PNWTIRC

Pacific Northwest Tree Improvement Research Cooperative

Annual Report 2004-2005

Oregon State University

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Pacific Northwest Tree Improvement Research Cooperative

2004-2005 Annual Report

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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 2004-05

- PNWTIRC personnel published 15 journal articles, PNWTIRC Reports, proposals, software programs, and abstracts, and gave 4 research presentations.
- We published the third and final *Pollen Contamination Study* paper as a PNWTIRC report, and the associated Pollen Flow program (PFL) is now available at the PNWTIRC website.
- We published the *Proceedings of the Genetics and Growth Modeling Workshop* on the PNWTIRC website, and Glenn Howe summarized workshop activities and conclusions at an IUFRO Conference entitled *Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future*, which was held in Charleston, South Carolina.
- We helped organize a workshop entitled *Cold Hardiness Testing in Advanced-generation Cooperative Tree Improvement Programs*. This workshop was jointly organized by the NWTIC and PNWTIRC. Marilyn Cherry gave two talks and Glenn Howe gave one talk at the workshop.
- Graft survival in the *Miniaturized Seed Orchard Study* is now 95% after minimal grafting and transplanting in the spring of 2005. We pruned the trees to control tree height and to remove rootstock branches.
- In the spring of 2005, we began a pruning study at the Roseburg Resources Regeneration Center Vaughn miniaturized seed orchard.
- In June of 2005, in conjunction with the PNWTIRC 2005 Annual Meeting, we held two field trips relating to the *Miniaturized Seed Orchard Study*. As member feedback on the field trips was positive, we are planning on having more annual meeting field trips in the future.
- At the 2004 annual meeting, the PNWTIRC decided to concentrate new activities on wood quality and genetics research. Glenn Howe and Marilyn Cherry developed and distributed a detailed wood quality research proposal to members for review. Members voted to approve this study at the 2005 Annual Meeting. The study commenced during the summer of 2005.
- Vikas Vikram arrived in June 2005 to pursue his M.S. with the PNWTIRC. Vikas comes from India, and has a Bachelors of Science in Forestry from Kerala Agricultural University, India. Vikas will be working on the wood quality study for his thesis.

Message from the Director

During the past year, we made a great deal of progress developing our wood quality research, culminating in the distribution of a research proposal in June of 2005, and a decision to move ahead with a multi-faceted research project at the 2004-2005 annual meeting. We obtained a great deal of help from Dan Cress (Olympic Resource Management), Bob Ohrn (BLM), Jim Mosley (BLM), and Rich Kelly (BLM) during the development of our proposal. We also brought a new Master's student on board—Vikas Vikram will be supported by the PNWTIRC to help implement our wood quality research.

During this process, two themes emerged: integration and collaboration. The main objectives of our new wood quality research are to (1) understand the genetics of Douglas-fir wood stiffness and strength, and (2) make recommendations for incorporating wood stiffness and strength into applied tree breeding programs. In our wood quality proposal, entitled *Genetics of Douglas-fir wood stiffness (MOE) and strength (MOR)* (Cherry, Howe, Briggs, Neale, St. Clair, Cress), we proposed a comprehensive research program that integrates direct measurements of wood stiffness and strength, careful evaluation of non-destructive and other indirect measures of wood quality, and genome-scale analyses of wood quality candidate genes. Because of the depth and breadth of this project, we are collaborating with other research organizations in the region to accomplish this work. First, we are partnering with the Stand Management Cooperative (SMC) at the University of Washington (David Briggs, Director) to investigate the use of new acoustic tools (e.g., Fibregen HM-200 and ST-300) that can be used to obtain indirect measures of wood stiffness from logs or standing trees. Our work with the SMC is being supported by a research grant to the SMC from the USDA-Forest Service Agenda 2020 Program. This SMC project is entitled *Non-destructive evaluation of wood quality in standing Douglas-fir trees and logs* (Briggs, Lowell, Turnblom, Lippke, Carter). A second component of our wood quality research is being funded by another grant from the USFS Agenda 2020 Program entitled *Discovery of genes controlling wood property traits in Douglas-fir* (Neale, St. Clair, Howe, Cherry). Our collaborators on this grant are David Neale, a former geneticist with the USFS Institute of Forest Genetics who is now at the University of California at Davis, and Brad St. Clair, the Genetics Team Leader with the USFS Pacific Northwest Research Station (USFS-PNWRS). This genomics research relies heavily on other research being conducted by members of the Douglas-fir Genome Project (http://dendrome.ucdavis.edu/dfgp/).

