (Pseudotsuga menziesii).* The citations for these papers can be found in Appendix 1.

Workshops and meetings

During the past year, Glenn Howe gave talks on conifer genomics at the International Conference on Conifer Genomics in Cold Spring Harbor, NY; crown management of miniaturized seed orchards at the Northwest Seed Orchard Managers Association Annual Meeting in June; Douglas-fir breeding at the Western Forest Genetics Association meeting in Galveston, TX; incorporating genetics into process and hybrid growth models at the CAFS Planning Meeting in Atlanta, GA; and gene discovery for adaptive traits at the USFS national workshop on forest productivity and technology. Marilyn Cherry reported on the Wood Quality Study at the Northwest Tree Improvement Cooperative Annual Meeting in September, 2006, and Vikas Vikram reported on the Wood Quality Study at the Stand Management Cooperative Biannual Meeting in April, 2007.

Technology transfer plans for 2007-08

We plan to host a workshop on Douglas-fir wood stiffness in collaboration with the Northwest Tree Improvement Cooperative (OSU) and the Stand Management Cooperative (UW).

Wood Quality Study

Introduction

Wood stiffness is one of the most important properties of structural lumber, and is particularly important for Douglas-fir, which is used mostly for dimension lumber and is renowned for its strong, stiff, and dense wood. The use of wood quality traits in tree improvement programs requires rapid measurement techniques that are preferably non-destructive and applicable to small trees. Direct estimates of wood stiffness (or modulus of elasticity, MOE) can be obtained by bending the wood and measuring its elasticity, but these tests are time consuming, costly, and usually destructive. Indirect estimates of MOE (dynamic MOE) can be obtained by measuring wood density and the velocity of acoustic (or stress) waves in wood. New tools for estimating wood acoustic velocity of standing trees or logs are now becoming available, providing new opportunities to enhance wood stiffness via tree breeding and stand management. These indirect measures of wood quality may be valuable selection criteria in tree improvement programs, allowing tree breeders to improve wood stiffness.

Objectives of the Wood Quality Study

Our objectives are to:

- Estimate potential genetic gains for direct measures of Douglas-fir wood stiffness (modulus of elasticity, MOE)
- Determine which indirect measurements of MOE are useful for improving wood stiffness in operational tree improvement programs, and to estimate the relative gain efficiencies of the various indirect measures tested
- Determine whether the wood properties of seed orchard parents can be used to predict the wood properties of their progeny
- Identify molecular genetic markers that are associated with desirable wood properties

The Fibre-gen Director HM200™ (HM200) tool can be used to measure acoustic velocity and estimate the stiffness of logs. These indirect estimates of MOE are highly correlated with direct estimates of MOE obtained from bending tests (Andrews 2002; Carter et al. 2007). In contrast to the HM200, the Fibre-gen Director ST300™ (ST300) can be used to measure acoustic velocity of standing trees. The ST300 estimates acoustic velocity from a single pass of the soundwave (time-of-flight), whereas the HM200 estimates the acoustic velocity from the resonant frequencies created by repeated acoustic echoing between the log ends. Different acoustic wave propagation mechanisms are in effect for these two tools (Wang et al. 2007). Because the ST300 measures acoustic velocity in the outer, generally denser wood of the tree, it overestimates the stiffness of the entire tree. In contrast, the HM200 measures acoustic velocity across the entire log, thereby capturing information on the stiffer outerwood and less stiff juvenile core. Finally, because the repeatability of the HM200 is high, only one measurement is typically needed for each tree. For best results, the ST300 should be used on multiple sides of the tree, and more than one measurement may be needed at each location.

