



# PNWTIRC

*Pacific Northwest Tree Improvement  
Research Cooperative*



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*Annual Report 2003 - 2004*

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# PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

*Annual Report*

*2003-2004*

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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 2003-2004

- Gancho Slavov completed his Ph.D. in June of 2004. His dissertation was entitled "Development and Application of SSR markers for Measuring Gene Flow in Douglas-fir."
- PNWTIRC personnel published 12 journal articles and abstracts (i.e., published or submitted) and gave two other presentations that deal with PNWTIRC research projects.
- We used nine SSR markers to measure pollen contamination and characterize mating patterns based on seed samples collected in three years (1999, 2000, and 2003) from one block of a non-isolated, open-pollinated, clonal seed orchard of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) in western Oregon. Pollen contamination was consistently high across the three years (mean = 35.3%, range = 31.0-41.3%), and appeared to result primarily from cross-pollination among the orchard blocks. Levels of pollen contamination varied substantially among clones, and were higher in clones with early female receptivity (mean = 55.5%) than in those with either mid (mean = 36.4%) or late (mean = 28.3%) female receptivity. There was a clear pattern of positive assortative mating with respect to floral phenology. This study illustrates that SSR markers are powerful tools for characterizing seedlots and improving the design and management of Douglas-fir seed orchards.
- We completed the early flowering study. Results from this study indicate that cone and seed production can be enhanced by stimulating very young grafts with a combination of girdling and gibberellic acid (GA). During the past year, we investigated the effects of our flower stimulating treatments on tree growth, cone abortion, number of seeds per cone, and seed weight. These analyses indicate that the flower stimulating treatments have no major adverse effects on tree health or seed traits, although GA concentrations higher than the recommended rates may have some detrimental effects on very young grafts. We treated some of the trees with insecticides and estimated seed yields at age 6. Using our recommended treatment every other year, it should be possible to obtain 4.5 kg of seed/hectare per year in the orchard we studied (i.e., 8 x 13 foot spacing). Extrapolating our results to a micro-orchard situation (i.e., 1 x 3 m; 3.3 x 9.8 foot spacing), it might be possible to harvest as much as 14.4 kg filled seed/ha per year at age 6 (i.e., 28.8 kg/ha every other year).
- Grafting continued in the Miniaturized Seed Orchard Study. The number of new grafts in 2004 was 1,177 in the main plots and 262 in the supplemental blocks. In addition, 173 trees were transplanted to replace dead trees and allow new clones to be incorporated into the experimental design. Overall graft survival in the fall of 2004 now stands at 95.0%.
- We held a technology transfer workshop entitled "Genetics and Growth Modeling Workshop" on November 4-6, 2003. This workshop was organized in collaboration with the USFS Pacific Northwest Research Station, Northwest Tree Improvement Cooperative, Stand Management Cooperative, Port Blakely Tree Farms, and the Department of Forest Science at Oregon State University.

## MESSAGE FROM THE DIRECTOR

In the spring of 2004, we completed the Pollen Contamination Study, one of our most important research projects. Gancho Slavov defended his Ph.D. dissertation, entitled "Development and Application of SSR markers for Measuring Gene Flow in Douglas-fir," and gave his final presentation to the PNWTIRC members at the 2003-04 Annual Meeting. A summary of his most recent results is included in this annual report. Tom Adams and Steve Strauss (OSU Department of Forest Science) deserve a great deal of the credit for Gancho's success, both for initiating the Pollen Contamination Study and providing valuable advice during its implementation. At the annual meeting, Gancho gave the PNWTIRC members a heartfelt "thank you" for all their support, and specifically acknowledged Jim Smith for all the work he did to make Gancho's research at the Plum Creek seed orchard a success. Gancho also worked closely with Valerie Hipkins to transfer the technology associated with his SSR markers to the USFS National Forest Genetics Laboratory (NFGEL). Valerie gave an overview of NFGEL at the annual meeting and emphasized that genetic analyses with Gancho's markers can now be done on a contract basis through NFGEL. I am very pleased that we were able to take Gancho's work all the way from theory to practice. Finally, Gancho completed his career as a PNWTIRC graduate student by giving us a wonderful overview of forestry in Bulgaria. Since the annual meeting, Gancho has returned to Bulgaria, married, and is now looking for a job in Bulgaria as a forest geneticist. We wish him all the success he deserves!

Now that the Pollen Contamination Study is completed, we will be shifting our emphasis into new research areas. During the next year, we will focus heavily on designing and initiating new research on Douglas-fir wood quality (see New Research Directions), completing the Early Flowering Study, and continuing the Miniaturized Seed Orchard Study. Over the past 20 years, graduate students have played a critically important role in PNWTIRC research, so I hope we can continue to attract high-quality graduate students to work with the PNWTIRC in the future.

*Glenn Howe*

## INTRODUCTION

### *Research Overview*

Our recent focus has been on seed orchard research, including (1) pollen contamination in wind-pollinated seed orchards, (2) early flowering, and (3) miniaturized seed orchards.

**Pollen Contamination Study.** Pollen contamination can be a problem if improved genotypes are pollinated by pollen from outside of the seed orchard—either from trees in nearby native stands or adjacent seed orchard blocks containing parents from other breeding zones. The ultimate goal of the Pollen Contamination Study is to increase genetic gains by reducing pollen contamination in wind-pollinated seed orchards. For the past three years, Gancho Slavov has been developing molecular genetic markers (SSRs) and studying pollen contamination in an operational Douglas-fir seed orchard as part of his Ph.D. program. The SSR markers he developed can be used by seed orchard managers to measure pollen contamination, SMP success, selfing, and within-orchard patterns of mating. Furthermore, once pollen contamination can be measured precisely, it will be easier to test strategies for reducing pollen contamination—strategies such as bloom delay, selective harvesting of seed orchard seed, or pollination control (e.g., controlled mass pollination or supplemental mass pollination). The final results of Gancho’s research are described on page 11.

**Early Flowering Study.** The goal of the Early Flowering Study is to speed genetic gains from tree improvement by promoting seed production on very young orchard grafts. We tested the ability of GA (gibberellic acid) and girdling to stimulate seed production in young orchards. Our results suggest that operational amounts of improved seed can be obtained from high-density seed orchards as young as 6 years from grafting with little adverse effect on the trees. These results are described on page 17.

**Miniaturized Seed Orchard Study.** Miniaturized seed orchards (MSOs) are attractive alternatives to conventional wind-pollinated seed orchards. In MSOs, the 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. Progress on our Miniaturized Seed Orchard Study is described on page 21.

### *New Research Directions*

At the annual meeting in July 2004, we decided to move ahead on two related wood quality projects. The first project is a collaborative study that will be funded jointly by the PNWTIRC and USFS Agenda 2020 program entitled “Discovery of Genes Controlling Wood Property Traits in Douglas-fir.” Our collaborators on this project are David Neale, a geneticist with the USFS Institute of Forest Genetics and University of California at Davis, and Brad St. Clair, who is the Genetics Team Leader with the USFS Pacific Northwest Research Station (USFS-PNWRS). For the second project, we will develop and expand on a pre-proposal that we wrote as part of the PNWTIRC five-year planning process. The tentative title of this project is “Genetics of Douglas-fir Wood Stiffness (MOE) and Strength (MOR).” We will develop a full proposal for this project that will be presented to the PNWTIRC membership for review and approval.



# Technology Transfer

## Introduction

Over the past three years, the PNWTIRC placed a renewed emphasis on technology transfer. Our newest initiative is the organization of technology transfer workshops on relevant and timely tree improvement topics. In November 2003, we held the third of these workshops entitled “Genetics and Growth Modeling Workshop” (Tables 1 and 2). These workshops augment other ongoing technology transfer activities, including publishing cooperative research reports, meeting with cooperators, holding annual meetings, and publishing annual reports.