Our wood quality research is an extension of earlier research conducted by the PNWTIRC. Research by Jesus Vargas-Hernandez and Tom Adams focused on the genetics of Douglas-fir wood density, and resulted in a series of valuable publications (Vargas-Hernandez 1990; Vargas-Hernandez and Adams 1991, 1992, 1994; Vargas-Hernandez et al. 1994; see Literature Cited). By integrating quantitative genetics, wood products research, and genomics, we hope to build upon this foundation and make new contributions to our understanding of Douglas-fir wood quality, including information that can be used to advance Douglas-fir breeding programs. It is important to stress that this comprehensive program would not be possible without the help of many dedicated and enthusiastic individuals and organizations.

Glenn Howe

Research Overview

Miniaturized Seed Orchard Study

In miniaturized seed orchards (MSOs), trees are planted at close spacings in clonal rows, and then maintained at a height of only 2 to 4 m. The potential advantages of MSOs are (1) increased genetic gains by facilitating controlled mass pollination and reducing pollen contamination, (2) speeding genetic gains (and financial returns) by producing operational amounts of improved seed at an earlier age (because of the large number of trees per hectare), and (3) decreasing seed orchard costs because the crowns are closer to the ground, thereby facilitating management techniques such as seed collection, pest management, and bloom delay. Our MSO research has two important components: (1) a test of three alternative MSO designs that we established at the Plum Creek seed orchard complex beginning in 2002, and (2) early flowering and pruning experiments that we initiated in 2001 in two existing MSOs managed by Roseburg Resources. Progress on our *Miniaturized Seed Orchard Study* is described on page 17.

Wood Quality Study

Wood quality has played an important role in breeding programs of forest trees, including Douglas-fir (Zobel and Jett 1995; Howe et al 2005). Historically, most research has focused on wood specific gravity because it is easy to measure in breeding programs, and because high specific gravity is associated with stiff and strong wood, as well as increased pulp yields. For example, the PNWTIRC studied the physiological and quantitative genetics of Douglas-fir wood density in the early 1990s (Vargas-Hernandez 1990; Vargas-Hernandez and Adams 1991, 1992, 1994; Vargas-Hernandez et al. 1994). Although specific gravity plays an important role in breeding programs, we still know little about the genetic relationships between specific gravity and lumber quality. Therefore, we recently initiated a comprehensive project to understand the genetics of Douglas-fir wood stiffness and strength. Wood stiffness is the most important property of structural lumber, and because juvenile wood is less stiff than mature wood, the quality of Douglas-fir lumber may decline as stand rotations decrease. Key components of this project include (1) a milling study to directly measure the stiffness and strength of lumber derived from a Douglas-fir genetic test, (2) evaluation of nondestructive and other indirect measures of wood stiffness (e.g., Fibre-gen HM-200 and ST-300 acoustic tools), (3) a comparison of wood quality measured in parental seed orchard trees vs their progeny growing in genetic test plantations, and (4) a study of associations between wood quality phenotypes and wood quality candidate genes. Ultimately, we will make recommendations for incorporating wood stiffness and strength into Douglas-fir breeding programs, evaluate the relationships between fundamental wood properties (e.g., specific gravity and microfibril angle) vs wood stiffness and strength, and determine whether candidate gene markers can be used to predict wood quality phenotypes. These studies will provide valuable information for designing optimal strategies for improving Douglas-fir wood quality and value. Our plans for the *Wood Quality Study* are described on page 13.

Technology Transfer

Introduction

The PNWTIRC has placed a renewed emphasis on transferring research to tree improvement practitioners. Our technology transfer efforts include distribution of cooperative research reports, meetings with cooperators, annual meetings, and annual reports. One important function of the PNWTIRC is holding workshops for our members on relevant and timely tree improvements topics. In December 2004, the PNWTIRC helped to organize a workshop on *Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs* with the Northwest Tree Improvement Cooperative. We presented the results of former cold hardiness research carried out by the PNWTIRC, and discussed what should be taken into account when deciding on whether to include cold hardiness testing in advanced generation breeding programs.

