20 YEARS

PNWTIRC

Pacific Northwest Tree Improvement Research Cooperative

OREGON STATE UNIVERSITY
FOREST SCIENCE

annual report 2002 - 2003

PNWTIRC Participants

Regular Members

- *Boise Corporation*
- *Longview Fibre Company*
- *Menasha Forest Products Corporation*
- *Olympic Resource Management*
- *Oregon Department of Forestry*
- *Oregon State University*
- *Plum Creek Timber Company*
- *Port Blakely Tree Farms*
- *Roseburg Resources*
- *Simpson Timber Company*
- *Stimson Lumber Company*
- *USDI Bureau of Land Management*
- *Washington State Department of Natural Resources*
- *Weyerhaeuser Company*

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- *South Coast Lumber Company*
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- *Lone Rock Timber Company*
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- *Inland Empire Tree Improvement Cooperative*
- *Northwest Tree Improvement Cooperative*
- *University of British Columbia*
- *University of Washington*
- *USDA Forest Service, Pacific Northwest Research Station*

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annual report 2002-2003

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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: Gancho Slavov

Highlights of 2002-2003

- The PNWTIRC is 20 years old!
- Marilyn Cherry began as Assistant Director of the PNWTIRC in March 2003.
- Boise Corporation joined the PNWTIRC.
- We completed five journal articles and abstracts (i.e., published or submitted) and 11 presentations that deal with PNWTIRC research projects.
- In the Pollen Contamination Study, we mapped 20 of our 22 highly variable SSRs to 10 linkage groups in Douglas-fir. We submitted a manuscript and distributed a PNWTIRC report describing the development of these markers. We used our SSRs to (1) measure seed and pollen contamination and (2) determine the relative maternal and paternal contributions of the clones in the orchard. In collaboration with the National Forest Genetic Electrophoresis Laboratory (NFGEL), we developed a protocol and measured the success of supplemental mass pollination for 6 crosses from the Weyerhaeuser program. Our results indicate that SSR markers are a valuable tool for Douglas-fir tree improvement programs.
- We identified two areas of future research that will be added to our mix of research topics: (1) how to incorporate genetic improvement into growth models and (2) the genetics of wood quality.
- A second year of grafting was carried out in the Miniaturized Seed Orchard Study in March and April of 2003. Forward and backward selections were grafted into three orchard types (macro, mini, and micro) and two supplemental blocks (mini and micro).
- We measured the results of the early flowering treatments that were applied in spring 2002 (Early Flowering Study). Gibberellic acid and girdling treatments were applied to 3- and 5-year-old grafts in the Vaughn and PNWCTA seed orchards. The combined GA/girdling treatment significantly increased the number of female cones on both the 3- and 5-year-old grafts, and increased the number of pollen cones and proportion of trees bearing seed cones in the 5-year-old orchard. Large clonal responses were observed. Flower stimulation treatments did not impede growth. Our results indicate the potential for obtaining commercially harvestable crops in just a few years after orchard establishment.
- We held a workshop entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation* in collaboration with the Institute of Forest Genetics—USFS Pacific Southwest Research Station and the USFS Pacific Northwest Research Station.

Message from the Director

Happy anniversary—the PNWTIRC is 20 years old! The PNWTIRC was officially established on July 1, 1983 after a lot of hard work by Tom Adams, representatives from private industry, and administrators in the College of Forestry at Oregon State University (see PNWTIRC History, page 22). Six of the original contributing members are still active—Oregon State University, Bureau of Land Management, Menasha Corporation, Sun Studs (Lone Rock Timber), Washington Department of Natural Resources, and Weyerhaeuser Corporation. Three other organizations no longer participate in the cooperative or no longer exist in the Pacific Northwest. These include Champion International, Crown Zellerbach, and International Paper. Today, the PNWTIRC has grown to 16 contributing members (see inside front cover).

The PNWTIRC has made a large contribution to forest genetics research in the Pacific Northwest. This is due to the strong leadership of Tom Adams, the co-op's Director for nearly 20 years; PNWTIRC staff, including Glenn Howe, Dennis Joyce, Sally Aitken, and Thimmappa Anekonda; and graduate students, including Brad St. Clair, Peng Li, Jesus Vargas-Hernandez, Lisa Balduman, Christine Lomas, Andy Bower, Nicolas Schermann, Greg O'Neill, Fatih Temel, and Gancho Slavov. This year, we are pleased to welcome Marilyn Cherry to the cooperative as the new PNWTIRC Assistant Director.

In addition to celebrating the past, we also need to reflect on the present and look to the future. During the past year, we gained one new PNWTIRC member—Boise Corporation. Beginning in 2000, we began a campaign to attract new members, ultimately adding Pope Resources (Olympic Resource management), Port Blakely Tree Farms, and now, Boise Corporation to the PNWTIRC membership. Another recent goal has been to promote technology transfer. Last year, we organized a workshop entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation*. This workshop was co-sponsored by the PNWTIRC; Institute of Forest Genetics—USFS Pacific Southwest Research Station; and the USFS Pacific Northwest Research Station. Next year, we will hold a workshop on the impacts of genetics on growth modeling.

At the annual meeting in July, 2003, we decided to focus future research on two new topics: (1) how to incorporate genetic gain into growth models and (2) the genetics of wood quality. During the next year, we will initiate work in each of these areas, augmenting our ongoing projects on pollen contamination, early flowering, and miniaturized seed orchards. In this year's annual report, we describe the progress we made during the past 12 months and celebrate the first 20 years of the PNWTIRC.

Glenn Howe

Introduction

Research Overview

Most of our current research focuses on seed orchards. This research falls into three main areas: (1) pollen contamination, (2) early flowering, and (3) miniaturized seed orchards. An overview of these topics is presented below. As our pollen contamination research winds down, we will be taking on new research topics—how to incorporate genetic gain into growth models and the genetics of wood quality (see New Research Directions).

Pollen contamination. Pollen from unimproved trees (or poorly adapted genotypes) surrounding seed orchards can significantly reduce genetic gains by fertilizing orchard seed. Pollen contamination in conventional orchards often exceeds 40% and can adversely affect both realized genetic gains and adaptability (Wheeler and Jech 1986; Adams and Burczyk 2000). The ultimate goal of the Pollen Contamination Study is to increase genetic gains by reducing pollen contamination. By developing powerful DNA-based genetic markers for measuring pollen contamination, seed orchard managers will have new tools for testing strategies designed to reduce pollen contamination—strategies such as bloom delay, selective harvesting of orchard seed, or pollination control (e.g., controlled mass pollination or supplemental mass pollination). Progress on our Pollen Contamination Study is described on page 8. We also describe how the molecular markers we developed for Douglas-fir can be used to identify genotypes in tree improvement programs, measure the relative maternal and paternal contributions of seed orchard trees in seedlots, and measure the success of supplemental mass pollination.

Early flowering. The goal of the Early Flowering Study is to speed genetic gains from tree improvement by promoting seed production on very young orchard grafts. Many first-generation Douglas-fir orchards took 10 to 15 years to produce useful amounts of seed (Cress and Daniels 1990). The long time lag between seed orchard establishment and seed production represents a huge 'opportunity' cost. Results from our early flowering study suggest that operational amounts of improved seed can be obtained from high-density seed orchards as young as 2-4 years from grafting. Progress and results from our Early Flowering Study are described on page 12.

Miniaturized seed orchards. In miniaturized seed orchards (MSOs), the trees are planted at close spacings, then maintained at a height of only 2 to 4 m (Sweet and Krugman 1977, Webber and Stoehr 1998). One advantage of miniaturized (i.e., high-density) orchards is that it may be possible to obtain operational amounts of improved seed at much earlier ages. In addition, with trees planted in clonal rows, it should be easier to control pollen contamination and produce elite crosses to increase genetic gains because the trees are short and easily accessible, making controlled mass pollination and supplemental mass pollination easier. Finally, the costs and efficiencies of some management techniques may be lower because fewer acres are needed, and the crowns are closer to the ground, thereby facilitating management techniques such as seed collection, pest management, and bloom delay. Progress on our MSO Study is described on page 15.

New Research Directions

At the annual meeting in July, 2003, we decided to add two new topics to our mix of PNWTIRC research topics. These are (1) how to incorporate genetic gain into growth models and (2) the genetics of wood quality. This decision culminates a careful analysis and prioritization of potential PNWTIRC research topics (see PNWTIRC Annual Report, 2001-2002). These topics will be added to our list of ongoing research topics: pollen contamination in Douglas-fir, early flowering in grafted seed orchards, and miniaturized seed orchards for Douglas-fir.

Technology Transfer

In 2002, we began offering occasional workshops on important tree improvement topics. We held the second of these workshops in November 2002. This workshop was entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation* (Table 1). The goals of this workshop were to (1) educate forest scientists, policy makers, and tree breeders about genomics and (2) discuss how genomics might be used to increase forest productivity and promote gene conservation in the Pacific Northwest. Special attention was paid to summarizing genomics research in Douglas-fir, integrating genomics with applied tree improvement, and charting a course for the future. Next year, we plan to hold our third workshop since 2002, entitled *Genetics and growth modeling.*

Table 1. Agenda of a workshop entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation.* This workshop was held November 14, 2002, and was jointly sponsored by the PNWTIRC; Institute of Forest Genetics – USFS Pacific Southwest Research Station; and the USFS Pacific Northwest Research Station.

Speaker	Affiliation	Title of presentation
Glenn Howe	PNWTIRC, OSU	*Introduction: What is 'Genomics'?*
Gancho Slavov	PNWTIRC, OSU	*Molecular Markers: An Overview*
David Neale	Institute of Forest Genetics	*Gene Mapping and the Genetic Dissection of Complex Traits in Conifers*
Kostya Krutovskii	Institute of Forest Genetics	*Comparative Gene Mapping between Loblolly Pine and Douglas-fir*
Steve Strauss	TGERC, OSU	*Genetic Engineering: Its role in Genomics Research*
Richard Cronn	USFS PNWRS	*Population Genomics and Gene Conservation*
Glenn Howe	PNWTIRC, OSU	*Using 'Gene Chips' to Understand Tree Physiology*
Randy Johnson	USFS PNWRS	*Integrating Genomics with Applied Tree Breeding*
All	Many	*Conclusions and Recommendations*

Pollen Contamination Study

Introduction

Pollen contamination is measured as the proportion of seeds fertilized by pollen coming from outside of the seed orchard or orchard block. Pollen contamination often exceeds 40% in conventional Douglas-fir orchards (Adams and Burczyk 2000), which could reduce genetic gains by 20% or more. Therefore, efficient methods for measuring and managing pollen contamination are needed.