The workshop entailed a 1-day series of presentations that (1) covered key concepts in tree improvement and growth modeling and (2) discussed ways to incorporate genetics into growth models. This session was attended by 55 participants. A smaller group of 29 people attended the following 2-day discussion session that explored genetics and growth modeling issues in much greater depth.

Table 1. Organizing committee and financial sponsors of the Genetics and Growth Modeling Workshop

Organizing committee	Affiliation
Glenn Howe	PNWTIRC, Oregon State University
Marilyn Cherry	PNWTIRC, Oregon State University
Brad St. Clair	USFS Pacific Northwest Research Station
Keith Jayawickrama	NWTIC, Oregon State University
David Briggs	SMC, University of Washington
David Marshall	USFS Pacific Northwest Research Station
Greg Johnson	Weyerhaeuser Company
Paul Anderson	USFS Pacific Northwest Research Station
David Walters	Roseburg Resources
Mike Mosman	Port Blakely Tree Farms
Financial sponsors	
Pacific Northwest Tree Improvement Research Cooperative	
USFS Pacific Northwest Research Station	
Northwest Tree Improvement Cooperative	
Stand Management Cooperative	
Department of Forest Science, Oregon State University	
Port Blakely Tree Farms	

### The goals of the Genetics and Growth Modeling Workshop were to:

- Promote discussion among forest geneticists and growth modelers
- Promote discussion among researchers who have specifically studied the impacts of genetics on growth and yield models
- Develop specific recommendations for incorporating genetic gain into Douglas-fir growth and yield models
- Develop a list of research priorities to better understand the effects of genetics on growth and yield models
- Inform foresters about the potential effects of genetics on growth and yield models

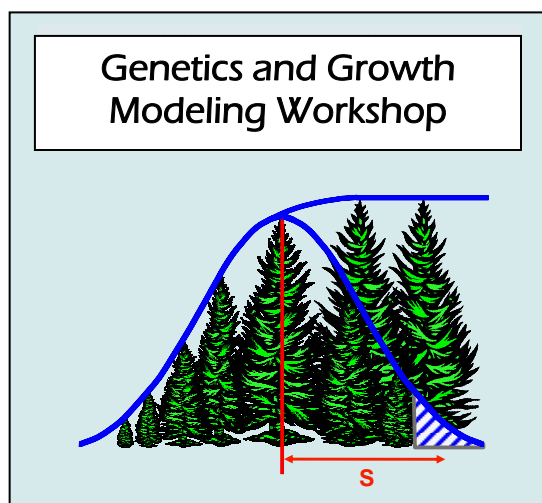
A more detailed summary of the meeting is available online at the PNWTIRC web site (Cherry et al. 2004). This publication contains the presentations from the 1-day general session and summarizes the questions addressed, workshop goals, and relevant literature.

Table 2. General workshop session agenda.		
Presentation	Speaker	Affiliation
Workshop introduction	Glenn Howe	PNWTIRC, Oregon State University
Introduction to forest genetics	Randy Johnson	USFS Pacific Northwest Research Station
An introduction to growth models	David Marshall	USFS Pacific Northwest Research Station
Incorporating genetics into growth models: a geneticist's perspective	Sam Foster	Mississippi State University
Incorporating genetics into growth models: a modeler's perspective	Greg Johnson	Weyerhaeuser Company
Genetic effects in growth and yield models: what do model users think?	Wade Harrison	Forest Technology Group
Integration of genetics into growth models: state of the art in B.C.	Jim Goudie	British Columbia Ministry of Forests
Modeling genetic gain in growth in New Zealand: 1986-2003	Sue Carson	Carson Associates
Incorporating genetics into growth and yield models: stand dynamics considerations	Marilyn Buford	USFS Vegetation Management and Protection Research
Integration of genetics into growth models: state of the art and challenges in the southern U.S.	Steven Knowe	University of Tennessee
Process models and tree breeding	Joe Landsberg	CSIRO, Australia (retired)
Merging genetics and forest growth modeling	Robert Monserud	USFS Pacific Northwest Research Station

## Key points from the “Genetics and Growth Modeling Workshop”

### *Why incorporate genetics into growth models?*

Plantations throughout the world are being established with improved tree varieties that have different growth characteristics than those used to develop current growth models. New growth models that incorporate the effects of genetic improvement are needed to conduct realistic financial analyses of forest operations and guide tree improvement programs. The “Genetics and Growth Modeling Workshop” addressed these needs from both the geneticist’s and the growth modeler’s perspective.



The approaches that geneticists use to select superior genotypes and estimate genetic gains are usually inadequate for predicting growth superiority at rotation age. These approaches include the use of small plots (mostly single-tree plots in the Pacific Northwest), selection of superior genotypes at an early age (perhaps  $\frac{1}{4}$  rotation age), and incomplete sampling of field environments. Although these approaches work well for ranking genotypes at an early age and (presumably) maximizing genetic gain per year, suppression of slow-growing genotypes in single-tree plots, imperfect age-age genetic correlations, and genotype by site interactions can affect estimates of heritabilities and genetic gains. The methods used by tree breeders are highly efficient, but large-plot genetic experiments must also be established.

Forest biometricians use various types of models to mathematically represent the dynamics of natural forests. Growth models may describe the growth of individual trees or stands, or may combine growth processes at both scales. Process/mechanistic models are based on growth processes at the physiological, physical, and biochemical levels, but are not predictive. Hybrid models are a complementary merging of well-understood processes and reliable tree/stand empirical elements. In any case, models should be based on the growth characteristics of the trees being planted, most of which are genetically improved. Furthermore, the link between genetic improvement and growth models is weak because most models are designed for stands greater than 10 to 15 years old (i.e., after vegetative competition has been overcome), but genetic tests in the Pacific Northwest are rarely measured beyond age 15. While young stand models do exist, there is a discontinuity when information from one growth model is fed into another.

### *Geneticists and modelers view growth and genetic gain differently*

The traits of interest to geneticists and growth modelers often differ. Geneticists typically focus on individual-tree traits such as tree height, diameter, volume, crown size, mortality, stem taper, and branch size. Although these traits are consistent with the architecture of individual-tree growth models, modelers are often interested in other stand-level traits and growth functions, such as volume or basal area per hectare, dominant stand height, height and diameter frequency distributions, etc. Process models, on the other hand, may incorporate changes in photosynthetic or water-use efficiency, carbon allocation, or the architecture of crowns or roots.

Another distinction between geneticists and growth modelers is that geneticists often measure genetic gain at a particular point in time, whereas growth modelers may be interested in measuring genetic differences in growth curve parameters. If growth differences are present, then it becomes important to know how long these differences persist. Our ability to predict future differences in volume per hectare is largely dependent on our ability to estimate these genetic differences in growth curves on a stand basis.

Whereas site index curves are generalizations over many sites, geneticists want to know how much additional volume to expect at rotation age by planting a seedlot of a certain breeding value on a specific site. This would require fine-scaled knowledge of genotype by site interactions. Furthermore, geneticists want to be able to model the effects of competition and stand structure, and to understand how to alter silvicultural practices in conjunction with improved planting stock to optimize yields.

### *How to incorporate genetics into growth models?*

Ideally, geneticists and modelers would work together to design trials and gather data that will provide information they both need. The best way to incorporate genetics into growth models would be to derive entirely new models based on long-term measurements of superior genotypes in large-plot experiments. If the new models had the same form as earlier models, then it would also be possible to see how the growth functions changed in response to genetic

improvement. Because there is not enough data to do this, other approaches have been used. These include (1) site index adjustment, (2) effective age adjustment, and (3) growth modifiers. Each of these methods is described in the workshop proceedings (Cherry et al. 2004).