Accomplishments for 2004-05

Key points from the cold hardiness workshop entitled *Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs*

Twenty-six people attended the workshop on December 7, 2004. The purpose of the workshop was to discuss whether (1) cold injury might be more prevalent in second-generation Douglas-fir progeny testing zones (which are larger than the first generation zones), and (2) whether cold hardiness testing should be incorporated into testing programs. Table 1 lists the presentations given.

Table 1. Presentations given at the workshop entitled *Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs.*

Keith Jayawickrama	OSU	Introduction and overview
Marilyn Cherry	OSU	Conifer cold hardiness: a little background
Marilyn Cherry	OSU	PNWTIRC operational frost hardiness testing protocols
Dana Howe	OSU	Demonstration of frost hardiness testing protocol
Sylvia L'Hirondelle	BCMoF	Cold hardiness testing in the BCMoF tree improvement program: theory and implementation
Glenn Howe	OSU	Genetics of cold hardiness in temperate conifers (with emphasis on PNW conifers)
Brad St. Clair	PNWRS	Genecology of cold hardiness in coastal Douglas-fir in Oregon and Washington: results of common-garden studies
Nick Wheeler	MTBS[1]	Experiences and recommendations on implementing cold hardiness testing in an applied tree improvement program
Keith Jayawickrama	OSU	General discussion

[1] Molecular Tree Breeding Services

In December 2004, the PNWTIRC helped to organize a workshop on Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs *with the Northwest Tree Improvement Cooperative.*

Marilyn Cherry and Sylvia L'Hirondelle pointed out that the physiology of cold hardiness is complex, although numerous advances in testing protocols and increased knowledge have occurred over the past few years. Seedlings are typically more susceptible than older trees to frost damage. After giving some background on conifer responses to cold, Marilyn described the testing protocols used by the PNWTIRC to study cold hardiness.

Sylvia followed up with a description of the cold hardiness testing program used by the B.C. Ministry of Forests (BCMoF). She described the advantages of using chlorophyll fluorescence to monitor cold hardiness. With the decreasing price of high-quality fluorimeters (currently around US $6,000), this method will probably become more popular for answering many of the questions regarding breeding programs in the Pacific Northwest. The BCMoF is currently using chlorophyll fluorescence in their genecology studies. Dana Howe described her Master's thesis research in which she hopes to identify candidate genes associated with cold hardiness in Douglas-fir.

Glenn Howe discussed the genetics of cold hardiness. The timing of acclimation differs greatly between and within populations, with interior and high-elevation populations acclimating earlier than coastal and low-elevation populations. Heritabilities of cold hardiness traits are moderate to high. Glenn proposed that a single test in the fall would be adequate for assessing population differences in fall cold hardiness. Because of the strong relationship between bud flush and spring cold hardiness, bud flush could be used to indirectly determine which families deacclimate sooner. Glenn Howe recommended capturing data on damage from naturally occurring frost events in progeny tests, and testing at least a subset of families in second-generation tests.

Brad St. Clair described cold hardiness testing in relation to his Douglas-fir genecology work. His findings indicate that considerable genetic variation occurs within breeding zones, but current second-generation breeding zones generally account for much of the variation in cold hardiness traits. For example, clinal variation in cold hardiness may be observed within the Trask region, and clinal variation may also occur within the Washington Cascades.

Nick Wheeler described a wide-adaptability study in the Pacific Northwest. The strategy of the agency that implemented the study is to manage for cumulative damage, not rare, extreme events. Decisions are made at the family level, not at the regional level. Early-flushing families are generally considered to be undesirable except when planted on very stress-free sites. Nick cautioned that prior cold hardiness testing is critical when deploying clones on an operational basis. He felt that nursery-stage cold hardiness testing is preferable because it is easier than field testing, and cold damage is more severe at the nursery stage. QTL maps of cold hardiness traits may ultimately lead to approaches for marker-assisted selection.