Pollen contamination is typically measured with genetic markers. The goal of the Pollen Contamination Study is to develop improved, DNA-based genetic markers called simple sequence repeats (SSRs) for estimating pollen contamination in Douglas-fir seed orchards.

What are SSRs?

SSRs (simple sequence repeats) are stretches of DNA composed of many short repeats (e.g., repeats of 2-3 nucleotides, such as 'AC' or 'ATC') that are aligned end-to-end (in tandem). Because the number of repeats often varies between chromosomes and individuals, SSRs are good genetic markers. For example, an SSR locus with 12 repeats of 'AC' (i.e., $(AC)_{12}$ = ACACACACACACACACACACACAC), might mutate to $(AC)_{13}$, or 13 tandem repeats of 'AC.' SSRs can be scored by isolating DNA, amplifying the SSR region with DNA primers and the polymerase chain reaction (PCR), then measuring the length of the resulting DNA band after it is pulled through a gel with an electric current (electrophoresis). Because the $(AC)_{13}$ DNA fragment is slightly longer than the $(AC)_{12}$ fragment, it will migrate a little more slowly through the gel. Therefore, each different SSR allele appears as a band at a different location on the gel. A good SSR marker is genetically variable (e.g., has 8-15 alleles in the test population), has a low frequency of null (i.e., 'missing') alleles, and amplifies a single locus in each PCR reaction.

SSRs are more informative than traditional isozyme markers

SSRs are more informative because they are highly variable (often having more than 10 alleles per locus; Goldstein and Pollock, 1997) and abundant in the genomes of most higher organisms (Powell et al. 1996). Therefore, SSRs have become the markers of choice for measuring pollen contamination, evaluating the relative contributions of the parents to the orchard seed, identifying genotypes, verifying controlled crosses, and measuring the success of supplemental mass pollination (SMP) (Stoehr and Newton 2002; Chaix et al. 2003).

The objectives of the Pollen Contamination Study are to (1) develop 7-10 SSR marker loci for Douglas-fir, (2) confirm the inheritance of the markers and measure their genetic variability, (3) use the most variable markers to measure pollen contamination in a conventional seed orchard, (4) optimize testing and estimation procedures, and (5) determine how pollen contamination varies with flowering phenology and location of the ramets within the orchard. Our study site is one orchard block of the Plum Creek seed orchard in western Oregon, hereafter referred to as the *Test Block*. In 2001-2002, we developed and characterized 22 highly variable SSR markers for Douglas-fir (i.e., we completed objectives 1 and 2).

Accomplishments for 2002-2003

This year, we completed objectives 3 and 4 of the Pollen Contamination Study. We determined the location of 20 of our 22 SSR markers on a Douglas-fir genetic map. We also demonstrated the potential applications of SSR markers in Douglas-fir tree improvement programs. First, we tested different analytical methods for measuring pollen contamination using SSR markers. Second, we measured seed and pollen contamination in the *Test Block*. We also determined the relative parental contributions of the clones in that block. Finally, we developed and applied a step-by-step protocol for measuring the success of SMP in Douglas-fir seed orchards.

A suite of highly variable and fully characterized SSR markers is available for Douglas-fir

In collaboration with Konstantin Krutovskii and David Neale from the Pacific Southwest Research Station, we mapped 20 of our 22 highly variable SSR markers to 10 Douglas-fir linkage groups. The 20 markers that we mapped are well dispersed throughout the Douglas-fir genome. The genetic distances between adjacent SSRs on the same linkage group were at least 10 cM[1] in all cases but one (in which the two markers were 4 cM apart). Therefore, most of our mapped SSRs can be treated as independent loci, which enhances their usefulness for genetic fingerprinting.

Tree breeders can use SSRs for genetic fingerprinting

We demonstrated the broad applicability of these markers in several small-scale projects involving genetic fingerprinting, and used our SSR markers to measure pollen contamination in the *Test Block*. We also collaborated with Valerie Hipkins and Robert Saich from the National Forest Genetic Electrophoresis Lab (NFGEL) on transferring the technology of using our SSRs to NFGEL. Presently, NFGEL staff is ready to apply our highly variable SSR markers to a variety of projects in Douglas-fir (information on how to submit projects to the NFGEL is available at http://dendrome.ucdavis.edu/NFGEL/pjsubmission.html).

SSRs will enhance Douglas-fir tree improvement

A set of Douglas-fir SSR markers is now available to:

- Verify clonal IDs
- Certify seedlots
- Measure pollen and seed contamination
- Analyze the relative parental contributions of parents and the frequency of specific crosses in seed orchard seed
- Measure the success of SMP

[1] One cM corresponds to 1% chance that an allele at one locus will be separated from an allele at another locus due to recombination in a single generation. In large, outcrossed populations, such as those of most conifers, markers located more than 1-2 cM apart can be treated as independent loci (Epperson and Allard 1987).

First, we used our SSRs to verify the identity of two Douglas-fir parents that were selected for grafting into a new seed orchard. These two trees were thought to be field selections that performed well in progeny tests, but their original markings were gone. Using only 3 of our SSRs, we fingerprinted the 2 trees and a number of offspring of the desired plus trees (i.e., offspring growing in progeny tests), and concluded that one of the parents was indeed the desired plus tree, but the other one was not the desired plus tree. The correct parent was included in the new seed orchard, but the incorrect parent was not, thereby preventing loss of genetic gain (this project is described in more detail in the 2001-2002 PNWTIRC Annual Report).

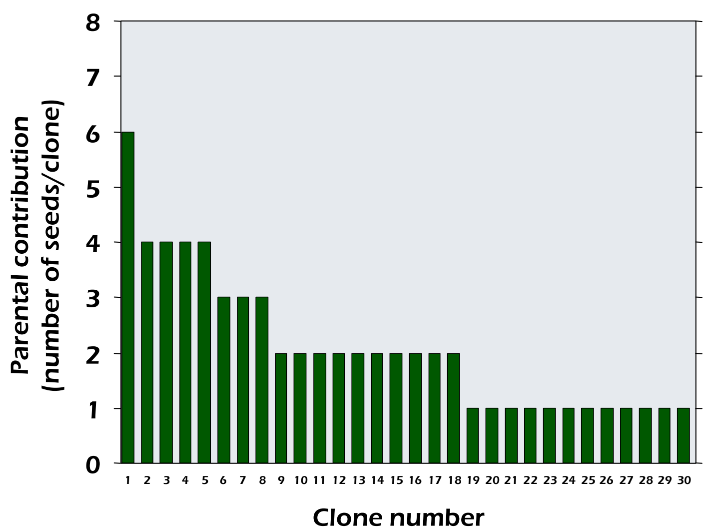

Figure 1. *Relative paternal contributions of 30 clones from the Test Block: 63 out of 102 seeds analyzed were fathered by clones from the Test Block.*

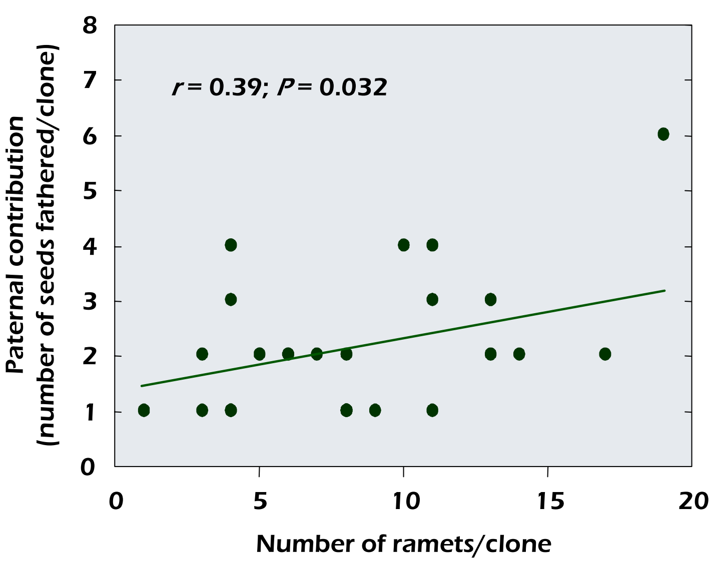

Figure 2. *Relationship between the number of ramets of 30 clones from the Test Block and the number of seeds fathered by each of these clones.*

Second, we developed a protocol for efficiently verifying the clonal identities of ramets in Douglas-fir seed orchards. In the 2001-2002 PNWTIRC Annual Report, we described the detection of one mislabeled ramet out of the 152 that we had sampled in the *Test Block*. Last year we sampled all 342 ramets in the block and detected another 4 mislabeled ramets. This rate of labeling errors (1.5%) is still relatively low compared to labeling errors found in other seed orchards (e.g., Adams 1983). As in the first example, 3 SSR markers were enough to match the ramets of the same clone or detect a mislabeled ramet. At least five times as many isozyme loci would have been needed to make decisions with the same level of confidence.

Third, we performed extensive computer simulations to test different sampling scenarios and analytical approaches for measuring pollen contamination. Based on these simulations, we chose an experimental design for measuring pollen contamination in the *Test Block*. Using 7 of our most informative markers, we analyzed 192 seeds from a bulked seed sample collected in 2000. We detected high seed contamination in the sample (50.3±3.9%), which probably resulted from a mistake made by seed extractors during the preparation of the sample. After accounting for seed contamination, pollen contamination was also high (41.0±5.2%). This result is not surprising because the *Test Block* is not spatially isolated from the other blocks in the orchard and the surrounding native Douglas-fir stands. Previous studies based on isozyme markers showed that if trees from the same species are closer than 1-2 km from the seed orchard, pollen contamination can be as high as the 41% we measured, or even higher (Adams and Burczyk 2000). On the positive side, analyses based on our SSR markers have an excellent ability to detect seed contamination and measure pollen contamination with high precision. Thus, the accuracy of seedlot labeling and the efficacy of pollen management techniques (e.g., bloom delay and SMP) can be readily quantified and eventually improved.