Although site index and effective age adjustments are commonly used to incorporate genetics into growth models, these methods are not optimal. The growth modifier approach seems to be the most promising short-term solution. This would entail developing a multiplier for each growth trait in the model that is correlated with the breeding value of that trait. In New Zealand, research results support the following conclusions: (1) growth multipliers are an effective way to incorporate genetics into growth models; (2) increases in growth rate (growth multipliers) are proportional to genetic superiority; (3) increases in growth rate are constant across stands ages, regions, and tree stocking levels (i.e., thinning regimes); (4) genetic gains estimated from progeny tests are similar to actual diameter increases in large-plot trials; and (5) diameter and height distributions do not differ among improved seedlots. Although it is important to validate genotype performance using large block-plot experiments, few of these experiments exist or are old enough to provide useful data. Therefore, methods to predict genetic gains on an area basis from individual-tree data would be valuable. Ample progeny test data are available in the Pacific Northwest, but this approach has not been fully developed. In this region, an important first step would be to use data from single-tree plot progeny tests to develop growth modifiers that can be fed into the ORGANON growth model. This approach should be easy to implement and capable of providing short-term solutions in the near future. A case was made for standardizing the procedures used to estimate breeding values in the Pacific Northwest. The frequency of scheduled progeny test measurements may need to be adjusted. After crown closure, heritabilities are inflated by intergenotypic competition, but it may be possible to remove this bias using growth models.

In the long term, the challenge of incorporating genetics into growth models should be addressed from numerous standpoints. One option is to establish large block-plot experiments of paired treatments as part of an operational planting program. By using ongoing, operational planting programs, it should be feasible to install these experiments on a large scale across many sites. These experiments could be used to compare one checklot and one seedlot of known genetic worth. Furthermore, crown measurements should be incorporated into progeny test analyses. Better site and genotype by site characterization is desirable, and could form the basis for refining operational planting guidelines for the deployment of genetically improved materials so their genetic potential is optimized. The importance of a seedlot certification system for genetically improved seedlots was also recognized. Additionally, exploration of the potential ramifications of climate change is needed. Major additions that could be incorporated into current models such as ORGANON include young stand development, climate, and site characterization. Hybrid models could be used to investigate the physiological and morphological differences between genotypes as related to tree and stand growth.

We encourage interested individuals to read the full proceedings of the "Genetics and Growth Modeling Workshop," which can be found at the PNWTIRC web site ([http://www.fsl.orst.edu/pnwtirc/publications/pnwtirc\\_pubs\\_date.htm](http://www.fsl.orst.edu/pnwtirc/publications/pnwtirc_pubs_date.htm)).

## **Plans for Technology Transfer in 2004-2005**

The PNWTIRC will participate in a workshop entitled "Cold Hardiness Testing in Advanced-Generation Genetic Improvement Programs" to be held jointly with the Northwest Tree Improvement Cooperative in December 2004. We will present the results of former cold hardiness research carried out by the PNWTIRC, and factors to consider when deciding whether to use cold hardiness testing in advanced generation breeding programs.

# POLLEN CONTAMINATION STUDY: FINAL RESULTS

## *Introduction*

The goal of the Pollen Contamination Study was to develop improved, DNA-based genetic markers called simple sequence repeats (SSRs) that can be used to estimate pollen contamination and other mating parameters in Douglas-fir seed orchards. Pollen contamination is measured as the proportion of seeds fertilized by pollen coming from outside of the seed orchard, and has the potential to reduce genetic gains and increase the maladaptation of seed orchard crops. Highly variable SSR markers make it possible to accurately measure pollen contamination and characterize patterns of within-orchard mating by directly identifying the male and female parent of each seed produced in the orchard. The goal of the final phase of the Pollen Contamination Study was to take the SSR markers we developed and measure pollen contamination and mating patterns in an operational Douglas-fir seed orchard. The specific objectives of this phase of the study were to (1) estimate pollen contamination in three seed crops of one seed orchard block, (2) test whether pollen contamination levels vary among clones with different floral phenologies, (3) determine the relative paternal contributions of the clones in the block, and (4) test for assortative mating with respect to floral phenology. In previous annual reports, we described the development of our SSR markers and showed how the paternal contributions to the seed orchard seed varied dramatically among seed orchard parents (PNWTIRC Annual Report 2001-2002 and 2002-2003).

## *Accomplishments for 2003-2004*

### **Materials and methods**

We used SSR markers to measure pollen contamination and characterize mating patterns based on seed samples collected in three years (1999, 2000, and 2003) from one block of a non-isolated, open-pollinated, clonal seed orchard of Douglas-fir (Figure 1). In addition to the seed orchard block we studied (referred to as the "Test Block"), there are four other seed orchard blocks at this seed orchard complex. All blocks are subdivided into three sections of approximately equal size (Figure 1). Each year, flower stimulation is carried out on all ramets in one of the three sections in each block. Therefore, each section is stimulated and harvested once every three years, and pollen production is approximately equal in all blocks each year.

**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 alleles, and amplifies a single locus in each PCR reaction.

We used 7-9 SSR markers to genotype (1) all ramets in the Test Block and (2) seeds from four samples collected in 1999, 2000, and 2003. We also measured reproductive bud phenology in 2000 and 2003, and used pollen traps to measure relative pollen abundances throughout the seed orchard complex during the spring of 2000.

Two types of seed collections were tested: 'bulk' and 'individual-ramet' samples. Two 'bulk' samples (1999 and 2000) were constructed by mixing approximately equal numbers of seeds from all ramets for which cones were operationally harvested. For the bulk samples, the haploid megagametophytes and diploid embryos were genotyped separately using seven SSR loci, and the identity of the female parent was inferred by comparing the maternal haplotype to the clonal genotypes in the orchard. We used these bulk samples to (1) estimate mean pollen contamination per ramet and (2) evaluate the relative paternal contributions of the clones in the Test Block.

In the individual-ramet samples, the seedlots were collected by individual tree. Therefore, the identity of the female parent for each seed was known, and only the genotype of the embryo was measured using DNA analysis. Furthermore, the parents of the individual-ramet samples were classified into three female receptivity classes to compare pollen contamination among ramets with different timings of female cone receptivity. We used nine highly variable SSR loci to analyze the individual-ramet seed samples.