A general discussion followed the presentations. Participants compared the costs of conducting a cold hardiness test against the merits, especially for second-generation families. At the time of the workshop, bud flush or cold hardiness data had not been collected for second-generation breeding programs coordinated by the NWTIC, nor had there been screening for elite families that flush early in the spring or families that are less cold hardy than other lower-gain families. Spring bud flush may be a good indirect measure of spring cold hardiness. One cold hardiness assessment carried out in the fall, perhaps using a subset of families from a selected test site of a series, may be desirable for

Glenn Howe recommended capturing data on damage from naturally occurring frost events in progeny tests, and testing at least a subset of families in second-generation tests.

Figure 1. Annual meeting 2005 field trip to the miniaturized seed orchard at Plum Creek.

Figure 2. Annual meeting 2005 field trip to the miniaturized seed orchard at Roseburg Resources Regeneration Center

breeding programs. However, the lack of available freezers, measuring equipment, and trained workers could be a challenge.

Annual Meeting 2005 field trips

We held two field trips in conjunction with the PNWTIRC 2005 Annual Meeting. On the afternoon of June 28, 2005, we visited the *Miniaturized Seed Orchard* at Cottage Grove. On the afternoon of June 29, 2005, we visited the Vaughn miniaturized seed orchard at Lebanon to view the pruning trials which were initiated this spring.

Genetics and growth modeling

Proceedings of the *Genetics and Growth Modeling Workshop* held in 2003 were published on the PNWTIRC website:
http://www.fsl.orst.edu/pnwtirc/publications/pnwtirc_pubs_date.htm.

Glenn Howe presented a talk entitled *Accounting for genetic gain in growth models: bridging the gap between geneticists and growth modelers* at the IUFRO conference on Forest Genetics and Tree Breeding in Charleston, SC in November 2004. This talk highlighted some of the key components arising from the *Genetics and Growth Modeling Workshop*.

Additional PNWTIRC reports

We published a PNWTIRC report entitled *Breeding Douglas-fir*. The contents are described in Table 2.

The third and final paper from the pollen contamination study was published as a PNWTIRC report (available online). The Pollen Flow (PFL) computer program was also published on the PNWTIRC website.

Plans for 2005-06

We will be hosting the 2005 annual meeting of the *Western Forest Genetics Association* in Corvallis, OR, in conjunction with the U.S. Forest Service Pacific Northwest Research Station.

Table 2. Breeding Douglas-fir Table of Contents

Wood Quality Study

Introduction

Wood stiffness is the most important property of structural lumber. Wood characteristics are particularly important for coastal Douglas-fir, which is used primarily for dimension lumber and is renowned for its high strength and dense wood. Maintaining wood stiffness and strength are essential for ensuring Douglas-fir's niche in the domestic and international timber markets. Because juvenile wood is less stiff than mature wood, the quality of Douglas-fir lumber may decline as stand rotation lengths decrease. Unfortunately, destructive testing, in which trees are felled in order to obtain wood quality measurements, has been the standard procedure for evaluating wood stiffness and strength. Therefore, the timber owner does not have a reliable way to assess the value of the final product prior to harvest. Post-harvest methods for measuring wood strength, such as visual grading and machine stress rating, are also not ideal.

Wood traits of coniferous species are typically highly heritable, and may be improved through selection and breeding. Because of the importance of wood quality to timber value, and the inadequacy of current methods for assessing wood quality prior to harvest, a number of agencies within the region are interested in studying the genetics of wood quality. At the 2004 PNWTIRC Annual Meeting, members approved the development of a research proposal to study the genetics of Douglas-fir wood stiffness and strength. For example, members would like to know whether nondestructive tools can be used to assess wood properties in their tree improvement programs. The University of Washington Stand Management Cooperative (SMC) was recently awarded a USDA Agenda 2020 research grant to investigate non-destructive evaluation of Douglas-fir wood quality. At the same time, the USDA Forest Service Pacific Southwest Research Station (PSWRS), USDA Forest Service Pacific Northwest Research Station (PNWRS), University of California Davis (UC Davis), and the PNWTIRC were awarded a USDA Agenda 2020 research grant to study genes that influence wood property traits in Douglas-fir. The *Wood Quality Study* was designed to address these and other questions in collaboration with the SMC, PNWRS, and PSWRS.