In addition to measuring seed and pollen contamination, we used the data from the 2000 bulked seed sample to determine the relative parental contributions (maternal and paternal) of the clones in the *Test Block*. Fig. 1 shows the paternal contributions of 30 clones from the block. After eliminating the seed contaminants from the sample, we matched each seed to exactly one mother, and for those seeds fathered by parents in the *Test Block*, to exactly one father (i.e., no ambiguous parentage was present).

This level of precision in distinguishing parents is practically impossible to achieve with isozyme markers. Information on the relative parental contributions can be used to monitor the genetic diversity of seedlots and study mating patterns (e.g., assortative mating with respect to flowering phenology, or crosses whose frequencies are highly disproportionate to the distances between the parents). For example, Fig. 2 demonstrates a simple test of the assumption that the parents in the *Test Block* mate at random. There was a moderate, statistically significant correlation between the number of ramets of clones from the *Test Block* and the paternal contribution of each clone, which is consistent with the assumption of random mating in the block.

Finally, we collaborated with NFGEL staff to develop and apply a step-by-step protocol for measuring the success of SMP using our markers. The goal of this project was two-fold. First, we used the protocol to analyze data for 6 crosses from the Weyerhaeuser program, and were able to measure the success of SMP using only 3 SSRs. Second, we successfully reproduced the performance of a subset of our markers in an independent laboratory. As a result of this collaboration, NFGEL added our SSRs to its kit of molecular markers, and will use them in its future work.

Plans for 2003-2004

This year, we will complete the *Pollen Contamination Study* and submit 3 new manuscripts for publication. The first manuscript will provide specific recommendations about the experimental design and methods needed to obtain reliable estimates of pollen contamination in different situations. In the second manuscript, we will characterize the genetic quality of seedlots from the *Test Block*, including overall and individual-tree estimates of pollen contamination and relative parental contributions in bulked seed samples. We will also investigate whether pollen contamination varies with flowering phenology and the location of the ramets within the *Test Block*. The third manuscript will be an overview on the various applications of SSR markers in tree improvement programs for Douglas-fir.

Early Flowering Study

Introduction

Early flower production in seed orchards is important for speeding the capture of genetic gains and for shortening the time between generations in breeding programs. Production of harvestable cone crops at an earlier age is therefore one way in which financial returns from tree improvement can be realized. Flowering may be enhanced in Douglas-fir orchards by girdling, gibberellic acid (GA) treatment, fertilization, and root pruning. However, most work to date has been done on trees 4 years old from grafting or older (Ross et al. 1980; Pharis et al. 1987). Very early seed production, i.e. in orchards as young as 2 years from grafting, has not been explored. The *Early Flowering Study* was designed to develop proven techniques for stimulating flowers on very young grafts. The purpose was to provide preliminary information on treatments which may be later used in the Miniaturized Seed Orchard Study once the establishment phase of that study is completed.

EF Study Objectives

- Develop improved methods for promoting early and sustained flowering on young Douglas-fir grafts
- Define the best age to begin floral stimulation treatments
- Measure the impacts of early flower stimulation on ramet health

Treatments

Flowering research is being carried out in 2 orchards managed by Roseburg Resources. The Vaughn orchard was planted in 1999 and the Pacific Northwest Christmas Tree Association (PNWCTA) orchard was planted in 1997. In the first experiment, study trees (9 clones/orchard, 4 ramets/treatment/clone) were initially treated in the spring of 2001. Treatments included girdling (G), gibberellin$_{4/7}$ stem injection (GA), girdling plus GA (G+GA), and a nontreated control (C). Using the manufacturer's recommended rate of GA, the flowering response was lower than expected. Therefore, the study trees were treated again in the spring of 2002. Each tree received the same treatment in both years, with one exception: trees treated with GA and G+GA were given 4 times (4X) the amount of GA in 2002 as they received in 2001 (Table 2).

In 2002, a second experiment was carried out in the Vaughn orchard to test how a range of GA application rates combined with girdling affects flowering and tree health. The same 9 clones were used as those tested in Experiment 1 in the Vaughn orchard, but 4 new (previously untreated) ramets per clone were selected. Treatments were as follows: C, G+GA 4X, G+GA 6X, and G+GA 8X (Table 2).

Table 2. Flower stimulation treatments. C = untreated control; G = girdled; GA = gibberellin stem injection; and 1X, 4X, 6X, and 8X are the relative stem injection rates, where 1X = 0.084 μl/mm^2 of scion cross-sectional area.

Expt.	Orchard	Treatment year	Graft age	Treatments
1	Vaughn	2001	2	C, G, GA 1X, G+GA 1X
1	PNWCTA	2001	4	C, G, GA 1X, G+GA 1X
1	Vaughn	2002	3	C, G, GA 4X, G+GA 4X
1	PNWCTA	2002	5	C, G, GA 4X, G+GA 4X
2	Vaughn	2002	3	C, G+GA 4X, G+GA 6X, G+GA 8X

Accomplishments in 2002-2003

Although we used higher rates of GA in 2002 than in 2001 (i.e., in both 2002 experiments), and the study trees in Experiment 1 had been stimulated for 2 years in a row, we found no evidence that any of the treatments in either experiment adversely affected ramet health (Fig. 3). In both experiments, very large clonal responses to treatments were found.

Experiment 1: Response to treatment reapplication in 2002

More cones were produced on trees after a second year of treatment, including control trees, than after the first year. Whereas treatments produced more pollen cones in the older (5 years post-grafting) PNWCTA orchard than in the younger (3 years post-grafting) Vaughn orchard (Fig. 5), only the G+GA 4X treatment produced more seed cones in the older orchard than in the younger orchard (Fig. 4).

Results after a second year of treatment generally showed the same patterns as those observed the previous year (PNWTIRC Annual Report 2001-2002). More seed cones and pollen cones were produced by applying GA in conjunction with girdling (Figs. 4, 5). In some cases (i.e., female cones in the Vaughn orchard, and male cones in the PNWCTA orchard), GA alone also produced significantly more cones than did the control. Only in the older PNWCTA orchard were there significantly more G+GA trees that bore female cones than there were in the control after 2 years of treatment (Fig. 6). Furthermore, there were no treatment differences in the proportion of trees with pollen cones.

Experiment 2: Response to a one-time treatment in 2002

In most cases, the control produced significantly fewer cones than the other treatments (Fig. 7). The highest numbers of female and male cones were produced using the G+GA 4X treatment. No differences were observed among treatments in the number of trees that flowered. The results of this study indicate that there does not appear to be any advantage to applying GA at rates above 4X.

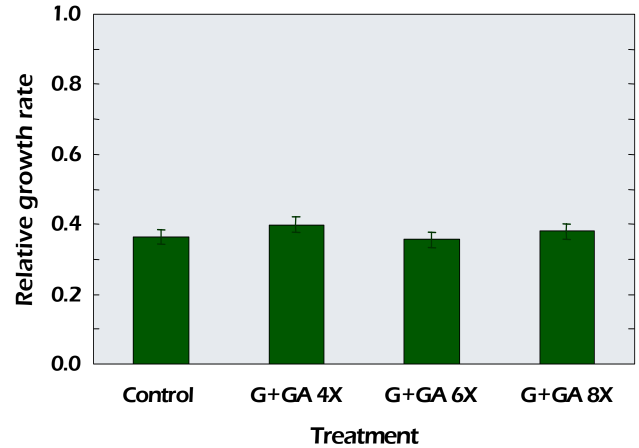

Figure 3. Experiment 2: flower stimulation treatments had no adverse effect on growth in the growing season following treatment.

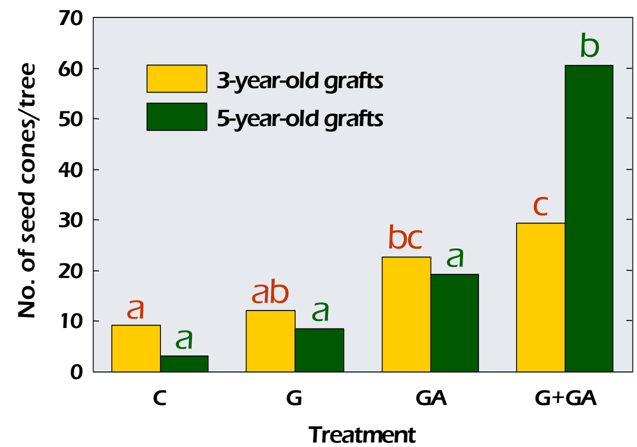

Figure 4. The combined girdling and 4X gibberellic acid treatment (G+GA) produced the most seed cones per tree on both the 3- and 5-year-old grafts.

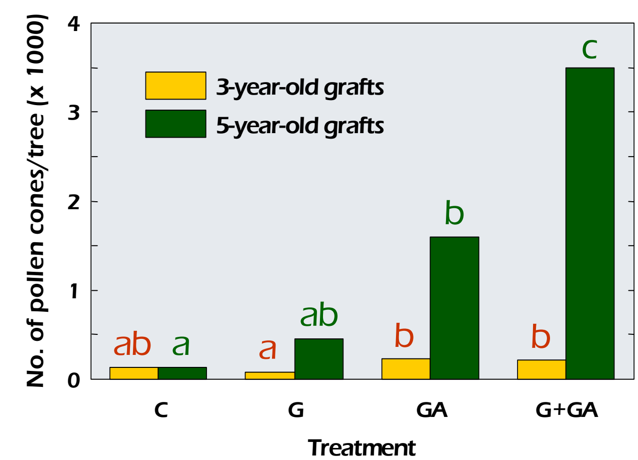

Figure 5. The combined girdling and 4X gibberellic acid treatment (G+GA) produced the greatest number of pollen cones per tree on 5-year-old grafts, but had little effect on the 3-year-old grafts.

