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OREGON STATE UNIVERSITY
COLLEGE OF FORESTRY

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- Menasha Forest Products Corporation
- Oregon Department of Forestry
- Oregon State University
- Plum Creek Timber Company
- Roseburg Resources
- Simpson Timber Company
- Stimson Lumber Company
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- Lone Rock Timber Company
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- Inland Empire Tree Improvement Cooperative
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- University of British Columbia
- University of Washington
- USDA Forest Service, Pacific Northwest Research Station

Pacific Northwest Tree Improvement Research Cooperative

Annual Report

2000-2001

Report authors

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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
Associate Director: Thimmappa Anekonda
Graduate Student: Gancho Slavov

Contents

HIGHLIGHTS OF 2000-2001

- Glenn Howe agreed to become Director of PNWTIRC and Assistant Professor of forest genetics in the Department of Forest Science.
- Gancho Slavov arrived in September of 2000 to begin a Ph.D. program with PNWTIRC. He will work on the *Pollen Contamination Study.*
- Nine journal articles and abstracts were completed by PNWTIRC staff (i.e., published or in press).
- 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 NWCTGA orchards. This study is designed to (1) develop improved methods for promoting early and sustained flowering on young Douglas-fir grafts, (2) determine the optimum age to begin flower stimulation treatments and (3) measure the impacts of early flower stimulation on ramet health. The best treatments will be used in the *Miniaturized Seed Orchard Study*.
- 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 onto the rootstock in February 2002.
- We developed 15 new promising 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*. Although very mild drought often enhances cold hardiness under routine nursery conditions, severe drought reduced the cold hardiness of 2-year-old seedlings in our study. There was a tendency for cold damage to be increased in families that set bud late in the fall, second flushed and grew taller. Family rankings for bud phenology, second flushing and cold hardiness were similar in the different moisture regimes. In addition, drought hardiness traits, such as drought-induced shoot damage, xylem cavitation and hydraulic conductivity were unrelated to bud set, second flushing and cold hardiness. Both of these observations suggest that selection and breeding for improved bud phenology and cold hardiness is unlikely to affect drought hardiness.

Message from the Director

After nearly two decades of outstanding leadership, Tom Adams resigned as Director of the PNWTIRC to become Head of the Forest Science Department at Oregon State University. Fortunately, OSU is the administrative home of PNWTIRC, so we will continue to benefit from Tom's expertise and leadership in both forest genetics and tree improvement.

Tom has contributed enormously to forest genetics in the region. During Tom's tenure, PNWTIRC supported 10 graduate students and published more than 45 scientific publications. This research included detailed genetic studies of adaptive traits like cold hardiness, drought hardiness and bud phenology, and the relationships between these traits and stem growth. Under Tom's leadership, PNWTIRC developed new measurement techniques and early testing strategies for increasing genetic gains in growth, cold hardiness, drought hardiness, bud phenology, stem quality and wood density. Recently, PNWTIRC has been leading the way toward developing and testing better ways to design and manage Douglas-fir seed orchards.

Tom has also been active in other areas of research. He used genetic markers (isozymes) to show that common methods of artificial and natural regeneration have little impact on the genetic diversity of managed stands of Douglas-fir. He also alerted tree breeders to the problems of genotype misidentification and pollen contamination in seed orchards. Tom has become one of the leading experts on mating systems in trees and was instrumental in organizing the Regional Forest Gene Conservation Committee (RFGCC). We are indebted to Tom for his overall leadership in setting the course for tree improvement in the Pacific Northwest and for mentoring many of the forest geneticists in the region. As Department Head, Tom is now helping to guide all aspects of forestry research in the region. More than anyone else, PNWTIRC members know that these efforts are in excellent hands.

Thimmappa Anekonda served as Interim Director of PNWTIRC until I became Director in August 2001. Thimmappa did an excellent job of juggling the research and administrative duties of the Cooperative for seven months and quickly got me 'up-to-speed' once I arrived. I also thank Tom Adams for his help and Gancho Slavov and Steve Strauss for helping with the *Pollen Contamination Study*. Finally, I thank the cooperators for spending valuable time with both me and Thimmappa during the late summer and fall. These meetings were extremely helpful for learning about forestry in the region and for brainstorming about how PNWTIRC can contribute to tree improvement programs in the future.