Accomplishments for 2004-05

Project development

Direct measurement of wood stiffness and strength requires destructive testing. Therefore existing, high-quality genetic tests that are about

Maintaining wood stiffness and strength are essential for ensuring Douglas-fir's niche in the domestic and international timber markets.

20 years old and due for thinning are ideal for this project. Glenn Howe and Marilyn Cherry visited numerous first-generation progeny test sites and cooperators in Washington and Oregon during the fall and winter of 2004 to evaluate the suitability of each series for inclusion in the proposed study. Potential series were narrowed down to Olympic Resource Management's Port Gamble series and its associated clonal seed orchard in Washington, and the BLM's Breeding Unit 13 series and its associated clonal seed orchards in Oregon. A research proposal was developed and presented to PNWTIRC members at the 2005 Annual Meeting. The four main objectives of the study, and approaches for addressing each objective, are described below.

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)

Direct measures of MOE and MOR from lumber will be used to estimate genetic parameters and heritabilities, and to predict genetic gains for each trait. We will also examine the genetic correlations among traits. The direct measures of MOE and MOR will be derived from a subsample of the Port Gamble progeny test (50 families, 8 trees per family). Lumber (1.5˝ x 3.5˝ x 8') will be cut from the logs using a portable sawmill, obtaining as many pieces of lumber as possible from each log. The lumber will be kiln-dried, and tested. MOE and MOR will be estimated using bending tests in which force is applied to the middle of a supported beam.

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

The use of wood quality traits in tree improvement programs requires the use of rapid measurement techniques that are preferably non-destructive and applicable to small trees. Various indirect measures of wood quality show promise, but data on Douglas-fir are limited. Therefore, it would be valuable to evaluate indirect measures of wood quality relative to direct measures of the same traits. Wood quality traits are difficult and expensive to measure directly compared to traits such as height and diameter, but indirect methods are becoming increasingly available. Indirect measures of MOE include various acoustic techniques using tools such as the Fibre-gen Director ST300™, hereafter referred to as the ST300, and Fibre-gen Director HM200™, hereafter referred to as the HM200. The ST300 is designed to be used on standing trees, wereas the HM200 is used on logs.

We will calculate genetic correlations between (1) the direct measures of MOE and MOR from lumber pieces and (2) indirect estimates of MOE obtained using the ST300 and HM200. These analyses will be used to calculate relative gain efficiencies 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 recommend nondestructive test procedures for tree improvement programs.

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

It may be possible to use parents in clonal seed orchards to efficiently and cheaply estimate the wood quality of their progeny. This would be desirable because seed orchard trees are normally easier to access and measure, but are

Wood quality is one of the key areas of interest for gene association mapping.

also open-grown and hence more likely to have larger growth rings and different wood properties than trees growing in progeny tests. The ability to measure fewer trees in an easily accessed seed orchard would be valuable because of the high costs of measuring wood quality traits. Therefore, we will compare genetic parameters and relative rankings of wood quality traits between clonal orchard parents and their progeny. We will determine whether gains in progeny wood quality can be predicted via indirect selection of the parents. Futhermore, we will estimate heritabilities using parent-progeny regression and compare these values to heritabilities derived from analyses of open-pollinated progeny tests.

Initially, analyses will focus on wood specific gravity and acoustic measures of wood stiffness. However, because we will retain the wood disk samples, it will be possible to analyze other wood properties in the future.

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

Wood quality is one of the key areas of interest for gene association mapping. A population genomic approach called association mapping can now be used to identify genes responsible for phenotypic differences in wood properties. This may allow promising genotypes to be selected without having to grow and measure them in long-term field tests.

In a previous study of loblolly pine (Groover et al 1994; Sewell et al 2000, 2002; Brown et al 2003), the number, effect size, and approximate location of quantitative trait loci (QTL) controlling wood properties were estimated, and significant associations between candidate genes and wood properties were detected. We will now verify these associations in a second species, Douglas-fir. Verification of these associations in a second conifer species will provide convincing evidence that the individual genes controlling conifer wood properties have been identified.