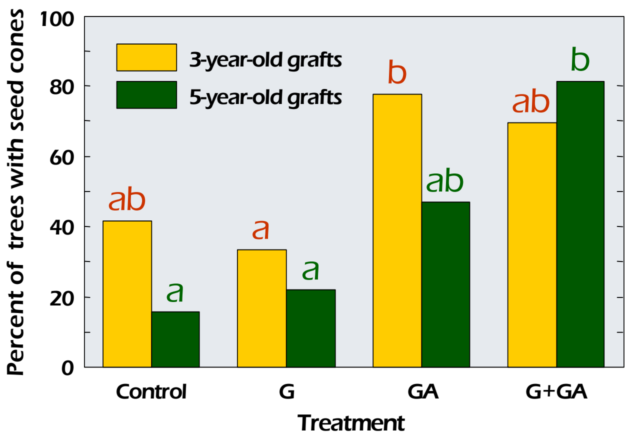

Figure 6. *The combined girdling and GA treatment (G+GA) increased the proportion of 5-year-old trees that had seed cones.*

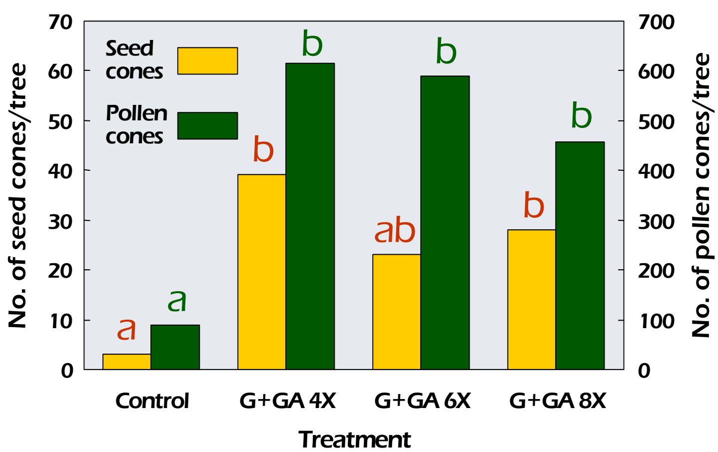

Figure 7. *A rate of gibberellic acid (GA) in the 4X range appears to be optimal for seed and pollen cone production when combined with girdling (G+GA).*

Recommendations

Based on our study, we recommend a combined girdling and GA 4X treatment for stimulating cone production in Douglas-fir clonal orchards as young as 2 years from grafting (Fig. 8). In light of the very large clonal differences observed in this study, we caution that very high GA rates (i.e., above those tested in this study) may potentially affect tree health of young grafts of other, untested clones. However, the 4X rate (with girdling) does not appear to be detrimental.

Figure 8. *5-year graft with substantial seed and pollen cone production following treatment with girdling and gibberellic acid (G+GA).*

'....we recommend a combined girdling and GA 4X treatment for stimulating cone production in Douglas-fir clonal orchards as young as 2 years from grafting.'

Plans for 2003-2004

The study will be completed during 2003-2004. The average number of seeds per cone and seed yields on an area basis will be estimated. Flower counts will be taken in the spring of 2004 to assess whether there are any remaining carryover effects of treatments—other researchers have observed that a flowering response to gibberellin treatments has sometimes been delayed for a year (Pharis et al. 1987). A final PNWTIRC report will be distributed, and a publication will be submitted to a peer-reviewed journal.

Miniaturized Seed Orchard Study

Introduction

Miniaturized seed orchards (MSOs), which are alternatives to conventional seed orchards, contain trees that are planted at close spacings and maintained at low heights. They are designed to facilitate intensive management on smaller, more easily managed trees (Sweet and Krugman 1977). As a result, management costs should be reduced, and activities such as cone collection, pest control, frost protection, and flower stimulation should be more efficient. Additionally, denser spacing means less land is needed. However, one drawback of MSOs is that it may be more difficult to use large equipment across rows (Copes and Bordelon 1994), unless leave strips are incorporated into the layout.

There is also the potential for increasing genetic gains. Using clonal rows (as in the PNWTIRC MSO), controlled pollination (CP) or supplemental mass pollination (SMP) techniques should be easier to apply, and elite crosses should be produced more easily. Pollen contamination may be reduced because of better control over pollen and an enhanced ability to use bloom delay. Using flower stimulation treatments, early seed production is also possible in MSOs. Because of the large number of trees per hectare, this should lead to earlier production of commercial amounts of improved seed.

The goal of the Miniaturized Seed Orchard Study is to compare management regimes on three alternative planting densities at an operational scale that will provide realistic estimates of management costs and seed production (Anekonda and Adams 1999). Table 3 describes the three orchard types in the study.

Objectives of the MSO Study

- 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 (CP, SMP)
- Evaluate clonal response to MSO management regimes

Table 3. Characteristics of three orchard types tested in the Miniaturized Seed Orchard Study.

Orchard type	Spacing (m)	Trees/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

'The goal of the Miniaturized Seed Orchard Study is to compare management regimes on three alternative planting densities ....'

Figure 9. An overhead sprinkler system has been installed at the miniaturized seed orchard site.

Orchard Management

The MSO study site is owned by the Plum Creek Timber Company. In addition to conventional dripline watering, an overhead sprinkler system was used during 2003 from the time of grafting through late summer to help prevent the new grafts from drying out while graft unions were being formed (Fig. 9). The overhead system will also facilitate frost protection and bloom delay in the future. In addition to irrigation, other site management this year included weeding, rootstock pruning, and graft maintenance.

Accomplishments in 2002-2003

Following low graft survival in 2002, all trees were regrafted in 2003. Because scion material for some clones was unavailable, a few substitute clones were needed to maintain 16 clones in the study. This year, two contracting companies were hired to carry out the grafting. Each company was assigned 4 of 8 replications in each spacing. One company used Parafilm strips to wrap the rubber ties of the grafts (Fig. 10A). The other company used either Tree Seal paint (Fig. 10B) or Buddy-Strips (Fig. 10C), which are similar to Parafilm strips but thicker and waxier. Grafting began the last week of March 2003 and took a month to complete.

Overall graft survival in 2003 was 62 %, although results varied greatly by grafting method. Grafts sealed with Tree Seal had a 95 % survival rate, whereas Parafilm grafts had a survival of 66 %, and Buddy-Strip grafts had a survival of only 39 %.

Unlike the other sealing types, the thick Buddy-Strip did not break down over time, and began constricting the graft unions. Therefore, the Buddy-Strips were cut open with a knife when the scions began to flush. Nonetheless, the Tree Seal method may have been more successful because the black Tree Seal kept the grafts warmer during the long, cold spring of 2003 (Don Copes, pers. comm.), thus enhancing cell growth activity and the formation of graft unions. In warmer, shorter springs, the survival differences between graft sealing methods may be less.

Figure 10A. *The Parafilm method of grafting had a survival rate of 66%*

Figure 10B. *The Tree Seal method of grafting had a survival rate of 95%*

Figure 10C. *The Buddy-Strip method of grafting had a survival rate of 39%*

Plans for 2003-2004

Fill-in grafting will be carried out in 2004 using the Tree Seal method only. We will reconvene the Seed Orchard Advisory Committee in fall 2003 to provide ongoing strategic direction for the study. One of the first tasks of the committee will be to assess whether we can keep (i.e., not replace) as many of the live grafts as possible by slightly altering the study design.

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Slavov, G.T., Howe, G.T., Yakovlev, I., Edwards, K.J., Krutovskii, K.V., Tuskan, G.A., Carlson, J.E., Strauss, S.H. and Adams, W.T. Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. Submitted to Theoretical and Applied Genetics.

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Presentations by PNWTIRC Personnel: 2002-2003

Aitken, S.N., Howe, G.T. and Neale, D.B. 2003. Cold hardiness in trees: complexities of quantitative, spatial and temporal genetic structure. Invited talk, International meeting entitled *Plant and Microbe Adaptations to Cold*, Quebec City, Canada, May 25-29, 2003.

Howe, G.T., Aitken, S.N., Chen, T.H.H., Neale, D.B., Jermstad, K.D. and Wheeler, N.C. 2003. Cold hardiness in trees: Unraveling genetic complexities using molecular approaches. Invited talk, International meeting entitled *Plant and Microbe Adaptations to Cold*, Quebec City, Canada, May 25-29, 2003.

Howe, G.T. 2003. Research priorities for western tree improvement: Where do we want to be in 2050? Invited talk, 30th Annual Meeting of the Inland Empire Tree Improvement Cooperative, *Seed: The Genetic Link to Future Forests*, February 26, 2003, Coeur d'Alene, ID.

Howe, G.T. 2003. What is genomics? Joint PNWTIRC/USFS workshop entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation*, Corvallis, OR, November 14, 2002.

Howe, G.T. 2003. Using gene chips to understand tree physiology, Joint PNWTIRC/USFS workshop entitled *Genomics of Douglas-fir: Implications for applied tree improvement and gene conservation*, Corvallis, OR, November 14, 2002.

Howe, G.T. 2002. Are three heads better than one? How and why universities, government agencies, and forest industries pursue cooperative research in the Pacific Northwest. Invited talk, Department of Forest Science, Oregon State University, Corvallis, OR, October 24, 2002.

Howe, G.T. 2002. Forestry research cooperatives in the Pacific Northwest: An overview and case history. Invited talk, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain, September 20, 2002.

Howe, G.T. 2002. Forestry research cooperatives in the Pacific Northwest: An overview and case history. Invited talk, Universidad de Valladolid, Palencia, Spain, September 13, 2002.

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PNWTIRC Financial Support for Fiscal Year 2002-2003

Regular members [1]	$104,000
Associate members [1]	8,000
Contracts	8,000
Forest Research Laboratory, Oregon State University [2]	104,131
Total	$224,131

[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.

History of the PNWTIRC

The PNWTIRC developed out of concerns that research efforts were not adequately keeping pace with the rapid expansion of applied tree breeding programs. In March of 1982, an ad hoc committee was formed with representatives from four private companies and Oregon State University (OSU). Their mission was to evaluate the need for additional research and to explore methods of organizing tree improvement research in the region.