Most wood properties are highly heritable, yet it is often difficult to improve wood quality in tree breeding programs because wood properties are expensive to measure and difficult to assess on small trees. A potential solution is to measure genotypes that are clonally replicated in grafted seed orchards. Compared to open-pollinated progeny, far fewer trees should be needed to obtain precise estimates of genetic worth. Although measurements in seed orchards may be compromised by grafting, flower stimulation treatments, and poor experimental design (including open-grown trees), these sources of experimental error may be offset by the high heritabilities of most wood properties and clonal replication. Furthermore, seed orchards may be valuable for obtaining clonal molecular marker data and wood property phenotypes for use in genetic association studies aimed at understanding wood quality at the gene level. Therefore, we studied wood stiffness and other traits in a grafted, clonal seed orchard, and in three open-pollinated progeny test plantations. We used the HM200 and ST300 to measure dynamic MOE in the seed orchard and progeny test, and bending tests to measure static MOE on 1.5″x3.5″ lumber milled from a subset of the trees at one of the progeny test sites. In last year's annual report, we reported preliminary results from three progeny test plantations and one seed orchard (Cherry and Howe 2006). In this year's annual report, we focus more closely on the results from the Hood Canal Seed Orchard.

Acoustic tools can be used to estimate wood stiffness (MOE):

$$MOE_d = D * V^2 * 10^{-9} \text{ where:}$$

MOE_d = dynamic modulus of elasticity (GPa)

D = wood density (kg m^{-3})

V = acoustic wave velocity (m s^{-1})

Fibre-gen Director HM200

The HM200 is used to estimate the stiffness of logs. A hammer is used to strike the end of the log, creating a soundwave that travels back and forth between the two log ends. The HM200 uses the resonant frequency of the soundwave and the log length to estimate the acoustic velocity. Stiffness (MOE) can then be estimated from the acoustic velocity and wood density. For details visit http://www.fibre-gen.com/hm200.html.

Fibre-gen Director ST300

The ST300 is designed to be used on standing trees. The ST300 uses ultrasound technology to automatically measure the distance between two pins that are inserted into the bole about 1 meter apart. The transmitter probe is hit with a hammer, and the receiver probe measures the time for the soundwave to arrive. Because the pins are inserted only a small distance into the wood, the ST300 measures wood stiffness in the outer rings of the bole. For details visit http://www.fibre-gen.com/st300.html.

The main objectives of this study are to (1) estimate potential genetic gains for Douglas-fir wood stiffness, and (2) determine whether the HM200 and ST300 acoustic tools can be used to improve wood stiffness in operational breeding programs. The specific objectives of the seed orchard study were to determine whether (1) the wood properties of seed orchard parents can be used to predict the wood properties of their progeny, and (2) whether clonal seed orchard trees can be used to reduce the costs of incorporating wood properties into breeding programs. This research is being conducted in collaboration with the Stand Management Cooperative (SMC), University of California at Davis (UC Davis), Olympic Resource Management (ORM), and USDA Forest Service Pacific Northwest Research Station (PNWRS).

Plant materials

Parent trees were randomly selected from the Kitsap and Olympic Peninsulas of northwestern Washington, allocated into four sets by geographic region of origin, and then used to establish a grafted seed orchard and progeny test on three sites. Scions from the selected parents were grafted into the Hood Canal Seed Orchard located on the Olympic Peninsula, WA (47°52.6' N, 122°44.0' W, 140 m elevation). The orchard, which is owned by Olympic Resource Management, was grafted between 1981 and 1983, and rogued in the spring of 2005, when the trees were between 22 and 24 years from grafting. The trees in the orchard had received routine weed control and occasional flower stimulation treatments consisting of nitrogen fertilization and stem girdling. The frequency and timing of flower stimulation varied among clones and ramets.

Measurements and analyses

The rogued seed orchard trees were cut with a chainsaw above the graft union, and HM200 acoustic velocities (Vel_{HM}) were measured on the felled, delimbed logs. One measurement was recorded per log according to the manufacturer's instructions. ST300 acoustic velocities (Vel_{ST}) were measured on logs after they had been yarded to the landing site. Nine ST300 measurements were taken in each of 4 cross-sectional quadrants around the circumference of the log, using probes that were placed about 1 m apart. Wood disks were cut from the base and top of each 17' (5.18 m) basal log. These disks, which included the pith, were either whole (round) or half (semicircular), and about 5 cm thick. For each disk, we measured the diameter inside the bark, disk mass, and green volume using the water displacement method.