Candidate genes for adaptive traits and wood properties have already been identified in Douglas-fir. More than 11,000 expressed sequence tags (ESTs) were sequenced, and single nucleotide polymorphisms (SNPs) in candidate genes for adaptive traits and wood properties were discovered as part of a completed USDA-Forest Service Agenda 2020 project (Phase I; Neale 2002). These sequences

Expected Outcomes of the Wood Quality Study

- Obtain information that can be used to develop optimal strategies for improving Douglas-fir wood quality and value
- Identify wood properties that can be incorporated into breeding programs to improve wood stiffness and strength
- Make recommendations for using nondestructive testing to improve wood quality in tree improvement programs
- Make recommendations for using wood properties of seed orchard parents to predict the wood properties of their progeny
- Identify molecular genetic markers associated with desirable wood properties in Douglas-fir that may be used by Douglas-fir breeders and gene resource managers
- Determine whether associations between wood quality phenotypes and candidate genes detected in loblolly pine are also present in Douglas-fir

Figure 3. Logging the Olympic Resource Management Hood Canal Seed Orchard, March 2005.

Figure 4. Removing wood disks from both ends of a butt log (17' log cut above the graft union) at the Hood Canal Seed Orchard.

Figure 5. Collecting data from wood disks at the Hood Canal Seed

Figure 6. Measuring acoustic velocity of each log at the Hood Canal Seed Orchard.

are available at GenBank (http://www.ncbi.nlm.nih.gov) and TreeGenes (http://dendrome.ucdavis.edu/treegenes). Additional ESTs have been added to this database, and the expanded database is now being used to identify additional wood property candidate genes. SNPs in these genes will be used for genotyping and for testing wood property associations in Douglas-fir.

Foliage samples from seed orchard ramets will be used for DNA isolation. Samples will be forwarded to UC Davis for genotyping of wood quality candidate genes. Associations between wood quality phenotypes and candidate genes found in loblolly pine will then be tested using our Douglas-fir samples. We will test for associations between the wood quality genotypes of the seed orchard parents and the wood quality phenotypes of the seed orchard parents and their progeny.

Project implementation

The study proposal was approved by members at the 2005 Annual Meeting. Work commenced at the Hood Canal Seed Orchard in Washington in March 2005 when the orchard was rogued (Figures 3, 4, 5, and 6). Specific gravity, tree ring counts, and acoustic velocities of 17' butt logs were measured using the HM200 and ST300. At the same time, foliage was collected for the candidate gene work.

Plans for 2005-06

The candidate gene analyses are progressing. A new PNWTIRC graduate student, Vikas Vikram, will be working on a portion of this study for his Master's thesis. Plans are being made to harvest the Olympic Resource Management Port Gamble progeny tests. During the summer, trees will be measured for dbh and standing tree acoustic velocity. Harvesting will commence in the fall of 2005. Nine-foot butt logs will be measured for log acoustic velocity and wood disks will be measured for specific gravity and ring count traits. A subsample of logs from each of 50 families per site will be milled into 2 x 4 lumber, and tested for MOE and MOR using bending tests.

Miniaturized Seed Orchard Study

Miniaturized Seed Orchard at the Plum Creek Seed Orchard Complex

Miniaturized seed orchards (MSO) are promising alternatives to conventional seed orchards. MSOs are designed to be intensively managed in an efficient and cost-effective manner. Seed crops are produced on numerous small, closely-spaced trees instead of fewer, wider-spaced, larger trees found in conventional 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).

Our goal is to compare management regimes on 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).

Following the advice of the MSO Advisory Committee, the original study plan was slightly altered in the spring of 2004 so that we could retain as many established grafts as possible, and graft as few new grafts as possible during the spring of 2004. The MSO now contains 24 clones in each of the 3 spacings; 8 of these clones were newly established in 2004. After we completed transplanting and grafting in the spring of 2004, overall survival was 96 %. Spare field grafts held in a clone bank are available for filling in dead material as needed.

Accomplishments for 2004-05

We topped the tallest orchard trees on September 9, 2004 to control their height. This will allow the smaller trees the chance to catch up in size. Top pruning was carried out as needed in all main plots plus all spare plots and the clone bank. Rootstocks of established grafts were also cut back in 2004 and 2005, with the intention of removing all remaining rootstock branches over the next year. This pruning protocol was not designed to enhance future flowering sites, but was only intended to control height. Now that the grafts are fairly uniform in size, we can begin managing crowns for final desired heights.