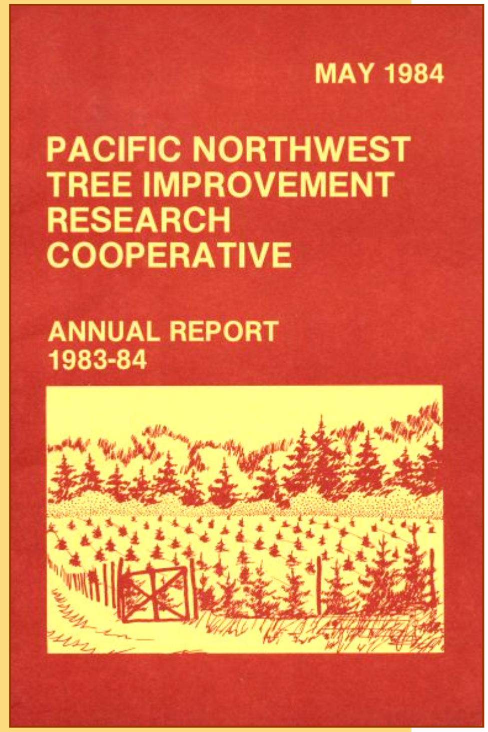
Based on an informal survey of regional tree improvement research and the results of a questionnaire by the Northwest Seed Orchard Managers Association, the committee concluded that additional tree improvement research was needed to augment ongoing programs. Furthermore, the committee concluded that the formation of a research cooperative was the best way to meet this need. It was recognized that further delay in acquiring needed information would only lead to inefficiencies in breeding programs and loss of revenue.

A proposal for a cooperative centered at OSU was developed by the ad hoc committee and circulated among forestry organizations in October of 1982. Extensive review of the proposal occurred over the next six months, culminating at a public forum in Corvallis on March 18, 1983.

Because the response to the proposal was enthusiastic, the PNWTIRC was officially established on July 1, 1983. Members have included representatives from public agencies and private companies in western Oregon, western Washington, and coastal British Columbia (see current Annual Report).

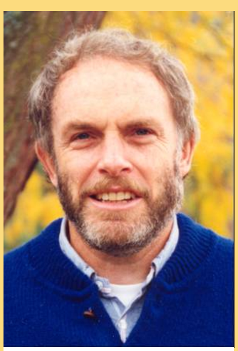
Highlights of 1983-84

- The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) is officially underway with the participation of 12 organizations this first year and more anticipated.
- Research priorities have been established by the Technical Committee.
- A full-time research assistant, Glenn Howe, was hired in April for the Cooperative.
- Our first project is to develop measurement methods in Douglas-fir progeny tests. Phase 1 data collection is scheduled to begin the summer of 1984, and significant progress is expected by next year.
- A pilot study for our next project, early evaluation of genetic potential, has been scheduled to begin in the spring of 1985.


MAY 1984

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

ANNUAL REPORT 1983-84



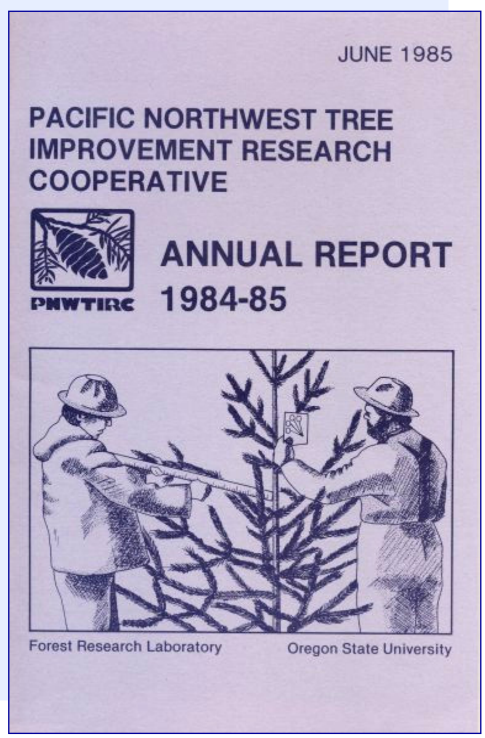
Tom Adams, Director

A happy crew — PNWTIRC Measurement Study

Highlights of 1984-85

- We completed the fieldwork and began the data analysis on the Douglas-fir Measurement Study.
- We released two preliminary reports on the Measurement Study results: "Measurement Strategies for Young Douglas-fir Progeny Tests- Study Plan" and "Stem Sinuosity Measurement in Young Douglas-fir Progeny Tests."
- Two new members joined the Cooperative; membership now stands at 14.
- We initiated the second Cooperative research project - on early testing of Douglas-fir. A study plan will be prepared by the fall of 1985.
- Two graduate students will begin work on Cooperative research projects in the fall of 1985.

Measuring branch angle in the Measurement Study


JUNE 1985

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

PNWTIRC

ANNUAL REPORT 1984-85

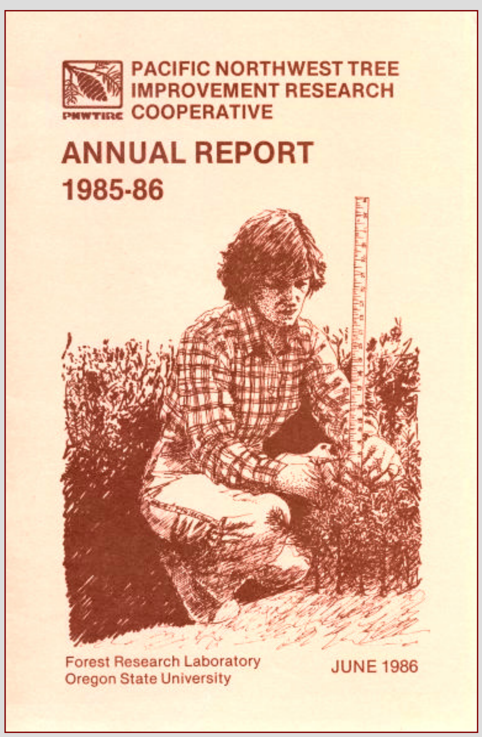
Forest Research Laboratory

Oregon State University

Highlights of 1985-86

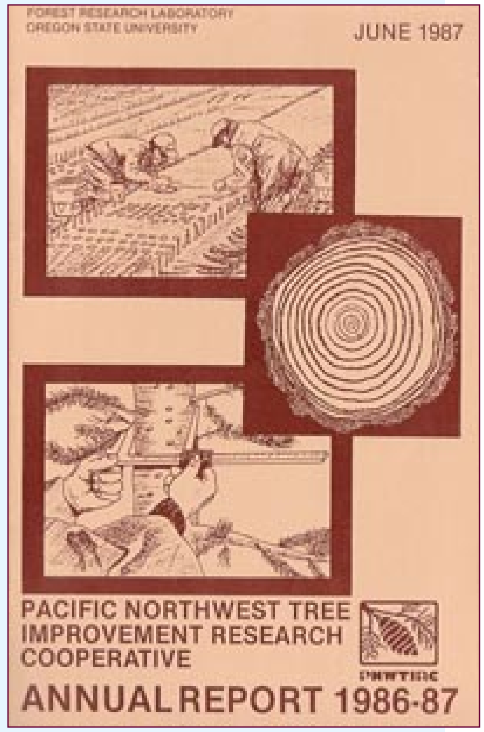
- We released the final study plan for the Early Testing Study and completed the sowing portion of the test at three Cooperative nurseries.
- Two graduate students completed their PhD study plans and began fieldwork on research that is closely related to early testing.
- We released two reports from the Measurement Study: "A comparison of measurements and selection indices for improving volume growth in young Douglas-fir progeny tests" and "Pole and clinometer estimation of height in Douglas-fir progeny tests."
- Two new members joined the Cooperative.

Henry Laik — Measurement Study



Highlights of 1986-87

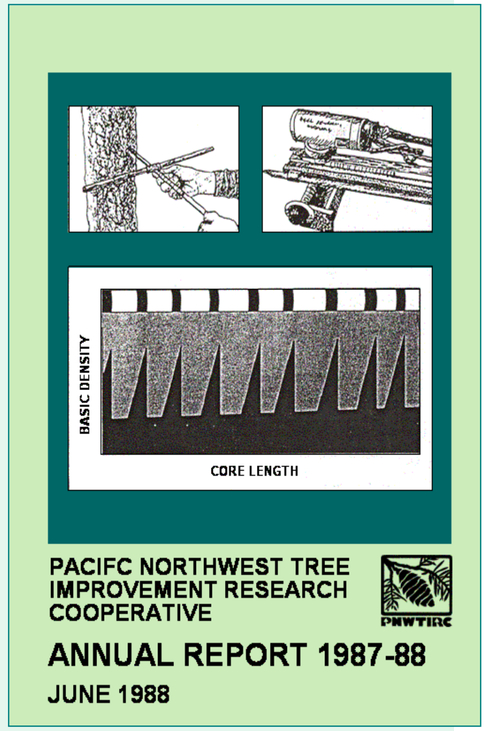
- The second year of the Early Testing Study was completed with the sowing of three sets of materials, two in greenhouses and one in a nursery, in the spring of 1987.
- Results from the Measurement Study indicate that substantial improvement in stem quality of young Douglas-fir can be accomplished through selection of branching traits, with little reduction in volume growth potential.
- Good progress was made in the two ongoing graduate student projects supported by the Cooperative.



- Plans were made to add genetics of wood quality in Douglas-fir as a major new project in the coming year. A third student has been added to the program to work on this project.
- With the resignation of Glenn Howe, who has returned to graduate school full time, Dr. Dennis Joyce was hired as the new Research Assistant for the Cooperative.

Nursery site - Early Testing Study

Highlights of 1987-88

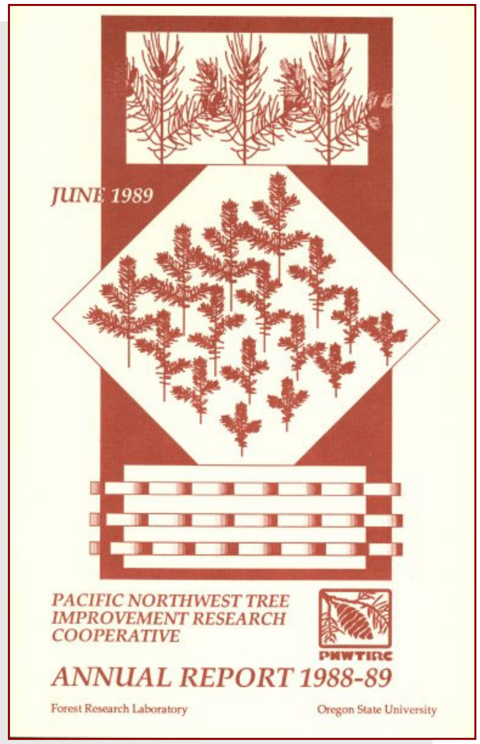


- Accomplishments of the initial 5 years of operation of the PNWTIRC were reviewed, and a new 5-year plan was formulated.
- The 1986 sowing of the Early Testing Study was harvested, and two 1987 greenhouse sowings were transplanted to two nursery sites for a second season of growth.
- Results from the Measurement Study indicate that the incidence of ramicorn branching in young Douglas-fir can be reduced by selection and breeding, but that it may be difficult to do so without also negatively affecting growth rate.
- Douglas-fir families that showed a tendency to break bud early in a 15-year-old field trial were readily identified in the nursery; thus, the timing of bud break appears to be amenable to early testing.