The disks were transported to Oregon State University, kiln-dried to less than 7% moisture content, and weighed. Wood green density (Den_{gr}, kg m^{-3}) and basic wood density (Den_{bd}, kg m^{-3}) were estimated for each tree as [disk mass (kg)] / [disk green volume (m^3)]. Moisture content (MC, %) was estimated as [$Den_{gr} - Den_{bd}$] / [Den_{bd} * 100]. Measurements for the top and basal disks from each log were averaged prior to some of the analyses. Dynamic MOE was estimated from both HM200 and ST300 acoustic velocities. The average Den_{gr} of the basal and top disks were used to estimate log dynamic MOE (MOE_{HM} and MOE_{ST}):

[1] $$\text{MOE (GPa)} = Den_{gr}\,(\text{kg m}^{-3}) \times [\text{acoustic wave velocity (m s}^{-1})]^2 \times 10^{-9}$$

These data were analyzed using standard quantitative genetic techniques (Cherry et al. 2007).

Results and discussion

Table 1 lists sample sizes, clonal mean descriptive statistics, variance components, and heritabilities for each measured trait. The number of clones sampled per trait ranged from 66 to 82, and the average number of ramets sampled per clone ranged from 1.4 for the ST300 measures to 1.9 for MC.

Table 1. Sample sizes, descriptive statistics, variance components, and broad-sense heritabilities of traits measured at the Hood Canal Seed Orchard.

	Vel_{HM} [a] ($m\ s^{-1}$)	**MOE_{HM}** (GPa)	**Vel_{ST}** ($m\ s^{-1}$)	**MOE_{ST}** (GPa)	**Den_{gr}** ($kg\ m^{-3}$)	**Den_{bd}** ($kg\ m^{-3}$)	**MC** (%)	**Diam** (cm)
Sample sizes								
Number of clones	82	82	66	66	82	82	81	82
Number of ramets	150	150	96	96	152	151	151	152
Ramets / clone	1.8	1.8	1.4	1.4	1.8	1.8	1.9	1.8
Clonal means								
Mean (± *s.e.)*	3,161 *(20.7)*	8.6 *(0.12)*	3,981 *(24.2)*	13.6 *(0.22)*	857.5 *(6.1)*	441.2 *(3.6)*	94.7 *(1.3)*	23.4
Minimum	2,620	6.4	3,525	9.9	727.5	325.8	63.8	15.4
Maximum	3,620	11.2	4,648	18.4	970.7	527.1	123.6	36.0
Coeff. of variation (%)	5.9	12.7	4.9	12.9	6.4	7.3	12.1	17.0
Variance components								
σ^2 Clone(Set)	11,920	0.56	5,982	0.45	387.3	623.3	37.8	1.76
Clone(Set) *p-value*	*0.020*	*0.002*	*0.223*	*0.241*	*0.228*	*0.0001*	*0.048*	*0.245*
σ^2 Set	0	0	0	0	0	0	0	0
σ^2 E	32,561	0.98	43,575	3.69	4,560.5	664.7	138.8	21.10
Coeff. of gen. var. (%)	3.4	8.7	2.0	4.9	2.3	5.7	6.5	5.7
Clonal heritabilities								
H^2_i	0.27	0.36	0.12 [b]	0.11 [b]	0.08 [b]	0.48	0.21	0.08 [b]
$H^2_{C(S)}$	0.40	0.51	0.16 [b]	0.15 [b]	0.13 [b]	0.63	0.33	0.13 [b]

[a] Vel_{HM} = acoustic velocity measured by HM200; Vel_{ST} = acoustic velocity measured by ST300; MOE_{HM} = modulus of elasticity estimated using Vel_{HM} and Den_{gr} measurements; MOE_{ST} = modulus of elasticity estimated using Vel_{ST} and Den_{gr} measurements; Den_{bd} = basic wood density; Den_{gr} = green wood density; MC = wood green moisture content; Diam = diameter inside bark.

[b] Denotes Clone(Set) variance component not significant at $p = 0.05$

The estimates of acoustic velocity were about 20% lower using the HM200 compared to the ST300, resulting in MOE values that were about 37% lower (Table 1). Our results are similar to those observed by Wang et al. (2007) for Sitka spruce, western hemlock, and jack pine. These differences may result from the different parts of the tree that were sampled by the two instruments. The ST300 measures acoustic velocity in the outer, stiffer mature wood of the tree, whereas the HM200 provides an integrated measurement of the entire log (Carter et al. 2007). The deviant wood grain associated with knots lowers the velocities and stiffness predictions obtained using the ST300 (Carter et al. 2007), but care was taken to avoid knots during measurement of the open-grown seed orchard trees.