Some of you may be unaware that I am returning to PNWTIRC after a long absence. I joined PNWTIRC in 1983, shortly after it was formed. After spending three enjoyable years with PNWTIRC, I left to get my Ph.D. in forest genetics from Oregon State University in 1991. After that, I spent time at both the University of Minnesota

and Ohio State University. Although I made many friends and learned a lot during the past 10 years, it's great to be back!

Next year will be particularly important for PNWTIRC. We will continue to focus on seed orchard research, renew our efforts to translate PNWTIRC research into practice via technology transfer and search for new members. Our most important task will be to develop a new long-term research plan (2001-2006). We have already begun to scrutinize the role of tree improvement in PNW forestry, assess research needs and formulate a new research plan that will guide our activities until 2006. This plan will require thoughtful input from PNWTIRC members and other forestry professionals in the region, and should be both challenging and fun. I am looking forward to working with you on this important task.

Glenn Howe

Introduction

Research

In recent years, seed orchard design and management has become a major focus of PNWTIRC research and will continue to be important for the foreseeable future. For Douglas-fir, the first round of parent tree collections, progeny testing and selection is now complete for most breeding programs and many first generation seed orchards are now in full production. As tree breeders consider their options for second-generation orchards and alternative deployment strategies, seed orchard research has risen high on the list of priorities. Our seed orchard research now includes the *Early Flowering Study* (page 9), *Miniaturized Seed Orchard Study* (page 12) and the *Pollen Contamination Study* (page 16). Seed orchard research has the potential for increasing genetic gains, improving adaptability and reducing the costs of seed orchard establishment and management. Because of these benefits, interest in seed orchard research remains high among our cooperators.

Adaptability research has been a long-term strength of the PNWTIRC. We began detailed physiological genetic studies of cold hardiness in 1991 and drought hardiness in 1996. We recently completed the analysis of bud phenology, second flushing and cold hardiness measurements from the *Seedling Drought Physiology Study* (page 21). These results will be submitted for publication during the next year. As we develop our new long-term research plan, we need to consider whether adaptability research will continue to be a major component of our program.

New Research Plan (2001-2006)

One of the most far-reaching activities of the next year will be the development of a new long-term research plan. This plan will summarize PNWTIRC research priorities and will guide our research activities until 2006. The last PNWTIRC research plan was written in 1995. At that time, high-priority topics included adaptability, seed orchard concepts, genotype x silviculture interactions, influence of forest management on genetic diversity, genetics of alternative species and stem growth and form. During the past five years, we developed in-depth projects on miniaturized seed orchards, pollen contamination and drought physiology. Although we began a study plan to validate cold and drought hardiness screening methods in field tests, this project has been postponed.

During the late summer and fall of 2001, Glenn Howe and Thimmappa Anekonda met with most PNWTIRC members to learn about their research needs. High-priority topics included seed orchard design and management, pollen contamination, early flowering, methods to prevent flowering in seed orchards, adaptability, alternative species, gene conservation, vegetative propagation, stem and wood quality, realized genetic gains, growth models, breeding zone delineation, genotype x environmental interactions and more. Our challenge is to reduce this list of research needs into a few key projects that will best meet the needs of our members.

We will begin this process at our annual meeting in December 2001. The first step is to bring our members together to exchange ideas, debate research needs and eventually identify a reasonable number of research projects that deserve careful consideration. This will be the most important outcome of our 2000-2001 annual meeting. The final step in this process is to write detailed study plans for a few high-priority topics and to get these approved by our members. The procedures that we will use to arrive at our final list of research topics will also be discussed at our annual meeting. This process will set the course for PNWTIRC research for the next five years and should be challenging, educational and ultimately, rewarding.

Technology Transfer

There have been many changes in PNWTIRC over the past 18 years. Few of the people who were around in 1983 are still around today. Although new perspectives are valuable for any organization, we do not want historical links to be broken and institutional memories to become short. How many of us have an intimate knowledge of the research we did 10 years ago? Fifteen years ago? Because most of this research is still relevant today, we plan to renew our efforts to translate our past research results into practice. We propose to organize a few technology transfer workshops in which PNWTIRC results and conclusions will be presented in a form that can be easily adopted by tree breeders and seed orchard managers. Workshop topics might include:

- Strategies for improving wood and stem quality.
- Strategies for improving cold and drought hardiness.
- How to integrate early testing into tree improvement programs?
- Seed orchard options.