Dennis Joyce — Early Testing Study

- Douglas-fir seedlings raised in pure family blocks were found to be more uniform (but slightly smaller) than the seedlings grown in mixed family plantings.
- Three research proposals dealing with the genetics of wood quality were written and reviewed, and work was begun on two of the projects.

Highlights of 1988-89



- The nursery phase of the Early Testing Study was completed with the harvest of 12,000 seedlings in the three remaining tests. Analysis of a portion of the data indicates that early testing could be effective for low-level culling of families for poor height growth, undesirable branch angle, and stem sinuosity.

- New analyses of data from the Measurement Study show that two-stage selection can be a very efficient means of selecting for bole volume in Douglas-fir progeny tests, while minimizing the need for expensive height measurements.

Family Composition Study

- Shoot phenology appears to be under strong genetic control in coastal Douglas-fir, but cambial phenology does not. Selection for greater volume is expected to extend the length of the growing season.

- Sowing of germinants, rather than ungerminated seed, does not appear to reduce the influence of seed weight on growth of families in early tests. Seedling families of Douglas-fir vary in both crown form and in partitioning of biomass.

- Although the correlation between wood density and volume growth rate in coastal Douglas-fir is negative, substantial genetic improvement can be made in both traits when appropriate selection indices are employed.

Highlights of 1989-90

Pilodyn study

- Dennis Joyce resigned to take a tree improvement position in Ontario. His replacement is Dr. Sally Aitken, who recently completed a PhD at the University of California, Berkeley.
- Preliminary results indicate that early testing for wood density in Douglas-fir is promising. Wood density assessed at age one had a strong genetic correlation with wood density of field trees at age 15.
- The Pilodyn wood tester is a fairly efficient, low-cost option for ranking individuals or families for wood density in Douglas-fir progeny tests.
- The genetic relationships between wood density and growth rhythm in Douglas-fir are weak; thus, selection for wood density should have little adverse effect on adaptability as influenced by growth rhythm.

- Age trends in relative earlywood density may be useful for identifying families that undergo early transition from juvenile to mature wood in Douglas-fir.
- The composition of nursery seedling stock sown with mixtures containing equal proportions of families was little changed after 2 years of growth and operational culling.
- In the Early Testing Study, performance of families was relatively consistent across nursery trials of the same type.

Highlights of 1990-91

- Jesus Vargas-Hernandez and Peng Li both completed their PhD degrees.
- Results from the Early Testing Study indicate that early selection for field growth in the nursery or greenhouse is effective:

Sally Aitken

✓ Using first-year seedling height to select for 12- or 15-year height or volume in the field is about 50% as effective as using selection in the field.

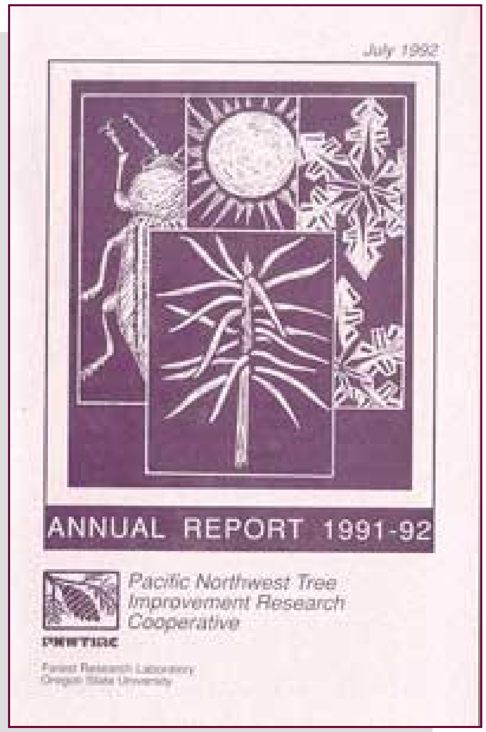
✓ Results are consistent for bare-root and container-sown seedlings, and for sowings in different years.

✓ Early seedling height growth is more efficient for early selection than biomass, branch, or phenology traits.

✓ Two-stage selection using early testing is a very effective option for breeding programs.

- The Provenance Architecture Study, a cooperative project between OSU and INRA (National Agronomic Research Institute of France), will investigate differences in quantitative genetic structure of breeding populations from different geographical locations (i.e., different breeding zones).
- A proposal to study adaptability in Douglas-fir and its implications for global climate change has been submitted to the USDA National Research Initiative Competitive Grants Program.
- Efforts to develop a long-term Cooperative project on adaptability and stability in coastal Douglas-fir continue.

Highlights of 1991-92



- Analysis of the economic benefits of using early testing to cull inferior families prior to field-testing in a second-generation breeding program indicated that the total cost of testing could be decreased by 20% without affecting genetic gain.
- Preliminary results from the Provenance Architecture Study suggest that there are no major differences among three contrasting breeding zones in amounts of variation and degree of genetic control of individual traits, or in the genetic relationships between traits.
- Significant genetic variation in fall cold injury to needles, stems, and buds was observed in the preliminary Cold Hardiness Study. Shoot phenology, however, appears to be only weakly predictive of fall cold hardiness.
- Study plans have been completed for two major adaptability research projects focusing on screening for cold hardiness:


Measuring cold hardiness

  - ✓ A study of cold hardiness and growth rhythm in six-year-old trees in operational progeny tests in one coastal and one Cascade Range breeding zone was initiated this spring.
  - ✓ A proposal to study genetic variation in annual developmental cycle traits in Douglas-fir seedlings, including cold hardiness, was submitted to the USDA National Research Initiative Competitive Grants Program.

Highlights of 1992-93

- Preliminary results from the Field Cold Hardiness Study indicate that there is substantial family variation for fall cold hardiness in coastal Douglas-fir, but that the amount of variation and degree of genetic control is dependent on the date of assessment and the type of tissue tested.

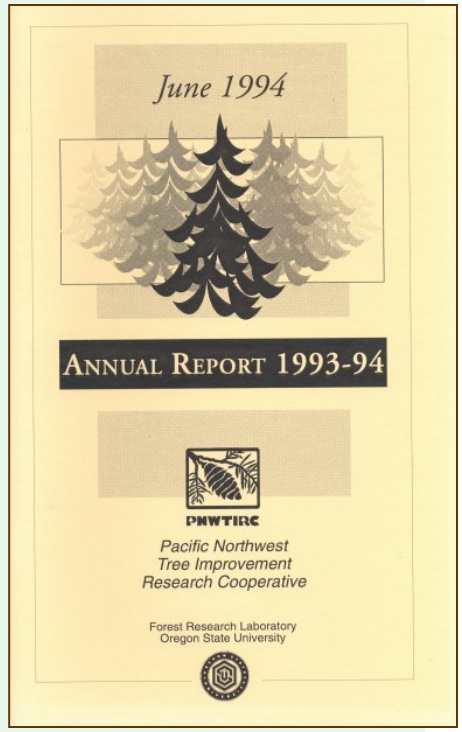
- Limited assessment of materials from a young progeny test in France indicates that the vulnerability of provenances and (to a lesser extent) families to spring frost injury can be predicted from laboratory freeze testing.

Damage caused by cold

- Results from the Provenance Architecture Study indicate that stem form defects are genetically associated with the propensity to produce lammas shoots.

- A study of repeatability of Pilodyn measurements indicates that careful control of measurement conditions, including crews, dates and instruments, is crucial to the effective use of the Pilodyn for ranking families for wood density.

Highlights of 1993-94



- Preliminary results from the Field Cold Hardiness Study indicate that spring hardiness prior to budburst is under relatively strong genetic control and is highly correlated with the timing of budburst; however, fall cold hardiness is under relatively weak genetic control and is not predicted well by bud phenology or by the timing of shoot elongation.

- Spring hardiness and the timing of budburst are unfavorably correlated with tree size in the Cascade breeding zone, but not in the coastal zone. Fall cold hardiness, however, is not significantly correlated with tree size in either the coastal or the Cascade zones.

The frequency of second flushing is positively correlated with forking and ramicorn branching

- A study of Douglas-fir in a progeny test in France indicates that the frequency of second flushing is positively correlated with forking and ramicorn branching frequency, and that larger trees have a greater tendency to second flush.

- Stable carbon isotope ratios (a measure of water-use efficiency) vary among varieties and populations within varieties of Douglas-fir. Surprisingly, populations from wetter source environments have higher water-use efficiencies than populations from drier environments.

Highlights of 1994-95

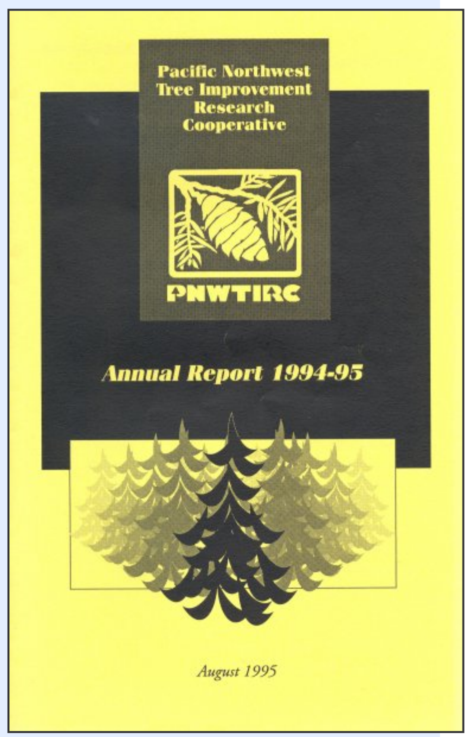
- Cooperative membership increased in 1994-95 from 18 to 22 members. MacMillan Bloedel, Ltd. returned to the Cooperative and Longview Fibre Company, Plum Creek Timber Company, Stimson Lumber Company, and South Coast Lumber Company joined as new members.