Our clonal means for Vel_{HM}, MOE_{HM}, Den_{bd}, Den_{gr}, and MC are similar to those obtained from a study of the Douglas-fir Nehalem breeding program (Johnson and Gartner 2006). The clone-within-set variation was significant at the 0.05 level of probability for Vel_{HM}, MOE_{HM}, Den_{bd}, and MC, but not for Vel_{ST}, MOE_{ST}, Den_{gr}, and Diam (Table 1). Set differences were not significant for any trait.

Variation and heritability

Individual-tree broad-sense heritabilities were estimated from clonal repeatabilities (discussed below). Estimated heritabilities were moderate for Den_{bd}, MOE_{HM}, and Vel_{HM}, moderately low for MC, and nonsignificant for the ST300 traits, Den_{gr}, and Diam. Clonal mean heritabilities for the HM200 measures, Den_{bd}, and MC were moderate even though the numbers of ramets per clone were low (Table 1). Den_{bd} had the highest heritability of all seed orchard traits, followed by MOE_{HM} and Vel_{HM}. The coefficient of genetic variation (CGV) was highest for MOE_{HM}, followed by MC, Den_{bd}, and Diam. Clonal variation was nonsignificant for certain traits, probably because of the low sample size (1.4 to 1.9 ramets per clone). Diam was specifically measured to examine the phenotypic relationships between wood properties and tree size (Table 2), and we did not expect to detect significant clonal variation for this trait. Error variation for Diam was large because the trees were grafted at different times and because of the uneven spacing of the seed orchard trees. Furthermore, growth may have been affected by the flower stimulation treatments that also varied among trees. Clonal variation in the ST300 traits may have been obscured by the inherently lower precision of this tool compared to the HM200, the large knots on the seed orchard trees, and logistical constraints that necessitated using the ST300 on felled logs, rather than on standing trees.

Table 2. Type A genetic (r_G), environmental (r_E), and phenotypic (r_P) correlations *(± s.e.)* between traits at the Hood Canal Seed Orchard.

Trait 1	Trait 2	r_G	r_E	r_P
Vel_{HM} [a]	Vel_{ST}	—	—	0.64 *(0.04)*
MOE_{HM}	MOE_{ST}	—	—	0.76 *(0.03)*
Vel_{HM}	MOE_{HM}	0.99 *(0.06)*	0.76 (*0.05*)	0.83 *(0.01)*
Vel_{ST}	MOE_{ST}	—	—	0.84 *(0.03)*
Vel_{HM}	Den_{bd}	0.73 *(0.26)*	0.19 *(0.12)*	0.36 *(0.06)*
MOE_{HM}	Den_{bd}	0.83 *(0.14)*	0.58 *(0.09)*	0.67 *(0.04)*
Vel_{ST}	Den_{bd}	—	—	0.43 *(0.04)*
MOE_{ST}	Den_{bd}	—	—	0.67 *(0.05)*
Vel_{HM}	Diam	—	—	-0.03 *(0.05)*
MOE_{HM}	Diam	—	—	-0.24 *(0.05)*
Vel_{ST}	Diam	—	—	0.07 *(0.04)*
MOE_{ST}	Diam	—	—	-0.20 *(0.03)*
Den_{bd}	Diam	—	—	-0.28 *(0.06)*
Den_{base}	Den_{top}	0.80 *(0.11)*	0.52 *(0.08)*	0.64 *(0.04)*

[a] Vel_{HM} = acoustic velocity measured by HM200; Vel_{ST} = acoustic velocity measured by ST300; MOE_{HM} = modulus of elasticity estimated using Vel_{HM} measurements; MOE_{ST} = modulus of elasticity estimated using Vel_{ST} measurements; Den_{bd} = basic wood density; Den_{gr} = green wood density; Den_{base} = basic wood specific gravity of basal wood disk; Den_{top} = basic wood density of top wood disk; Diam = diameter inside bark. Genetic and environmental correlations were not calculated when genetic differences were nonsignificant.