Our technology transfer activities will undoubtedly draw some resources away from our research. Nonetheless, if this concept is strongly supported by our members, we plan to organize the first of these workshops during the next year.

New Membership Drive

Changes in the forest industry, including recent mergers and new mergers on the horizon, will erode support for PNWTIRC unless we raise annual dues or attract new members. We are already loosing one dues-paying member next year because of the recent merger between two of our members. The best way to maintain stable funding is to attract new members. New members also bring with them new perspectives, expertise and enthusiasm—the kind of intangible benefits that are also critical to our success. During the next year, we will work hard to attract new members. We encourage all of you to help in this process. We will be contacting many of your colleagues during the next year—so please help us get our message out.

Current PNWTIRC Research

Seed Orchard Research

Douglas-fir seed orchards cover nearly 2,500 acres in the Pacific Northwest and supply most of the genetically superior seed used in the region. The cost of establishing and managing these orchards is substantial and will increase as new second-generation orchards are added. Thus, it is time to take a fresh look at seed orchard design and management, with an eye toward developing new approaches for increasing genetic gains, maintaining adaptability and reducing seed orchard costs.

Conventional Douglas-fir seed orchards have three problems that either reduce genetic gains or increase management costs. First, genetic gains are delayed (and financial returns sacrificed) because of the long time lag between seed orchard establishment and the production of genetically improved seed. Many of the first-generation orchards, for example, took 10 to 15 years to produce useful amounts of seed. Second, management is both difficult and costly because of the large size of the trees in conventional orchards. Third, pollen contamination from native trees and adjacent seed orchard blocks can reduce genetic gains. Pollen contamination in conventional orchards often exceeds 40% and can adversely impact both realized genetic gains and adaptability (Wheeler and Jech 1986; Adams and Burczyk 2000).

PNWTIRC has begun three closely related projects to address these problems. These are the *Early Flowering Study*, *Miniaturized Seed Orchard Study* and *Pollen Contamination Study*.

Early Flowering Study

Introduction

One way to increase financial returns from tree improvement is to speed the production of genetically improved seed from new orchards. Thus, improved methods for obtaining early and sustained flowering would be valuable. Flower stimulation can also cut management costs because more cones are produced on each tree, and on easily accessible branches close to the ground. Early flowering is also valuable for shortening the generation time in breeding programs.

Although techniques such as girdling, application of gibberellic acid (GA) and fertilization can be used to stimulate flowering of Douglas-fir trees, a number of important questions remain. Which techniques are best for maximizing very early seed production? How soon after grafting can these techniques be applied safely? How can the damage caused by GAs be minimized? How do flower stimulation techniques interact with the design and management of miniaturized seed orchards, including close spacing, heavy pruning and the application of growth regulators to control tree height? More fundamentally, what physiological mechanisms lead to flower bud differentiation in response to flower stimulation treatments?

We initiated the *Early Flowering Study* to obtain preliminary data on treatments that we intend to use in our *Miniaturized Seed Orchard Study* (see below). Nonetheless, our early flowering experiments will provide information that can guide the management of both conventional and miniaturized seed orchards. Therefore, we will discuss the *Early Flowering Study* as a separate avenue of seed orchard research.

The objectives of the *Early Flowering Study* are to:

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

…our early flowering experiments will provide information that can guide the management of both conventional and miniaturized seed orchards.

Accomplishments for 2000-2001

The *Early Flowering Study* was conceived and initiated during the past year. It is designed to test the effects of girdling and GA application on both 2- and 4-year-old grafts. We are using two young seed orchards for these experiments, both of which are managed by Roseburg Resources. The Vaughn seed orchard block contains trees that were grafted in early 1999. The second orchard, which is owned by the Northwest Christmas Tree Growers Association (NWCTGA), contains trees that were grafted in early 1997.