The protocol for orchard pruning in 2004 was as follows:

- Trees taller than ~1½ m (~5′) were topped to ~1½ m in height (except for a handful of very tall trees grafted in 2002, which were topped to around 1²/3 m)
- Terminal shoots were cut at a slight angle ~1 cm above a well-formed bud; this bud

should assume apical dominance and become the new terminal shoot

- If the uppermost remaining bud was one of a whorl of buds, then the other buds in that whorl were removed
- The uppermost lateral branches of the scions were pruned back to prevent them from assuming apical dominance
- On pruned laterals, as few buds as possible were removed from each branch, which should encourage the crown to fill out
- If the upper laterals immediately below the apical leader were far enough below the leader, then only the terminal cluster of buds at the tip of the lateral branch were removed, keeping one well-formed bud just below the cut
- If the lateral branches were very long or located just below the main leader, then they were pruned to about half their original length, just distal to a well-formed bud
- Dioryctria frass was later noted on the cut branches, but no damage has been observed

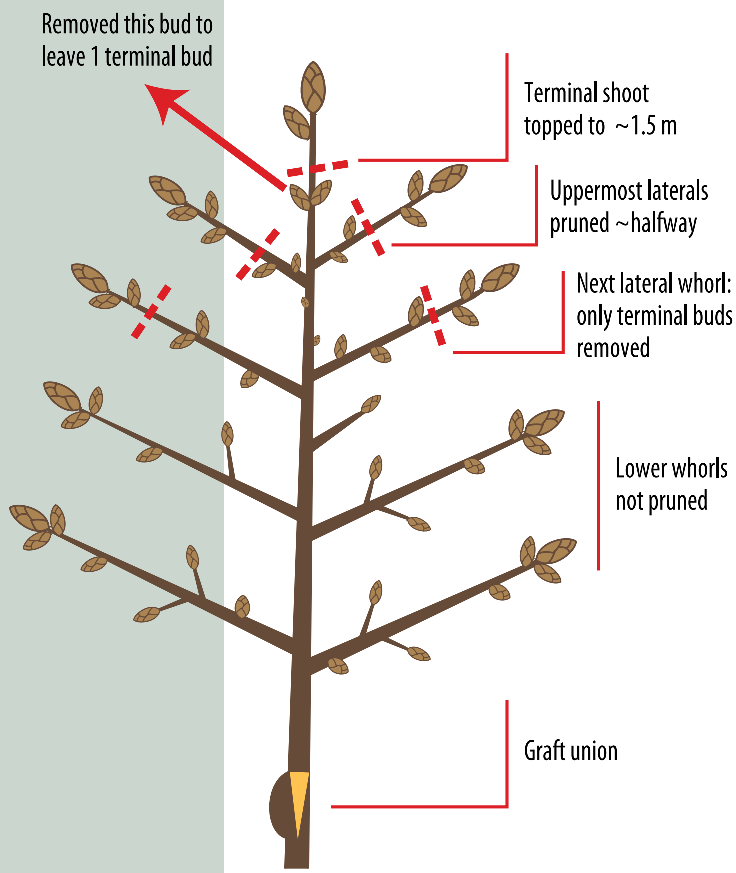

Figure 7. Plum Creek miniaturized seed orchard top height control protocols, fall 2004.

Site maintenance by Plum Creek is ongoing, including weed control, irrigation, and fertilization.

Plans for 2005-06

Now that the orchard establishment phase is almost complete, we will begin crown control. After the 2005 growing season, we will prune the tops of the taller trees to allow shorter trees to catch up in size. Trees with dead scions will be replaced with transplants from the clone banks. A study design will be developed, and operational crown management trials may begin as early as 2006.

Pruning Study at Roseburg Resources Regeneration Center

Introduction

A pruning study was initiated to test the effects of pruning timing and leader retention on crown form and cone production. Our goal is to learn about physiological responses to pruning prior to applying similar treatments at the Plum Creek Miniaturized Seed Orchard. This study uses slightly older, larger trees at the Vaughn Seed Orchard owned by Roseburg Resources. Our initial experiments will focus on the physiology of pruning and cone production, whereas later experiments will integrate operational concerns. We assumed that pruning will be carried out every 2 years, and that cone production will be affected by the timing of pruning.