We studied the extent to which larger sample sizes would provide higher heritability and gain estimates. Fig. 1 shows the expected increase in clonal mean heritabilities (Fig. 1A) and genetic gains (Fig. 1B) if sample sizes are increased from our actual sample size (n = 1.4 to 1.9 ramets per clone) to 50 ramets per clone. This figure shows that genetic gains in wood properties can be obtained by measuring relatively few ramets per clone. For example, using 10 ramets per clone, the $H^2_{C(S)}$ would be about 0.90 for Den_{bd}, 0.85 for MOE_{HM}, and 0.78 for Vel_{HM}. Heritability estimates stabilize at about 0.90 when the sample size is about 10 for Den_{bd}, 16 for MOE_{HM}, and 25 for Vel_{HM}.

Correlations

Table 2 lists the type A genetic correlations (r_G), environmental correlations (r_E), and phenotypic correlations (r_P) between traits. Only phenotypic correlations are listed if the clone-within-set variation was nonsignificant for one or more of the two traits (Table 1). The genetic correlation between Vel_{HM} and MOE_{HM} (0.99) was extremely high, suggesting that it is not necessary to measure wood density in order to rank genotypes effectively. However, because acoustic Vel_{HM} is used to calculate MOE_{HM}, this relationship is inflated due to autocorrelation. The genetic correlations between the HM200 traits and Den_{bd} were high (0.73-0.83), and much larger than the either the environmental or phenotypic correlations (0.19-0.67). The correlation between MOE_{HM} and Den_{bd} (0.83) is inflated because of autocorrelation (i.e., Den_{gr} was used to calculate MOE_{HM}, and Den_{gr} and Den_{bd} were measured on the same disks), but autocorrelation is not involved in the genetic correlation between Vel_{HM} and Den_{bd} (0.73). Therefore, the latter value may be better for evaluating potential genetic relationships between stiffness and wood density. We did not estimate genetic correlations for the ST300 traits (because of nonsignificant clonal variation), but the phenotypic correlation between Vel_{ST} and Vel_{HM} was 0.64, and the phenotypic correlation between MOE_{ST} and MOE_{HM} was 0.76. There was a strong genetic correlation (0.80) between the wood density of the disks sampled from base and top of each butt log (Table 2), indicating that a single disk from the base of a tree should be sufficient for estimating wood density, a practice that we used at the progeny test sites.