The same four treatments were applied to trees in both orchards. These treatments include girdling (G), $GA_{4/7}$ (GA), girdling plus $GA_{4/7}$ (G+GA) and an untreated control (C) (Figure 1). Thimmappa Anekonda, Mike Albrecht and Trilok Rathore (a visiting scientist from India) girdled the trees on April 18, 2001, then applied GA on May 16, near the time of bud burst. In the Vaughn seed orchard, the treatments were ap-

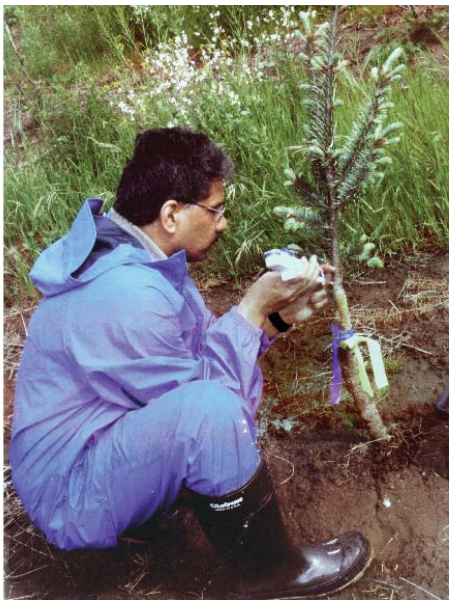

Figure 1. Girdling and/or $GA_{4/7}$ treatments were applied to either 2- or 4-year-old grafts in the Early Flowering Study. A. Girdling of a 2-year-old graft. B. $GA_{4/7}$ application to a 2-year-old graft.

plied to 2-year-old grafts (i.e., grafts that had already completed two growing seasons in the field). Nine clones were selected and each treatment was randomly applied to four ramets per clone. The same treatments were tested on 4-year-old grafts in the NWCTGA orchard, using nine different clones and four ramets per treatment.

Plans for 2001-2002

Tree health and shoot elongation will be measured in the winter of 2001, flowering will be measured in the spring of 2002 and cone and seed production will be measured in the fall of 2002. Flower stimulation treatments will be reapplied in the spring of 2002 to see how treatments in subsequent years affect flowering and ramet health. In the fall of 2002, we will begin to identify the best techniques for stimulating flowering in young Douglas-fir grafts. In the spring of 2002, the growth regulator treatments designed to control crown architecture will be tested on trees in the NWCTGA orchard. The information from these studies will be incorporated into the design of our *Miniaturized Seed Orchard Study* and may form the basis of new PNWTIRC studies on flowering in Douglas-fir.

Plans for future years

Early and sustained flowering is extremely valuable for both seed orchard management and advanced generation breeding. Therefore, our new 5-year research plan will include options for additional flowering studies. Areas of research might include studies on top grafting, maturation, methods to prevent flowering, the physiological basis of flower stimulation and research on alternative species.

Miniaturized Seed Orchard Study

Introduction

The trees in conventional Douglas-fir seed orchards are planted at wide spacings, ramets of the same clone are separated from one another to maximize outcrossing, and the trees are allowed to become quite large (15+ m). This design makes seed orchard management both difficult and costly. Because of the large trees, it is harder to apply pesticides, delay flowering using overhead irrigation (bloom delay), perform control pollination and supplemental mass pollination (SMP),[1] and harvest cones. Miniaturized seed orchards (MSOs) are an attractive alternative to conventional orchards that should solve some of these problems.

In MSOs, the trees are planted at close spacings in clonal rows, then maintained at a height of only 2 to 4 m (Sweet and Krugman 1977). Using this approach, seeds are produced close to the ground on many small trees, rather than on a few larger ones. MSOs are becoming increasingly popular for the production of horticultural tree crops and forest tree seed (Jackson 1989). For example, miniaturized seed orchards are now standard for producing radiata pine seed in New Zealand (Sweet 1995).

The potential advantages of MSOs include greater per-hectare seed yields and reduced land costs because of the greater planting density, reduced management costs because of the small size of the trees and more effective pest management. Genetic gains should also be greater because of better pollination control (i.e., SMP, control mass pollination, or bloom delay) and reduced pollen contamination. For example, SMP and control mass pollination are facilitated in MSOs because genetically identical scions are grafted adjacent to one another in clonal rows. Despite these potential advantages, MSOs are unproven. The costs of MSOs could be greater than the costs of conventional orchards because of the extra work needed to keep the trees small. In addition, it is unclear how seed production will be affected by the change in seed orchard design and management.

The goal of the *Miniaturized Seed Orchard Study* is to compare three alternative spacings and management regimes on a scale large enough to evaluate realistic management costs, seed yields and seed quality (Anekonda and Adams 1999; PNWTIRC Annual Report 1998–1999). The ramet spacings and target tree heights in our macro-, mini- and micro-orchards[2] are shown in Table 1.