- A five-year plan was developed to guide research activities of the PNWTIRC from 1995 through the year 2000. Priorities for this period will be continued emphasis on adaptability research, as well as projects addressing issues of Douglas-fir seed orchard design and management.

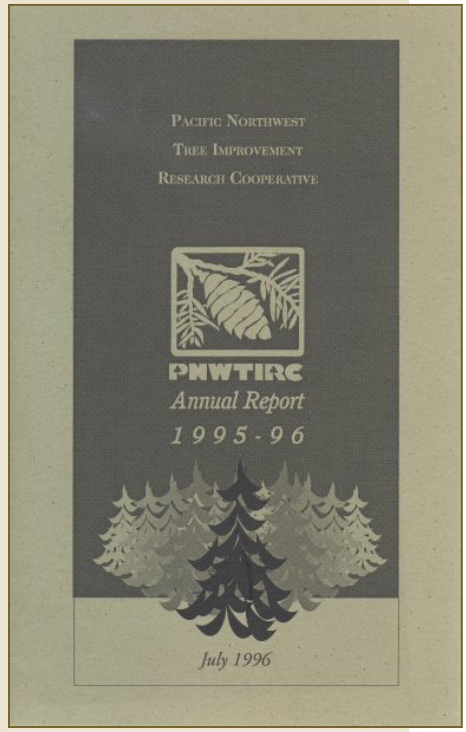
- Results from the Field Cold Hardiness Study indicate that variation among Douglas-fir families in spring and fall hardiness is primarily due to differences in the time when hardening and dehardening are initiated, rather than differences in rates of acclimation and deacclimation. There is little genotype-by-environmental interaction for fall or spring cold hardiness, either between sites or between years.

- Selection for height growth is expected to increase second flushing frequency in coastal Douglas-fir populations, and thus will increase the susceptibility of these trees to fall frost damage. Vulnerability to spring frost events may increase considerably in some Cascade populations in response to selection for increased growth rates, but no negative effects are anticipated in coastal populations.

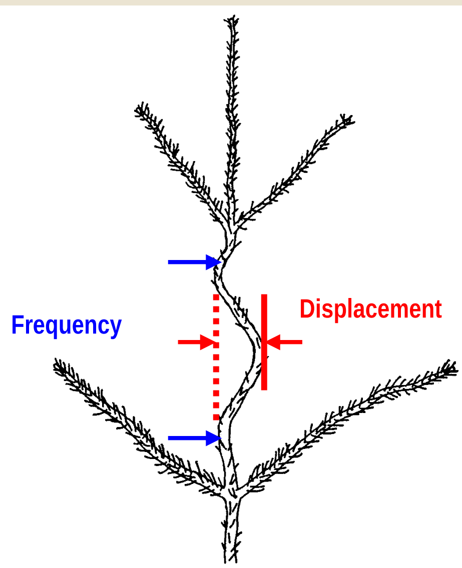
- Cold hardiness and bud phenology of Douglas-fir families at the sapling stage are associated with the temperature and moisture regimes of their parent trees, even when parent trees range over relatively short distances within breeding zones.



Highlights of 1995-96



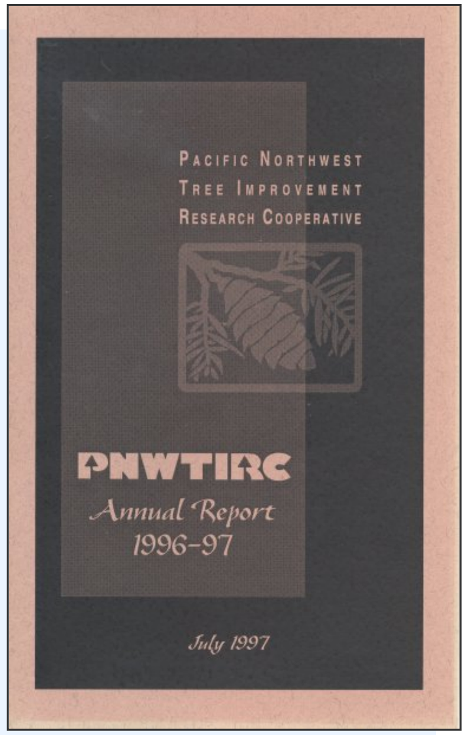
- Dr. Thimmappa Anekonda replaced Sally Aitken as Associate Director.
- Miami Corporation and Georgia-Pacific West joined the PNWTIRC.
- M.S. students Andrew Bower and Fatih Temel joined the Cooperative staff.
- The Seedling Drought Physiology Study was planned and established.
- A study of genetic response of growth ring components (earlywood and latewood width, proportion and density) to low soil moisture availability was planned and initiated.
- A project to evaluate age-age correlations in stem defects (sinuosity, forking and ramicorn branching) was initiated as a follow-up to the PNWTIRC's Measurement Study conducted in the mid-80s.



Age-age correlations were measured for stem sinuosity and other stem quality traits

- A fall frost event caused injury in the Seedling Cold Hardiness Study, providing an unanticipated opportunity to compare natural and artificial freeze injury.
- Several reports on the genetics of cold hardiness were prepared and published.

Highlights of 1996-97



- West Coast Forest Resources Limited and Willamette Industries became the newest members of the PNWTIRC.

- A new graduate student, Christine Lomas, joined the Cooperative staff.

- A recently completed study shows that second flushing in Douglas-fir delays hardening of shoots in early fall. Thus, foresters should avoid deploying families with high propensity for second flushing on sites prone to second flushing and fall frosts.

- The Cooperative joined an investigation to use molecular markers and a genetic linkage map to identify genes (quantitative trait loci) influencing growth phenology and cold hardiness traits in Douglas-fir.

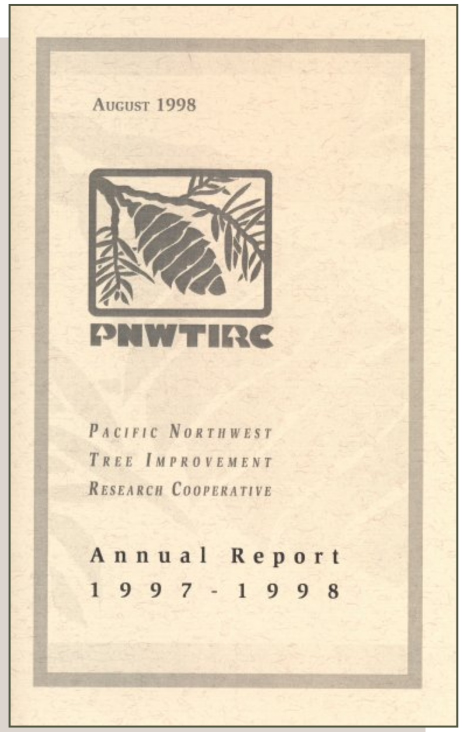
Ramicorn branches and forking were remeasured at age 24 in the Measurement Study plantations

- Results from the Seedling Cold Hardiness Study indicate that the quantitative inheritance of fall cold hardiness traits in seedlings of Douglas-fir (and genetic interrelationships among these traits) are quite similar to observations for the same traits in sapling-age trees.

- Latewood density and latewood mass of sapling Douglas-fir appear to respond to growing season moisture availability. Thus, these traits are candidates for assessing drought hardiness in field progeny tests.

- Reassessment of trees in 3 progeny test plantations indicated that forking defects (forks and ramicorn branches) observed at age 12 were still present 12 years later, although many forks at age 12 were classified as ramicorn branches at age 24.

Highlights of 1997-98



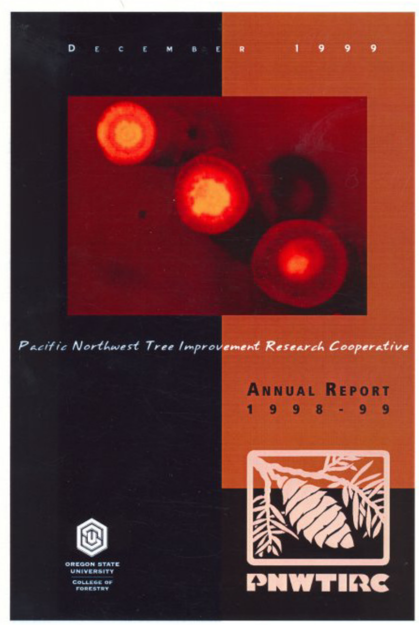
- The Cooperative completed its 15th year!
- Andrew Bower and Fatih Temel completed their Master's degrees.
- Moderate and severe summer drought treatments in the Seedling Drought Physiology Study had little influence on diameter and height growth of 2-year-old seedlings, although 20% of the foliage and 18% of xylem conduits were damaged (i.e., cavitated) in the severe drought treatment.
- Seedlings were less cold hardy in the fall after drought treatments than were the controls. Seedling hardiness to summer drought and to fall cold are nearly unrelated genetically.
- Families differed widely in the percentage of xylem conduits in the second-year growth ring damaged by severe drought (5-59%), suggesting that this trait may be a useful indicator of drought hardiness in seedlings.