Accomplishments for 2004-05

Eighteen clones were included in the pruning study, with 5 to 9 ramets per clone per treatment. Only ramets that had never been topped were chosen for the study. Clones had been grafted in either 1999 or 2000. Six pruning treatments were initiated (Table 3). Flower stimulating treatments using a combination of stem girdling and gibberellic acid (GA) stem injections were applied in late spring 2005.

The following pruning protocols were used:

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.

Branch pruning Treatments 2 and 3 were applied on March 23, 2005, and the trees in Treatment 3 were top pruned in July 2005. Lateral and leader pruning of Treatment 4 was carried out on July 12, 2005.

Plans for 2005-06

Treatments 5 and 6 will be carried out in 2006, one year after flower stimulation.

Table 3. Pruning treatments at the Roseburg Resources 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 top of the tree. 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.

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Appendix 1

Publications and Abstracts by PNWTIRC Personnel: 2004-05

Aitken, S.N., Howe, G.T., Neale, D.B., and Jermstad, K.D. 2004. Adaptation to cold in forest tree populations. Abstract in: 12th New Phytologist Symposium on Functional Genomics of Environmental Adaptation in *Populus*, Gatlinburg, Tennessee, October 10-13, 2004.

Boys, J., Cherry, M.L., and Dayanandan, S. 2005. Microsatellite analysis reveals genetically distinct populations of red pine (*Pinus resinosa*). Amer. J. Bot. 92(5): 833-841.

Cherry, M.L. and Howe, G.T. (eds.) 2004. Proceedings, Genetics and Growth Modeling Workshop. November 4-6, 2003, Vancouver, Washington. Pacific Northwest Research Cooperative Report #21, Oregon State University, 171 pp. (http://www.fsl.orst.edu/pnwtirc/publications/Electronic%20version%20of%20some%20pubs/Growth%20Modeling%20Proceedings%20-%20PNWTIRC.pdf)

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Greifenhagen, S. and Cherry, M.L. 2005. Estimating decay in living trees - an assessment of 2 decay detection instruments. Ontario Forest Research Institute Forest Research Report No. 153, Ontario Ministry of Natural Resources, 12 pp.

Howe, G.T. and Cherry, M.L. 2004. Accounting for genetic gain in growth models: bridging the gap between geneticists and growth modelers. Abstract in: IUFRO Conference on Forest Genetics and Tree Breeding in the Age of Genomics: Progress and Future, November 1-5, 2004, Charleston, South Carolina.

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Slavov, G.T., Howe, G.T., Gyaourova, A.V., Birkes, D.S., and Adams, W.T. 2004. Pollen Flow (PFL): A computer program for estimating pollen flow using highly variable markers and paternity exclusion (http://www.fsl.orst.edu/pnwtirc/publications/pnwtirc_pubs_date.htm).

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Slavov, G.T., Howe, G.T., and Adams, W.T. 2005. Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers. Can. J. For. Res. 35: 1592-1603.

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Appendix 2

Workshops and Presentations by PNWTIRC Personnel: 2004-05

Cherry, M.L. 2004. Conifer cold hardiness: a little background. Presentation in: Joint NWTIC/PNWTIRC Workshop entitled Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs, December 7, 2004, Oregon State University, Corvallis, OR.

Cherry, M.L. 2004. PNWTIRC operational frost hardiness testing protocols. Presentation in: Joint NWTIC/PNWTIRC Workshop entitled Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs, December 7, 2004, Oregon State University, Corvallis, OR.

Howe, G.T. 2004. Genetics of cold hardiness in temperate conifers (with emphasis on PNW conifers). Presentation in: Joint NWTIC/PNWTIRC Workshop entitled Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs, December 7, 2004, Oregon State University, Corvallis, OR.

Howe, G.T. and Cherry, M.L. 2004. Accounting for genetic gain in growth models: bridging the gap between geneticists and growth modelers. IUFRO Conference on Forest Genetics and Tree Breeding, Charleston, SC, November, 2004.

Appendix 3

PNWTIRC Financial Support for Fiscal Year 2004-05

Regular members[1]	$104,000
Associate members[1]	4,000
Contracts	2,000
Forest Research Laboratory, Oregon State University[2]	106,484
Total	216,484

[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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