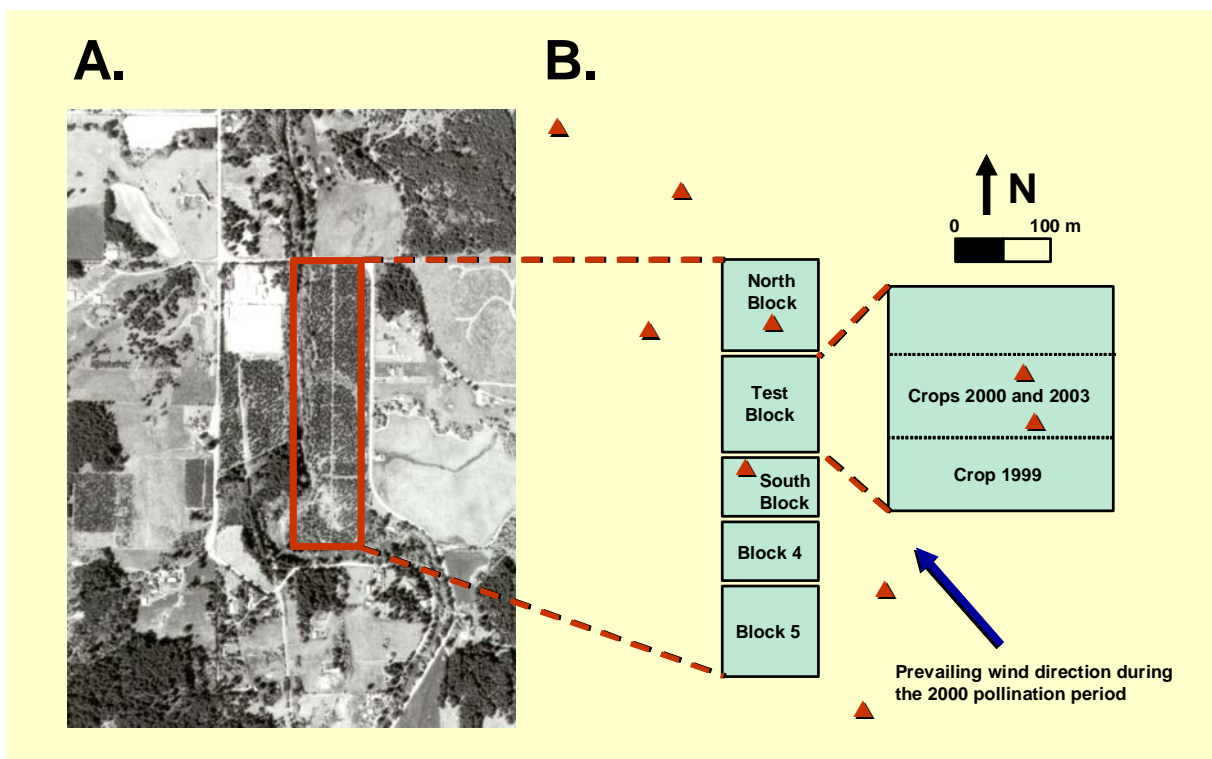


Figure 1. Douglas-fir seed orchard complex used in the Pollen Contamination Study. A. Aerial photograph showing the entire seed orchard complex. B. Schematic diagram showing the individual seed orchard blocks. The Test Block is 2.1 ha, with 342 ramets. Triangles indicate the approximate locations of pollen traps relative to the seed orchard blocks.

### *Pollen contamination was high*

Pollen contamination was high in all four seed samples that we analyzed (mean = 35%; range = 31-41%) (Table 3). This result is consistent with pollen contamination levels reported earlier for seed orchards of Douglas-fir and other conifers (Adams and Burczyk 2000; Pakkanen et al. 2000). In the absence of substantial spatial isolation (>1-2 km) from other orchard blocks or stands of the same species, reductions in pollen contamination in open-pollinated conifer seed orchards may require effective implementation of pollen management techniques such as supplemental mass pollination and bloom delay (Wheeler and Jech 1986; El-Kassaby and Ritland 1986; Adams and Burczyk 2000). Because SSR markers are so variable, the standard errors of our pollen contamination estimates were approximately three times lower than those reported in a study in which similar numbers of seeds were analyzed using 11 allozyme loci (Table 3; Adams et al. 1997).

Table 3. Seed and pollen contamination and self-fertilization in one block of a Douglas-fir seed orchard.

Year	Type of seed collection	No. of seeds analyzed	Observed seed contamination (%)	Observed self-fertilization (%)	Pollen contamination (%) $\pm$ SE
1999	Bulk	192 (190 <sup>a</sup> )	1.0	2.1	31.0 $\pm$ 3.5
2000	Bulk	192 (102 <sup>a</sup> )	46.9	1.0	36.8 $\pm$ 5.2
2000	Ind.-ramet	240	0	1.3	32.0 $\pm$ 3.2
2003	Ind.-ramet	336	0	2.3	41.3 $\pm$ 2.8
<b>Mean</b>					<b>35.3 <math>\pm</math> 2.4</b>

<sup>a</sup> Number of seeds used to estimate pollen contamination after accounting for seed contamination.

### *Seed contamination was detected*

Low levels of seed contamination have been reported in Douglas-fir seed orchards (e.g., Adams et al. 1997). The high seed contamination in the bulk sample from 2000 probably resulted from mixing the seedlot produced in the Test Block with a seedlot from a different orchard block during cone processing or seed extraction. Using highly variable SSR markers, seed contamination can be easily detected to avoid deploying maladapted seed.

### *Synchrony of pollen shed and female receptivity*

The period of peak female receptivity of the flower-stimulated section of the Test Block (Figure 2A) coincided with maximum pollen abundance in the Test Block (Figure 2B) and the North Block (Figure 2D). The early-flowering clones in the Test Block were receptive during maximum pollen abundance in the South Block (Figure 2C) and before pollen abundance in the Test Block had reached a stable maximum. Throughout the flowering period, the pollen abundance measured outside of the orchard was substantially lower than in any of the seed orchard blocks in which pollen traps were established (Figure 2E). Based on the relative pollen abundances measured in 2000, pollen contamination from the natural stands of Douglas-fir surrounding the orchard was only 6.4% (i.e., almost six times lower than the total rate of pollen contamination from all sources).

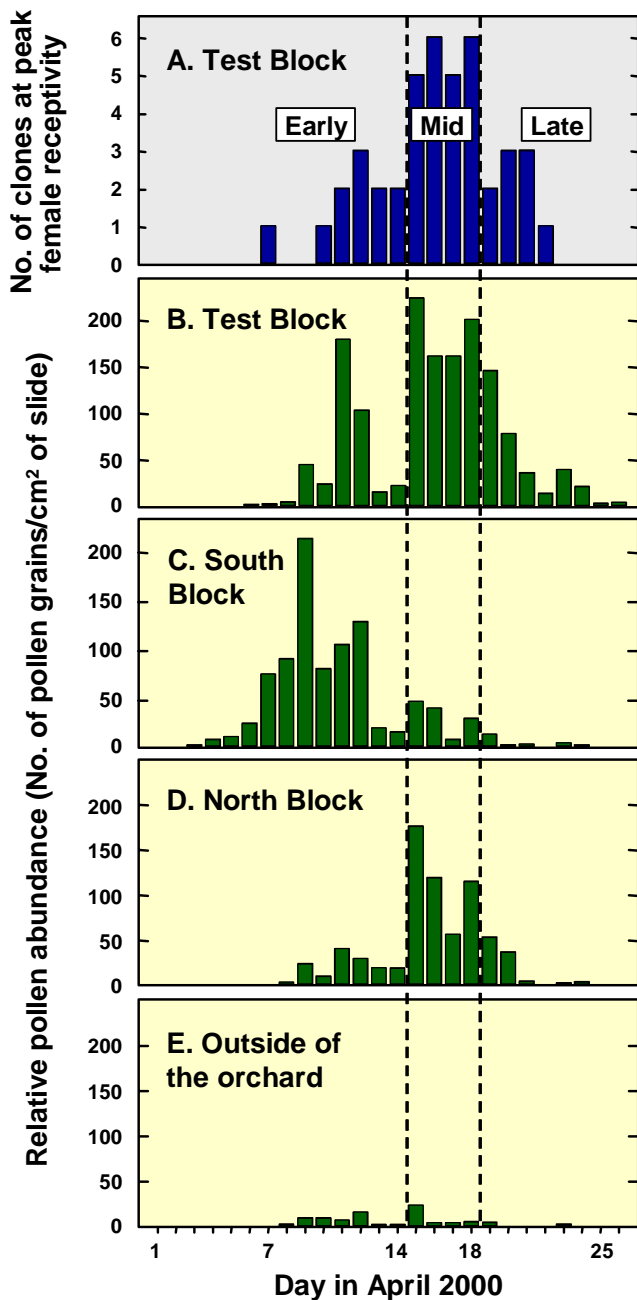


Figure 2. Synchrony between female cone receptivity and pollen shed in 2000. Timing of peak female receptivity of 42 clones from the Test Block (A) and relative abundance of pollen measured in the Test Block (B), South Block (C), North Block (D), and outside of the seed orchard (E). The dashed lines indicate the days in April that differentiate the early-, mid-, and late-receptivity classes.