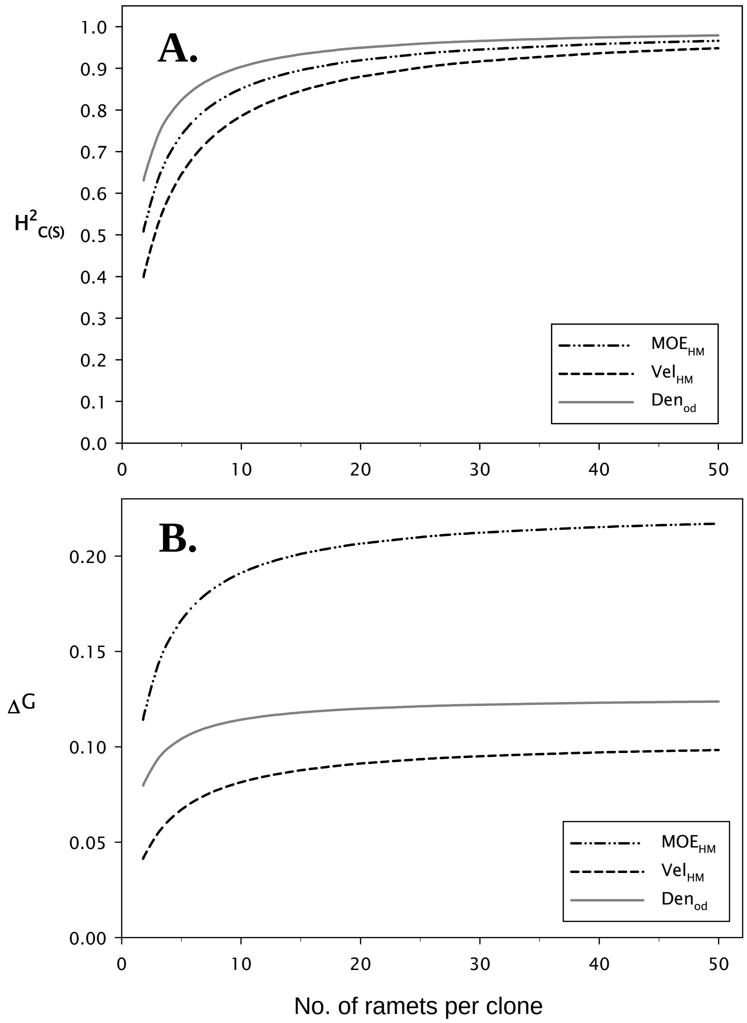

Fig. 1. Expected increases in clonal mean heritabilities ($H^2_{C(s)}$, Fig. 1A) and genetic gains (ΔG, Fig. 1B) when sample sizes are increased from our actual sample size (n = 1.4 to 1.9) up to 50 ramets per clone.

Summary and conclusions

Our results support the hypothesis that we can use seed orchard trees to measure wood properties and select genotypes with improved wood quality, at least for Vel_{HM}, MOE_{HM}, Den_{bd}, and MC. Furthermore, even slight increases in the number of ramets per clone should have a large effect on improving our ability to select superior trees. Therefore, the HM200 appears promising for use in Douglas-fir seed orchards (this study) and progeny tests (Johnson and Gartner 2006). Unfortunately, because trees must be cut down to use the HM200, the standing tree tool (ST300) would be much more flexible and useful. Although we did not detect significant clonal variation for Vel_{ST} and MOE_{ST}, we hypothesize that the ST300 will be useful in seed orchards that have more than a few ramets per clone. It may also be possible to use other approaches or acoustic tools on standing seed orchard trees. Although our overall results are promising, our estimates of clonal heritabilities, genetic gains, and genetic correlations are obviously imprecise because of the small sample sizes (Lynch and Walsh 1998). Our heritabilities may be inflated because of clonal 'C' effects and because for most clones, ramets were planted adjacent to one another. Finally, the seed orchard results are not directly transferable to open-pollinated or half-sib progeny tests because we measured clones. Hence, our genetic parameters include both nonadditive and additive genetic variation. Nonetheless, previous estimates of wood properties in Douglas-fir showed little evidence for substantial nonadditive genetic variation (King et al. 1988). Based on these promising, but somewhat ambiguous results (at least for the ST300), we recommend that additional comparisons of the HM200 and ST300 (or other standing tree tools) be conducted when trees are harvested in other seed orchards.

Acknowledgements

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Plans for 2007-08

We will continue testing lumber stiffness with the stress wave timer and longitudinal vibration tool. We will measure microfibril angle on a subset of the lumber, and all 2x4s will be visually graded. Candidate gene work will also continue. We will also develop specific recommendations and protocols for incorporating stiffness into tree breeding programs. For example, we will determine the optimal and minimum sample sizes needed for estimating stiffness using the acoustic tools, and we will develop breeding strategies such as multistage and multitrait selection indices that capitalize on the strengths of the log and standing tree acoustic tools, plus the added information provided by wood density.

Miniaturized Seed Orchard Study

Introduction

The Miniaturized Seed Orchard Study was undertaken to test alternatives to conventional Douglas-fir seed orchards. Miniaturized seed orchards (MSOs) mimic the management of fruit tree crops—seed crops are produced on many small trees instead of fewer, larger trees at wider spacings, which is typical of conventional Douglas-fir orchards. Intensively-managed MSOs have the potential to (1) increase genetic gains by facilitating controlled mass pollination and (2) reduce management costs because of the smaller trees. However, they are also likely to require more intensive crown management, perhaps involving specialized equipment.