Table 1. Characteristics of three orchard types tested in the *Miniaturized Seed Orchard Study*.

Orchard type	Spacing (m)	Trees/hectare	Total # 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

[1]Supplemental mass pollination is the broadcast application of pollen to non-isolated (i.e., non-bagged) female strobili.

[2]The names of the mini- and micro-orchard types were recently switched. 'Micro-orchard' now refers to the orchard with the smallest trees (2-m target height) and closest spacing (3 x 1 m) (Table 1).

The objectives of the *Miniaturized Seed Orchard Study* are to:

- Compare three orchard types for their (a) quantity of flowering and seed production, (b) ease and efficiency of management and (c) ramet health and seed quality.
- Define the optimum age to begin flower stimulation in MSOs.
- Determine whether small crowns can be maintained by controlling apical dominance with growth regulators.
- Compare methods of SMP and control mass pollination in MSOs.
- Determine whether clones respond differently to MSO designs and management regimes.

MSOs are becoming increasingly popular for the production of horticultural tree crops and forest tree seed.

Field design and management

Three orchard types are being compared at a site owned by Plum Creek Timber Company (Figure 2). Eight identical blocks (main plots) were established within each of the orchard types. Each of these blocks will contain the same 16 clones, consisting of eight forward selections (younger ortets) and eight backward selections (older ortets).[1] For the macro- and mini-orchards, the clones will be grafted into five ramet row-plots. For the micro-orchard, the row-plots will contain six ramets each, so that every other ramet can be removed, if necessary. The orchard rows were placed in a north-south orientation to increase the exposure of the crowns to sunlight. We also established two small 'supplemental blocks' for the mini- and micro-orchard types. These supplemental blocks will be used to test some treatments (e.g., growth regulator treatments) before they are tested in the main experiment. Each supplemental block will contain four clones replicated as two-ramet row-plots (27 row-plots per clone). We also established a holding block that will contain about 20 grafted trees per clone. Transplants from the holding block will be used to replace grafts that die in the main orchard blocks.

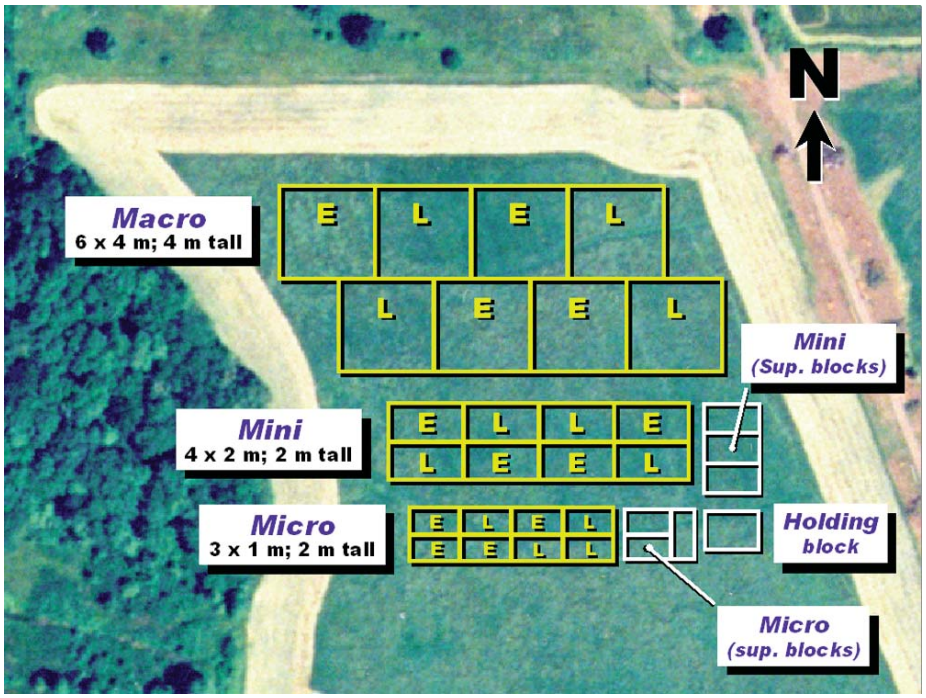

Figure 2. Aerial photo showing the field layout of the Miniaturized Seed Orchard Study.