### *Early flowering clones had the highest level of pollen contamination*

Levels of pollen contamination averaged over 2000 and 2003 were higher in clones with early female receptivity (mean = 55.5%) than in those with either mid (mean = 36.4%) or late (mean = 28.3%) receptivity (Figure 3). Given the relative pollen abundances inside and outside of the orchard complex (Figure 2), and the prevailing wind direction during the 2000 pollination period (Figure 1), the excessive pollen contamination in the early receptivity class probably came from the South Block.

### *Seed orchard mating is non-random*

There was a clear pattern of positive assortative mating with respect to floral phenology. Crosses among clones from the same female phenology class were more frequent than expected under the assumption of random mating (Figure 4). This deviation appeared to be stronger for the Early × Early (Figure 4A) and Late × Late crosses (Figure 4C), than for the Mid × Mid crosses (Figure 4B). Crosses among parents from the two extreme phenology classes were not observed. This pattern is of particular concern for clones with extremely early or late floral phenologies because these clones have little chance to mate with the majority of the other clones within the same orchard block.

### *Individual-ramet samples are more efficient than bulk samples for measuring pollen contamination*

Although bulk seed samples can be used to measure pollen contamination and other mating parameters, two assays are needed for each seed (embryo and megagametophyte). Compared to the bulk samples, the individual-ramet seed samples are more cost-effective than bulk samples because only the genotype of the embryo needs to be measured, thereby reducing the number of genotyping assays by 50%. This results in a large cost savings, even though two additional SSR loci must be used when only embryos are genotyped (Slavov 2004).



### Implications for seed orchard management

Our results have several practical implications. First, we confirmed that pollen contamination in non-isolated, open-pollinated conifer seed orchards could be high. Pollen contamination in seed orchards of Douglas-fir can be reduced using bloom delay and supplemental mass pollination (Wheeler and Jech 1986; El-Kassaby and Ritland 1986). The ultimate solution to this problem, however, may be to (1) establish seed orchards in areas isolated by at least a few kilometers from non-orchard sources of contaminating pollen and use appropriate regimes of flower stimulation, or (2) adopt alternative seed orchard designs that allow effective application of pollen management techniques, including controlled pollination (see Miniaturized Seed Orchard Study, p. 21).

Second, seeds from early clones may have pollen contamination levels that are 30-100% higher than seeds from clones with mid or late receptivity. Nonetheless, only a slight reduction in pollen contamination is expected if seedlots from clones with extreme floral phenology are not included in bulk seed crops. In the Test Block, for example, we estimated that if seeds from clones with early female receptivity had been excluded from bulk crops, the overall pollen contamination would have been reduced from 32.0 to 30.2% in 2000 and from 41.3 to 35.9% in 2003. Thus, variation in pollen contamination among clones with different phenologies is only practically important if individual-clone seedlots are to be deployed, and if some of the clones are receptive at times when little pollen is produced in the orchard block.

Third, our results suggest that the higher pollen contamination detected in early-receptive clones probably resulted from pollen produced in an adjacent seed orchard block. Furthermore, it appeared that most of the pollen contamination

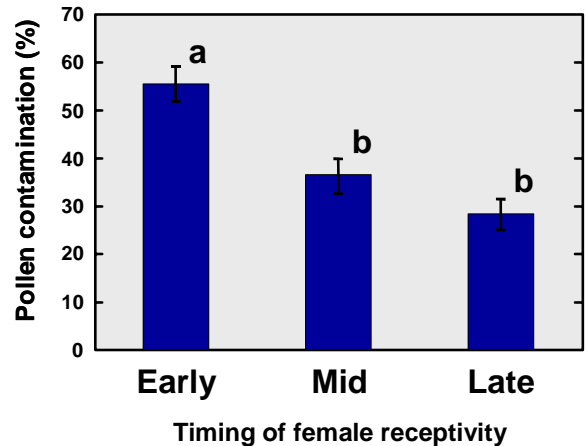


Figure 3. Mean ( $\pm$  s.e.) pollen contamination for parents with early, mid and late female receptivity in the Test Block.

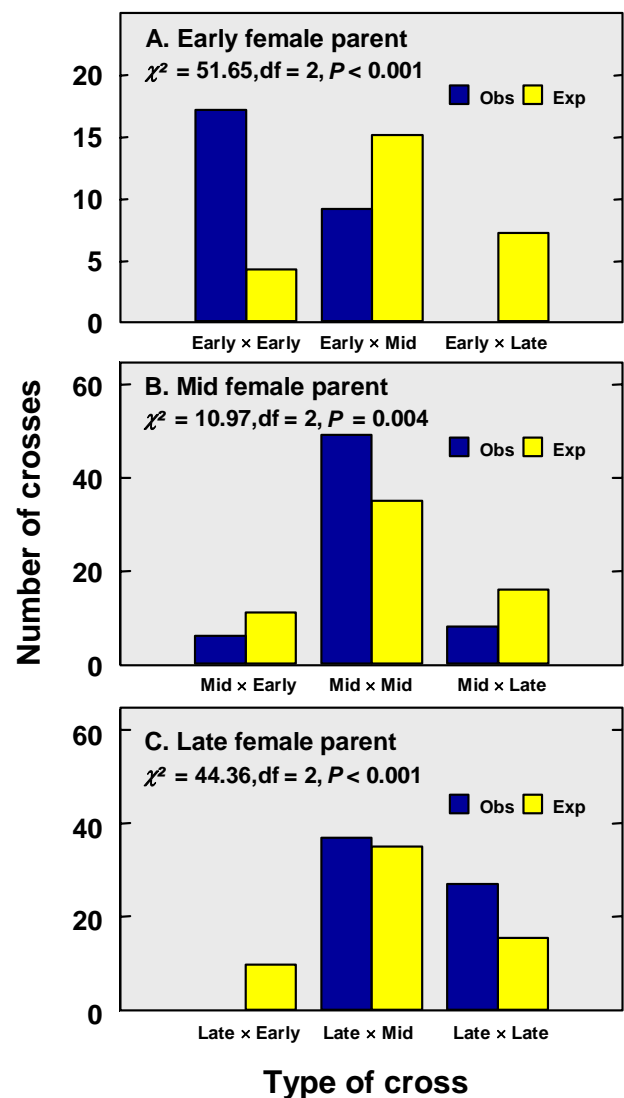


Figure 4. Goodness-of-fit-tests for observed and expected number of crosses within and among three floral phenology classes. (A) Crosses involving female parents with early receptivity. (B) Crosses involving female parents with mid receptivity. (C) Crosses involving female parents with late receptivity.

in all receptivity classes resulted from immigrant pollen produced in the other four blocks of the seed orchard. Cross-pollination between seed orchard blocks serving different breeding zones may adversely affect the adaptability of the resulting seedlots (Kylmänen 1980; Nikkanen 1982; Stoehr et al. 1994). If seed crops must be harvested from multiple blocks in the same year, the risk of compromising the adaptability of seed crops can be minimized by simultaneously stimulating blocks that serve ecologically similar breeding zones.

Finally, individual-ramet seed samples appear to be more cost-efficient than bulk seed samples for measuring pollen contamination and characterizing within-orchard mating patterns when highly variable SSR loci are used. This is because only a single genotyping assay is needed for the individual-ramet samples (i.e., the embryo), whereas two genotyping assays are needed for the bulk seed sample (i.e., the embryo and megagametophyte).

Overall, the Pollen Contamination Study demonstrated that fewer than ten SSR markers were needed to (1) measure pollen contamination and selfing rates, (2) measure the relative paternal success of the clones in the Test Block, and (3) detect deviations from random mating with respect to floral phenology. Because SSRs provide a way to measure genetic efficiency parameters with high accuracy, they will be useful tools for the future improvement of seed orchard design and management.

SSR genotyping is now available through the USFS National Forest Genetics Laboratory (NFGEL; (<http://www.fs.fed.us/psw/programs/nfgel/>)). A computer program for estimating pollen flow is available on the PNWTIRC website (<http://www.fsl.orst.edu/pnwtirc/publications/pfl.zip>).