Objectives of the MSO Study

Our objectives are to:

- Compare three orchard types for seed production and management efficiency
- Define the best age to begin floral stimulation in MSOs
- Evaluate crown control techniques
- Compare pollination methods (e.g., CP, SMP)
- Evaluate clonal response to MSO management regimes

Characteristics of three orchard types tested in the Miniaturized Seed Orchard Study

Orchard type	Spacing (m)	Trees per hectare	Total number of trees	Final height (m)
Macro	6 x 4	416	640	4
Mini	4 x 2	1,250	640	2
Micro	3 x 1	3,333	768	2

Plum Creek Miniaturized Seed Orchard

Design

Our study compares 3 tree spacings using 24 grafted clones. Grafts were established between 2002 and 2004. 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). Our goal is to compare management regimes on 3 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).

Accomplishments for 2006-07

The trees at the Plum Creek seed orchard are being managed and maintained until they are large enough to begin experimental treatments. In July 2006, the taller trees were topped, and lateral branches in the upper crowns of these trees were pruned to control crown size and shape, using a protocol similar to that used in previous prunings (Cherry and Howe 2005, 2006). Tree Seal was applied to all cut branches to prevent *Dioryctria* infestations. A cone crop was collected in the fall of 2006, and a few of the clones were quite prolific. Ongoing site maintenance by Plum Creek included weed control, irrigation, fertilization, and rootstock removal.

Plans for 2007-08

Plans for next year include topping the micro (1 x 3 m spacing) and mini (2 x 4 m spacing) orchards at a height of 7', but not pruning any side branches in order to maintain flowering sites on these trees. Trees in the 4 x 6 m spacing will be pruned according to the expected final desired height. The trees will soon be large enough to begin pruning treatments designed to enhance cone crop production. Based on results from our Early Flowering Study, we expect the trees to have reached sufficient size for initiating flower stimulation by the summer of 2008 or 2009. We may carry out exploratory testing of flower stimulation treatments in the supplemental plots adjacent to the MSO. Operational crown management trials will be designed and implemented. We determined that the Washington State Department of Natural Resources' sickle bar pruning equipment is not needed at this time to maintain the miniaturized seed orchard, so we will postpone transporting this equipment to the MSO for crown shaping experiments.

Pruning Study at Roseburg Forest Products Regeneration Center

Design

The pruning study at Roseburg's Vaughn Seed Orchard is testing 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. 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. Pruning will be carried out every 2 years. Eighteen clones with 5 to 9 previously untopped ramets per clone are included in each treatment. The study involves six pruning treatments that are listed below and described in more detail in Cherry and Howe (2005).

Accomplishments for 2006-07

During the summer and fall of 2006, Treatments 5 and 6 were topped and lateral-pruned. The following spring, Treatments 2 and 3 were topped and lateral-pruned. The top and branch pruning protocols are described in Cherry and Howe (2005). In the spring of 2007, male and female flowers were also counted. Crown types were assigned to every tree to qualitatively distinguish tight-branching, narrow crowns from lanky-limbed, sprawling crowns, etc.

Plans for 2007-08

Treatment 4 will be topped and lateral-pruned in the fall of 2007. The results from this study will be used to design pruning treatments at the Plum Creek MSO.

Pruning treatments at the Roseburg Resources Vaughn Seed Orchard

Treatment no.	Description
1	Control = no pruning
Treatments in the year of flower stimulation (begun Spring-Summer ’05)	
2	Top prune and prune branches before bud flush
3	Prune branches before bud flush; top prune in summer, after bud set
4	Top prune and prune branches in summer, after bud set
Treatments in the year of cone production (begun Summer-Fall ‘06)	
5	Top prune and prune branches in summer, after bud set
6	Top prune and prune branches in fall, after cone harvest

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Appendix 1

Publications by PNWTIRC Personnel: 2006-2007

Cherry, M.L., Anekonda, T.S., Albrecht, M.J., and Howe, G.T. 2007. Flower stimulation in young miniaturized seed orchards of Douglas-fir (*Pseudotsuga menziesii*). Can. J. For. Res. 37:1-10.

Cherry, M.L., Howe, G.T., Briggs, D., Cress, D., and Vikram, V. 2007. Genetic variation in wood quality in a clonal Douglas-fir seed orchard. PNWTIRC Report #26.