Drought damage in the nursery

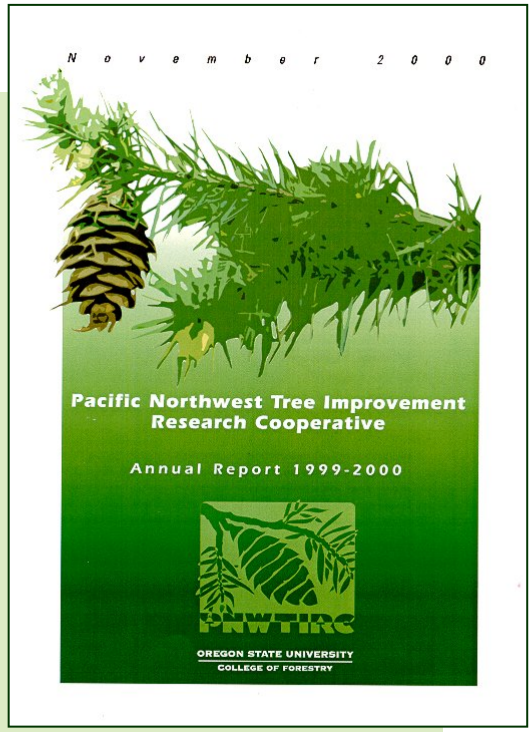
- Phase 1 of the Field Drought Study indicates that latewood width is decreased and latewood density is increased in summers with higher soil moisture deficits. Thus, latewood annual ring components may be useful for assessing the sensitivity of families to drought.
- Remeasurements of trees in the Cooperative's Measurement Study indicate that early selection (age 12) for stem diameter, branch size, and frequency of steep-angled branches (i.e., ramicorns and forks) would be nearly as effective as waiting to age 24 to make selections.
- A study plan was approved to develop microsatellite genetic markers for Douglas-fir, and then to apply these markers to investigate factors influencing pollen contamination in seed orchards.

Highlights of 1998-99

- Greg O'Neill completed his PhD dissertation in May, and Christine Lomas completed her M.S. thesis in October of 1999.
- The Cooperative staff has moved to brand new facilities in Richardson Hall.
- Shoot damage due to drought ranged widely among 39 full-sib families in the Seedling Drought Physiology Study, suggesting that selection among families for increased seedling drought hardiness would be quite effective. There appears to be little genetic association between drought hardiness in seedlings and growth potential in favorable moisture regimes.
- A calorimetric investigation based on a subset of families from the Seedling Drought Physiology study found that respiration parameters were generally reduced under moisture stress, but that families can differ in the degree of their response. Family differences in respiration traits expressed when seedlings are grown under well-watered conditions may be useful for predicting drought hardiness.
- The magnitudes of annual growth ring variables (e.g., earlywood width, latewood density, latewood proportion) are sensitive to moisture availability in the growing season in which a ring is produced. X-ray densitometry analysis of increment cores from sapling-age trees in one progeny test site showed that families differed widely in their response to past summer droughts.
- Nine potential microsatellite (SSR) marker loci were identified for use in Douglas-fir seed orchard studies. Preliminary analysis revealed as many as 10-12 different variants (alleles) at some loci in DNA samples from less than 20 individuals.
- A plan was approved for a 15-year study to evaluate alternative miniaturized seed orchard designs in Douglas-fir.



Highlights of 1999-2000

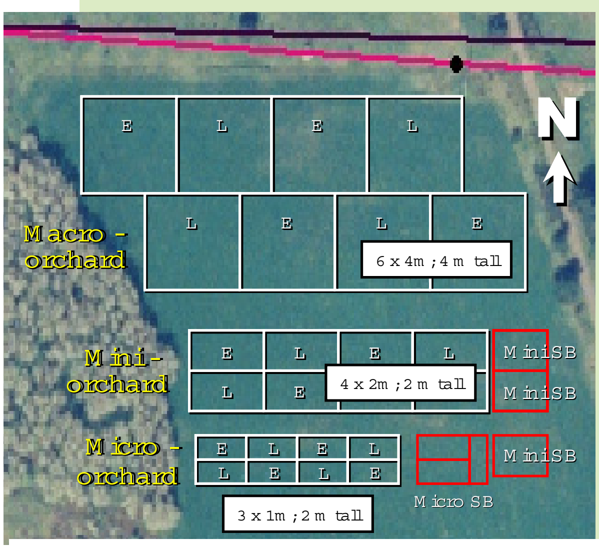


- Tom Adams resigned from the PNWTIRC's leadership to become Head of the Forest Science Department at OSU.

- Families varied considerably in their response to summer drought applied in the nursery. Growth of families under well-watered conditions is uncorrelated with drought hardiness, suggesting that selection for growth traits will not reduce drought hardiness of the selected trees.

- Due to their low heritability and difficulty of measurement, there seems to be little practical utility to using drought sensitivity coefficients (DRCs) to assess drought hardiness in older, field-grown trees.

- New results from the Early Testing Study showed unfavorable correlations between growth and stem form and branching traits in seedlings — selection for growth alone will increase the number of whorls with steep-angle branches and stem sinuosity in older trees. To avoid negative impacts, appropriate stem form and branching traits should be included, along with selection for growth.

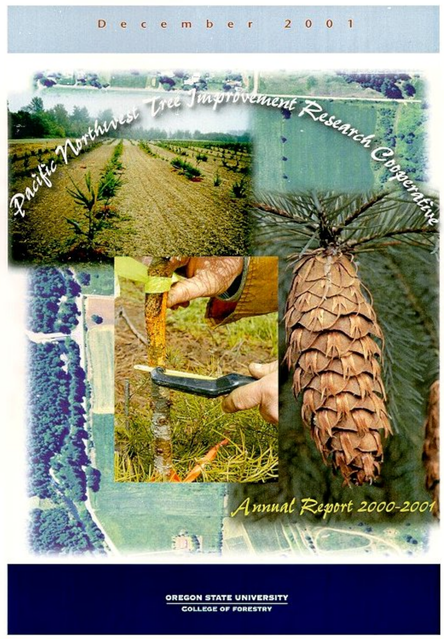


Layout of the Miniaturized Seed Orchard Study

- Microsatellite and minisatellite DNA markers are highly polymorphic. These markers should be very effective for mating studies, including measurement of pollen contamination.

- A site for the Miniaturized Seed Orchard Study was selected and prepared for planting rootstocks in the winter of 2001. An advisory committee of seed orchard managers and other experts was formed to guide the implementation of the study.

Highlights of 2000-01



- Gancho Slavov arrived in September 2000 to begin a PhD with the PNWTIRC. He will work on the Pollen Contamination Study.

- Flower stimulation treatments were applied in the Early Flowering Study. Gibberellic acid and girdling treatments were applied to 2- and 4-year-old grafts in the Vaughn and PNWCTA orchards.

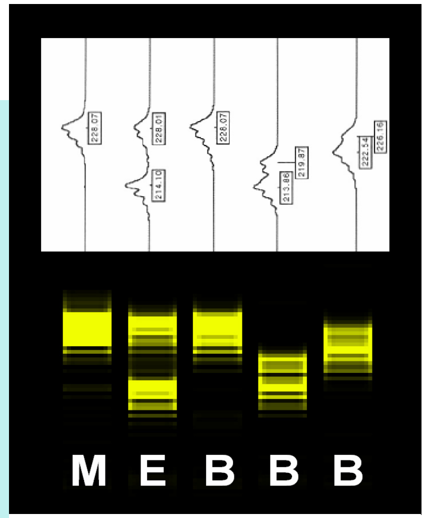
- Three orchard types (macro, mini, and micro), two supplemental blocks (mini and micro), and one holding block were established in the Miniaturized Seed Orchard Study. We planted the rootstock, installed the irrigation system, and applied nitrogen fertilizer. The scions will be grafted in February 2002.

- We developed 15 new SSR markers in the Pollen Contamination Study. These markers were derived from five new SSR libraries that were constructed using recently improved molecular techniques. At least 62 potentially promising SSR markers remain to be tested.

- We completed the analyses of bud phenology, second flushing, and cold hardiness for 39 full-sib families of Douglas-fir in the Seedling Drought Physiology Study. There was a tendency for cold damage to increase in families that set bud late in the fall, second flushed, or grew taller.

Mike Albrecht and Thimmappa Anekonda applying girdling and gibberellic acid treatments in the Early Flowering Study

Highlights of 2001-02



Douglas-fir SSR genetic markers

- Dr. Glenn Howe began as Director of the PNWTIRC and Assistant Professor of forest genetics in the Department of Forest Science at OSU in August 2001.

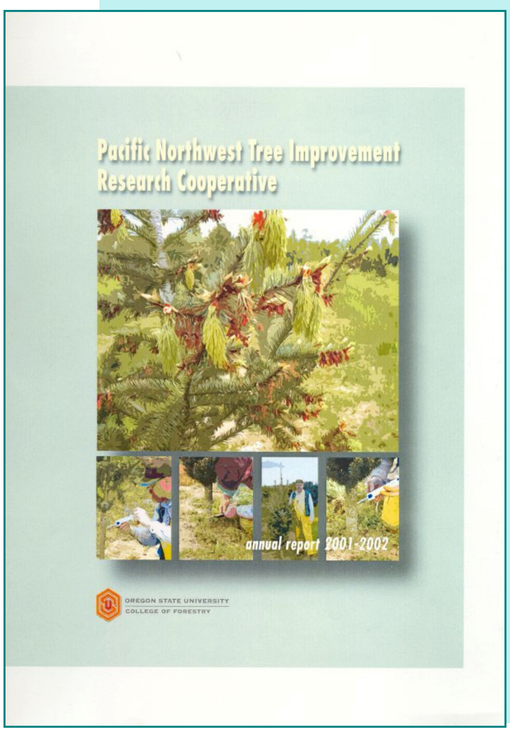
- Two new members joined the PNWTIRC — Port Blakely Tree Farms and Pope Resources (Olympic Resource Management).

- We developed 7 new SSR genetic markers and conducted detailed analyses of our full set of 22 SSRs in the Pollen Contamination Study.

- We identified 7 'high priority' research topics to be addressed in the new PNWTIRC 5-year plan.

- We completed the first grafting for the Miniaturized Seed Orchard Study in February and March of 2002.

- We held a workshop entitled *Genetic improvement of wood quality in coastal Douglas-fir and western hemlock*. This workshop was held on June 27, 2002 in collaboration with the Northwest Tree Improvement Cooperative (NWTIC).



- In the spring of 2002, we measured the results of the early flowering treatments that were applied in the spring of 2001 (Early Flowering Study). Gibberellic acid and girdling treatments were applied to 2- and 4-year-old grafts in the Vaughn and PNWCTA seed orchards. The combined GA/girdling treatment significantly increased female flowering on both the 2- and 4-year-old grafts, but only increased male flowering on the older trees. Treatments were reapplied in spring 2002, and new treatments were applied to test different levels of GA.


Glenn Howe

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