### *Plans for 2004-2005*

The pollen contamination study is now completed. We will be publishing two more papers from this study in scientific journals. These papers will be entitled "Estimating pollen flow using SSR markers and paternity exclusion: Accounting for mistyping" (Slavov, Howe, Birkes, Gyaourova, and Adams), and "Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by SSR markers" (Slavov, Howe, and Adams).

# EARLY FLOWERING STUDY: FINAL RESULTS

## Introduction

Early flower production in seed orchards allows breeders to capture genetic gains earlier and may shorten the time between breeding generations. The Early Flowering Study was designed to determine (1) whether girdling and gibberellin<sub>4/7</sub> (GA) treatments stimulate flowering in very young Douglas-fir seed orchard grafts, (2) the best GA application rates for stimulating flower production, and (3) whether treatments adversely affect tree growth or seed production. This study was intended to provide preliminary information for designing flower-stimulating treatments for later use in the Miniaturized Seed Orchard Study.

Two separate experiments were carried out (for complete details, please see the PNWTIRC Annual Report for 2002-2003). In Experiment 1, we investigated the effects of girdling and GA stem injection treatments, alone and in combination, in two separate Douglas-fir seed orchards over two consecutive years. The Pacific Northwest Christmas Tree Association (PNWCTA) seed orchard was grafted in 1997, whereas the Vaughn orchard was grafted in 1999. In the second year, GA rates were quadrupled (4X) due to the low response we observed after the first treatment year. In Experiment 2, we studied the effect of increased rates of GA (4X, 6X, and 8X) in combination with girdling in the Vaughn seed orchard.

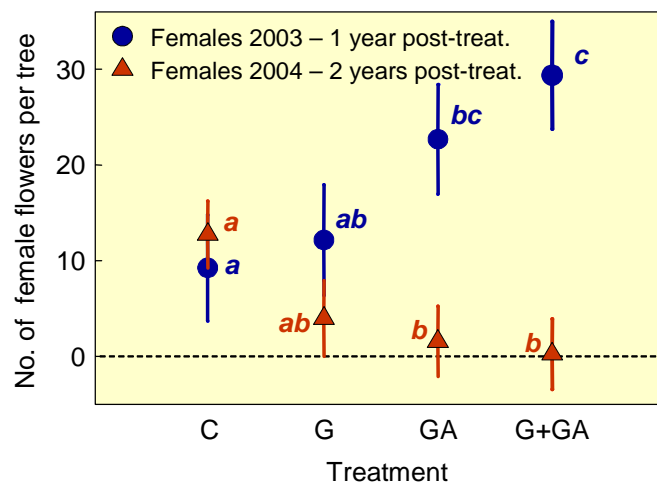
## Accomplishments for 2003-2004

Major results from this study were presented in last year's annual report. The most promising treatment is a combined girdling plus GA treatment, in which GA is applied at a '4X' rate of about 0.336  $\mu\text{l}$  ProCone<sup>®</sup> per  $\text{mm}^2$  stem diameter (4X is the manufacturer's recommended rate for Douglas-fir). If this treatment is used 4 or 5 years after grafting, commercially harvestable crops may be produced. Our subsequent focus was to investigate whether the treatments showed any (1) carryover effects (e.g. increased flowering two years after the last treatment), (2) adverse effects on tree growth and health, or (3) adverse effects on seed yields or seed weight.

## Treatment carryover effects

Treatment carryover effects have been noted in other Douglas-fir girdling trials (Woods 1989). In our study, girdling and GA did not increase flowering two years after the last treatment was applied in 2002 (Figure 5). In fact, there was a negative correlation between the amount of flowering in 2003 (one year after the last treatment) and flowering in 2004 (two years after the last treatment). Hence, it appears that high flowering and seed production in 2003 resulted in lower seed crops the following year.

Figure 5. Female flower counts in the spring of 2004 show an inverse relationship with flowering in 2003 in trees treated with girdling (G), gibberellic acid (GA), or a combination of girdling and gibberellic acid (G+GA) in the spring of 2002. GA = 4X rate. 'C' represents untreated controls.



## Tree health, survival, and cone abortion

We measured relative growth rate (RGR) to determine whether the treatments adversely affected tree growth. RGR is the amount of annual stem elongation relative to the height of the tree at the beginning of the growing season. Because some ramets in Experiment 2 had been topped before the Early Flowering Study was begun, RGR was measured only in Experiment 1. RGR showed no treatment effects during the growing season the treatments were applied. However, RGR was significantly lower in the trees treated with GA or girdling+GA the following year during cone development. Because cone production was higher in these trees, this suggests that there was a tradeoff between vegetative and reproductive growth in the stimulated trees (Figure 6).

In Experiment 1, post-treatment mortality was not significantly different between treatments in either orchard (Figure 7). However, in Experiment 2, significant treatment differences ( $p = 0.0058$ ) were observed (data not shown). When the 6X rate of GA was used in combination with girdling (i.e., the G+6X GA treatment), 25% of the treated trees died in subsequent years, but there was no mortality among the untreated controls.

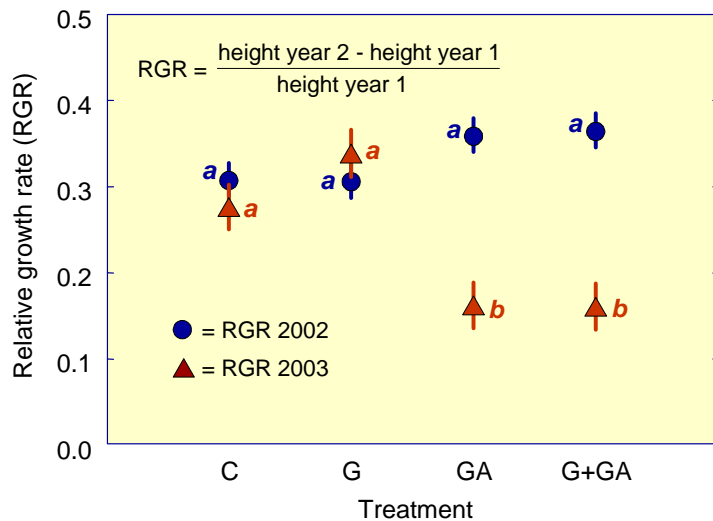


Figure 6. Relative growth rates (RGR) for trees in the Vaughn seed orchard (Experiment 1) in 2002 (the growing season after the second treatment) and 2003 (the growing season during cone development). Treatments are described in Figure 5. GA = 4X rate.

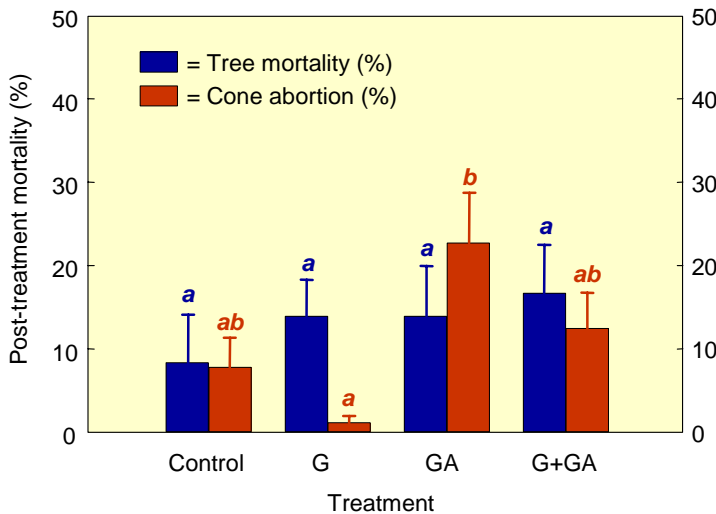


Figure 7. Post-treatment mortality and cone abortion rates in the Vaughn seed orchard (Experiment 1) in 2003, the growing season during cone development. Treatments are described in Figure 5. GA = 4X rate.