El-Kassaby, Y.A., Lstibůrek, M., Liewlaksaneeyanawin, C., Slavov, G.T., and Howe, G.T. 2006. Breeding without breeding: approach, example, and proof of concept. *Paper In*: Scientific Program of the IUFRO Division 2 Joint Conference on Low-input Breeding and Genetic Conservation of Forest Tree Species, October 9-13, 2006, Antalya, Turkey.

St.Clair, J.B., and Howe, G.T. 2007. Genetic maladaptation of coastal Douglas-fir seedlings to future climates. Global Change Biology 13:1441-1454.

Appendix 2

Presentations, and Abstracts by PNWTIRC Personnel: 2006-2007

Cherry, M.L., Howe, G.T., Briggs, D., Neale, D., St.Clair, J.B., Cress, D., and Vikram, V. 2006. Genetics of Douglas-fir wood stiffness (MOE) and strength (MOR). 2006. *In*: Proceedings of the Annual Meeting of the Northwest Tree Improvement Cooperative, September 21, 2006, Milepost 18, Oregon.

Howe, G.T. 2007. Importance of conifers: Who benefits from conifer genomics research? *Presentation in*: International Conference on Conifer Genomics, March 18-21, 2007, Banbury Conference Center, Cold Spring Harbor, NY.

Howe, G.T. 2007. Crown management in miniaturized seed orchards. *Presentation in*: Moving into the Future: What is Changing in Seed Orchard Management? Annual Meeting of the Northwest Seed Orchard Managers Association, Little Creek Conference Center, Shelton, WA, June 27-28, 2007.

Howe, G.T. and St.Clair, J.B. 2007. Douglas-fir breeding: Past successes and future challenges. *Keynote address and abstract in:* Proceedings, Tree Improvement in North America: Past, Present, Future. Joint meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association, June 19-22, 2007, Galveston, TX.

Howe, G.T., Anderson, P., St.Clair, J.B., and Cherry, M.L. 2006. Components and mechanisms of Douglas-fir productivity: Toward process and hybrid models of stand growth for the Pacific Northwest. *Presentation in*: NSF I/UCRC Center for Advanced Forestry Systems Planning Meeting, Sept. 13, 2006, Atlanta, GA.

Howe, G.T., Pande, B., St.Clair, J.B., Krutovsky, K.V., and Neale, D.B. 2006. Discovery of genes controlling adaptive traits in Douglas-fir. *Presentation in*: National Workshop on Forest Productivity and Technology: Cooperative Research to Support a Sustainable and Competitive Future, November 8-9, 2006, Washington, D.C.

Pande, B., Krutovsky, K.V., Howe, D., Jermstad, K.D., Hipkins, V, Howe, G.T., St.Clair, J.B., Wheeler, N.C., and Neale, D.B. 2006. Association genetics for adaptive traits in Douglas-fir. *Abstract in*: A joint conference of IUFRO Working Groups 2.04.01 (Population, ecological and conservation genetics) and 2.04.10 (Genomics), and Cost Action E-28 (Genosilva: European Forest Genomics Network), Oct. 1-6, 2006, Alcalá de Henares, Madrid, Spain.

St.Clair, J.B., and Howe, G.T. 2007. Using Tree Improvement to Mitigate Climate Change. *Keynote address and abstract in*: Proceedings, Tree Improvement in North America: Past, Present, Future. Joint meeting of the Southern Forest Tree Improvement Conference and the Western Forest Genetics Association, June 19-22, 2007, Galveston, TX.

St.Clair, J.B., Howe, G.T., Mandel, N., and Vance-Borland. 2006. Genecology of Douglas-fir. *In*: Proceedings of the Annual Meeting and 20th Anniversary of the Northwest Tree Improvement Cooperative, September 21, 2006, Milepost 18, Oregon.

Vikram, V., Howe, G.T., Cherry, M.L., Cress, D., and Briggs, D. 2007. Stiffness of Douglas-fir lumber: Effects of wood properties and genetics. *In*: Annual Meeting of the Stand Management Cooperative, April 25, 2007, Vancouver, WA.

Appendix 3

PNWTIRC Financial Support for Fiscal Year 2006-2007

Regular members[1]	$112,000
Associate members[1]	4,000
Contracts	2,000
Forest Research Laboratory, Oregon State University[2]	122,689
Total	**240,689**

[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.