Seed cone counts were made in the fall of 2003 to determine cone abortion rates (i.e., relative to female flower counts made in the spring of 2003). Cone abortion varied among treatments only at the Vaughn orchard in Experiment 1, where the 1X GA treatment resulted in significantly more cone abortion than the girdling treatment, but no treatments resulted in more cone abortion than the controls (Figure 7). Interestingly, three trees that were girdled two years in a row produced a total of 15 hermaphrodite strobili (Figure 8), with a male portion at the base and a female portion at the tip of each strobilus. These trees were all ramets of a single clone in the Vaughn orchard.

## Seed yields

Filled seed counts and filled seed weights were available from trees that had been treated for cone and seed insects using a foliar spray of Asana<sup>®</sup> XL. Cones were collected in the fall of 2003 from the control and 4X treatments in the Vaughn seed orchard (Experiment 2). The number of filled seeds per cone was not affected by the flower-stimulating treatments (Figure 9). However, the seeds from the trees treated with the 4X concentration of GA weighed slightly less than the seeds from the controls.

## Projections of seed yields per hectare

We estimated seed production for the test orchards (8 x 13 foot spacing) assuming that all trees are stimulated on a 2-year cycle beginning at age 5 from grafting using the G+GA 4X treatment. Beginning at age 6, an average of 62.7 seed cones could be harvested per tree, with 10.6 filled seeds per cone. Using a value of 35,000 Douglas-fir seeds per pound, the yield from this orchard is estimated to be about 4.5 kg/ha per year. As trees grow and crowns enlarge, seed yields should increase. In a miniaturized seed orchard situation, in which the trees are planted at a very close spacing (e.g., 1 x 3 m; 3.3 x 9.8 feet), it might be possible to obtain as much as 14.4 kg filled seed/ha per year.



Figure 8. Hermaphrodite strobilus in the Early Flowering Study.

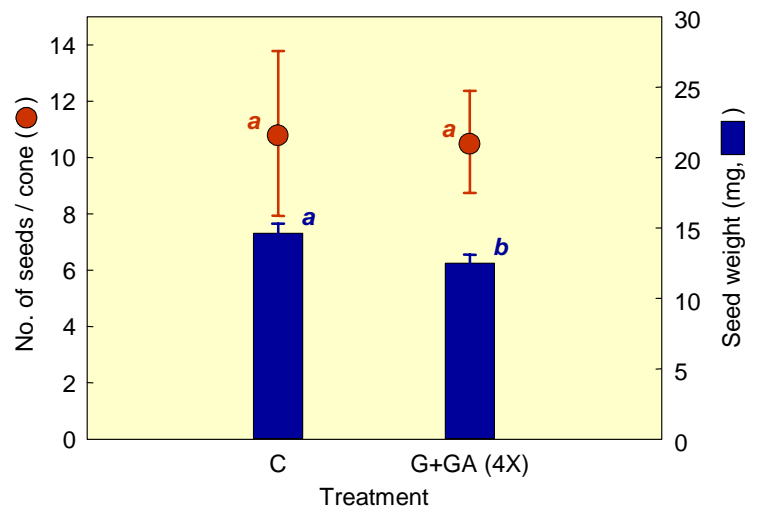


Figure 9. Number of seeds per cone and seed weight (mg) in 2003 for Vaughn Experiment 2.

## Summary

Cone and seed production can be enhanced by stimulating very young grafts with a combination of girdling and GA (at our 4X rate = 0.336  $\mu\text{l}$  ProCone<sup>®</sup> per  $\text{mm}^2$  stem diameter). Although we observed no major adverse effects on tree health or seed traits, orchard trees should be monitored because there were slight indications of adverse effects, particularly at the 6X rate of GA and higher. These effects included slightly increased tree mortality when the 6X rate of GA was used, and a slight reduction in seed weight using the 4X rate of GA. Despite these caveats, our girdling and GA treatments were very aggressive. In Experiment 1, for example, they were begun when the trees were 2 years old from grafting and were repeated in two successive years (1X GA followed by 4X GA). Because we observed no major adverse effects, these treatments should be reasonably safe, particularly if they are begun when the trees are 4 years old, and only applied every other year (discussed below).

Although cone crops can be increased on very young grafts, it may not be commercially viable to stimulate the trees until they are 4 or 5 years old from grafting. Cone production is limited by crown size and the number of shoot primordia that can differentiate into reproductive buds. Hence, it may be better to maximize vegetative growth in the first few years after orchard establishment, followed by floral stimulation at age 4 or later. Because young stimulated orchard grafts seem to require one year to recover from moderate to heavy cone production, we recommend stimulating the trees every other year (as is typically done in older trees) to maximize cone production over the long term.

Although non-precocious Douglas-fir clones responded to treatment, GA was most effective on clones that were predisposed to flower (Ross and Pharis 1976, 1986). Although we did not carry out extensive screening of clones, the high between- and within-clone variability that we observed, plus the higher tree mortality of the trees treated with 6x GA (G+GA 6X), leads us to caution against using dosage rates higher than our 4X rate on young untested clones. Management techniques including irrigation and fertilization during the year of treatment and the year following treatment are recommended for young stimulated orchards to partially mitigate the physiological stresses associated with the treatments and the ensuing heavy cone crops (Ross and Bower 1991).

## *Plans for 2004-2005*

The early flowering study is now complete. A draft paper has been written and will be submitted for publication in a peer-reviewed journal. A PNWTIRC Report will be sent to cooperative members. Findings may lead to further studies, such as studies to determine whether it is more efficient to use (1) root pruning instead of girdling or (2) foliar-applied GA instead of stem injections.

# MINIATURIZED SEED ORCHARD STUDY

## Introduction

The Miniaturized Seed Orchard Study is designed to test promising alternatives to conventional Douglas-fir seed orchards. In miniaturized seed orchards (MSOs), seed crops are produced on many small trees instead of fewer, larger trees at wider spacings, which is typical of conventional Douglas-fir orchards. Intensively-managed MSOs have the potential to (1) increase genetic gains by facilitating controlled mass pollination and (2) reduce management costs because of the smaller trees. More details on the objectives, potential advantages, and design of the MSO project can be found in the PNWTIRC Annual Reports for 2001-2002 and 2002-2003. The goal of the MSO Study is to compare various management regimes using three alternative planting densities at an operational scale that will provide realistic estimates of management costs and potential seed yields for Douglas-fir (Anekonda and Adams 1999).

## Accomplishments for 2003-2004

The grafting of the MSO study is essentially complete, with a graft survival rate of 95% measured in the fall of 2004. Because graft survival after the 2003 growing season was only 62%, it was necessary to do some re-grafting in the spring of 2004. Although scion material was readily available for the backward selections in the study (8 clones), insufficient material was available to make all necessary replacements for the forward selections (8 clones). If we had maintained the original field design, we would have needed to replace all forward selections in 2004 (i.e., including successful grafts), significantly adding to the cost of the study.

In January 2004, the MSO Advisory Committee was reconvened to discuss options for proceeding. The following committee members met in Salem: Mike Albrecht, Marilyn Cherry, Jeff DeBell, Randall Greggs, Glenn Howe, Sara Lipow, and Jim Smith. Don Copes was invited to join us to provide his perspectives to the group. At this meeting, we decided that one more year of orchard establishment was required, but that we would maintain as many successful grafts as possible by slightly altering the field design. In our original design, each replication had the same 16 clones. In the new design, replication is achieved using four, partially overlapping sets of clones (Sets A-D in Figure 10). The MSO Study now contains 24 clones replicated in each of the three spacings, with eight of these clones being new selections made in 2004. The new clones (i.e., new forward selections) that were added to the experiment were chosen based on a NWTIC advanced-generation BLUP analysis by Terrance Ye, and were subsequently field-verified by Jim Smith and Marilyn Cherry.

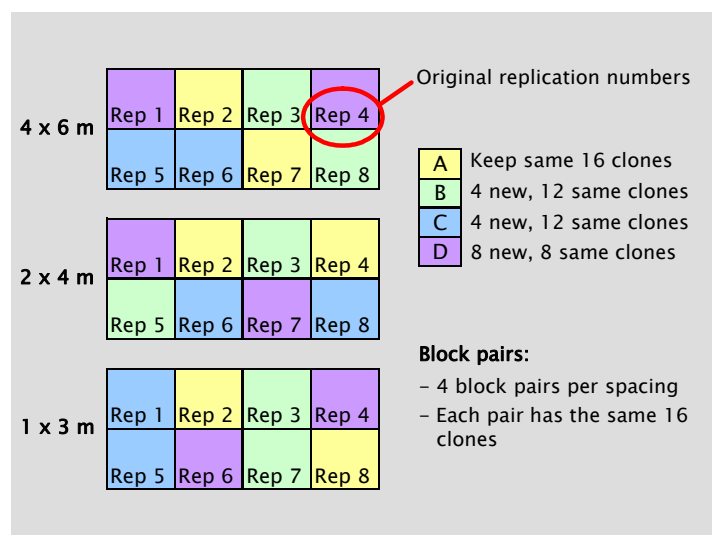


Figure 10. New field design for the Miniaturized Seed Orchard Study after grafting in the spring of 2004. Eight new clones were added to the existing set of 16 clones in the study.

A combination of transplanting and grafting was used to establish the new field design in 2004. Where available, grafts with a living scion were transplanted into spots within the main plots where that particular clone was required (Figure 11). In other cases, new scions were grafted onto existing rootstock. Scions were collected in late February and early March by Jim Smith, Mike Bramlett, and Marilyn Cherry. The best grafter from 2003 was contracted to carry out grafting in 2004 using Tree Seal<sup>®</sup> to seal the graft unions (Figure 12). This was the method that yielded the highest survival rate from the previous year.



Figure 11. A. Transplanting machine in action; B. Hand-transplanting in the 1 x 3 m micro-orchard spacing.

In 2004, 173 trees were transplanted and 1,177 rootstock were grafted in the main plots (Table 4). An additional 262 spare grafts were made in the supplemental blocks. The supplemental blocks were established to conduct preliminary experiments. Although we were unable to maintain a formal study design within the supplemental blocks, we still have enough grafts to carry out small preliminary operational studies in the supplemental blocks before we use these treatments on a full-scale basis in the main plots.

The grafting and transplanting in 2004 were both very successful. Of the trees transplanted into the main plots, only two died. Of the remaining transplants that lived, only nine scions died. Overall survival at the end of the 2004 growing season (including 2002 and 2003 grafts) was 95% (Table 4). Furthermore, we have enough spare grafts to be able to replace all grafts that die in the main plots in future years.

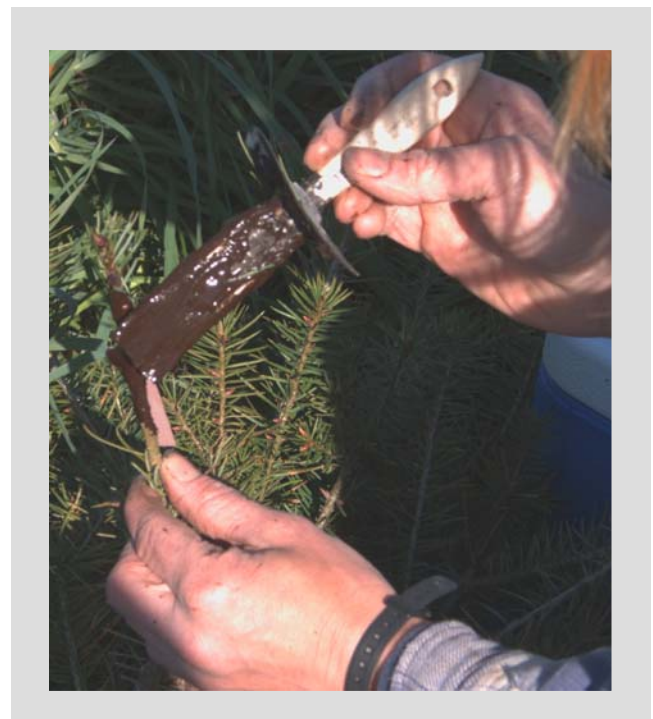


Figure 12. Applying Tree Seal<sup>®</sup>.



Table 4. Grafts, transplants, and survival for the Miniaturized Seed Orchard study in 2004

Total number of main-plot grafts in 2004 (including clonal substitutions)	1,177
Number of spare grafts in the supplemental blocks	262
<b>Total number of grafts</b>	<b>1,439</b>
Number of trees transplanted into the main plots	173
Graft survival in the main plots for grafts made in 2004	99.6%
<b>Overall graft survival in the fall of 2004 (including 2002 &amp; 2003 grafts)</b>	<b>95.0%</b>

Rootstock and graft maintenance was carried out by the grafting contractors and by Plum Creek throughout 2004. Site maintenance by Plum Creek is ongoing, including weed control, irrigation, and fertilization.

### *Plans for 2004-2005*

Now that the orchard establishment phase is almost complete, we will begin pruning the trees to control their size. After the 2004 growing season, we will prune the tops of the taller trees to allow the shorter trees to catch up in height. Dead scions will be replaced with transplants from the spare grafts in the supplemental blocks. A study design will be developed, and operational crown management trials may begin as early as 2005.

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## APPENDIX 1

### *Publications and Abstracts by PNWTIRC Personnel: 2003-2004*

- Cherry, M.L., and Howe, G.T. (eds). 2004. Proceedings, Genetics and Growth Modeling Workshop. November 4-6, 2003, Vancouver, Washington. Pacific Northwest Tree Improvement Research Cooperative Report #21, Oregon State University. 171 pp.  
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- Howe, G.T., Aitken, S.N., and St. Clair, J.B. 2003. Genomics and adaptation: Where do we go from here? Abstract in: Genomics and Adaptation: Proceedings of the Western Forest Genetics Association 2003 Conference, July 28-31, 2003, Whistler, B.C. p. 63.
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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. 2004. Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. *Theor. Appl. Genet.* 108:873-880.

## APPENDIX 2

### *Workshops and Presentations by PNWTIRC Personnel: 2003-2004*

#### **Workshops**

PNWTIRC/PNWRS/NWTIC workshop entitled "Genetics and Growth Modeling Workshop," Vancouver, WA, November 4-6, 2003 (55 attendees).

#### **Presentations**

Howe, G.T. 2004. Summary of the recent OSU genetics and growth modeling workshop. Meeting of the Growth Modelers Users Group, Feb. 25, 2004, Vancouver, WA.

Slavov, G.T. 2004. Development and application of SSR markers for measuring gene flow in Douglas-fir. Dissertation Defense, May 20, 2004.

## APPENDIX 3

### *PNWTIRC Financial Support for Fiscal Year 2003-2004*

Regular members <sup>1</sup>	104,000
Associate members <sup>1</sup>	4,000
Contracts	8,000
Forest Research Laboratory, Oregon State University <sup>2</sup>	104,414
<b>Total</b>	<b>\$220,414</b>

<sup>1</sup> Each Regular Member contributed \$8,000 and each Associate Member contributed \$4,000 excluding in-kind contributions of labor, supplies, etc.

<sup>2</sup> The contribution from Oregon State University includes salaries, facility costs, and administrative support.