[1]Forward selections are selections from progeny tests, whereas backward selections are parents that were selected based on progeny test results.

The MSOs are being irrigated to enhance survival and growth, increase seed yields and provide more effective control of frosts and flower phenology. Within each orchard, the target tree height will be maintained using either mechanical or chemical pruning, and flower stimulation will be used to obtain early and sustained seed production. We thank the Seed Orchard Advisory Committee (SOAC)[1] for their help in developing and implementing this research plan.

Accomplishments in 2000-2001

During the past year, the site for the *Miniaturized Seed Orchard Study* was prepared, the orchards were laid out, the rootstock was planted, and an irrigation system was installed. Jim Smith of Plum Creek Timber Company played a key role in accomplishing these important steps. We also thank Don Copes (USDA Forest Service Pacific Northwest Research Station) for providing the graft-compatible seed, and Mike Albrecht and Ken Kearny (Roseburg Resources) for producing the seedlings used as rootstock. Thimmappa Anekonda, Glenn Howe, Jim Smith and Jerry Barnes surveyed the rootstock in October 2001 and determined that nearly all of the trees are ready to graft this season.

Plans for 2001-2002

Additional work during the winter of 2001-2002 will prepare us for grafting the scions in the winter of 2002. First, a few of the smallest trees will be replaced by transplanting rootstock from the holding block. This holding block contains trees from the same graft-compatible families already in the experiment. Second, Plum Creek will select 16 clones to be grafted onto the rootstock. We will choose clones that survive well in seed orchards and avoid clones that flower either exceptionally early or exceptionally late in the spring. Finally, scions will be collected and grafted by Jerry Barnes, probably in February of 2002. Although we originally planned to do the grafting in late winter of 2002 and 2003, the trees are doing well enough that we can do all of the grafting in 2002.

Plans for future years

In future years, the MSOs will be used to test alternative management regimes. We will periodically compare the advantages and disadvantages of each orchard type over the next 15 years (Objective 1). Flowering, ramet health, seed yield and seed quality will be monitored yearly, and the costs of labor and supplies are being recorded so that we can compare the economics of the orchard types.

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[1]Mike Albrecht, Roseburg Resources; Jerry Barnes, Tree Improvement Enterprises; Don Copes, USFS PNWRS; Jeff DeBell, WDNR; Randall Greggs, Simpson Timber Co.; Glenn Miller, USDI BLM; Jim Reno, Weyerhaeuser Co.; Jim Smith and John Trobaugh, Plum Creek Timber Co.; Joe Webber, B.C. Ministry of Forests.

We plan to begin flower stimulation in the mini-orchard in the spring of 2004 (Objective 2). Each flower stimulation treatment (i.e., early or late) will be applied to four entire blocks within each orchard type (Table 2, Figure 2). Within each block, the flower stimulation treatments will be applied every other year, and the SMP and control pollination treatments will be tested in the intervening years.

Table 2. Timing of early and late flower stimulation treatments (i.e., $GA_{4/7}$ and/or girdling) in the *Miniaturized Seed Orchard Study*.

	Number of years after grafting when flower stimulation will occur[1]	
Orchard type	Early treatment	Late treatment
Macro	4	6
Mini	4	6
Micro	2	4

[1]In the Micro-orchard, for example, the early treatment will occur just before the ramet's third growing season in the field.

We will begin testing the other growth regulator treatments in the NWCTGA seed orchard in the spring of 2002 (Objective 3). Treatments such as the application of auxins or cytokinins may be useful for maintaining small trees without mechanical pruning, but it is unclear how they will affect flowering and seed yields. The growth regulator treatments in the MSO supplemental blocks will be scheduled after we see how they work in the NWCTGA orchard.

We will eventually compare methods of SMP and control mass pollination in the MSOs (Objective 4). Because these experiments must wait until we have sufficient flowering and seed production in the orchards, we do not know when these treatments will begin.

Finally, we plan to determine whether the clones respond differently to the MSO treatments (Objective 5). This information will be useful for judging the overall utility of the MSO treatments and for fine-tuning MSO management.

The *Miniaturized Seed Orchard Study* is a long-term research project that could significantly impact Douglas-fir seed orchard management in the Pacific Northwest. Thanks to our PNWTIRC members and to the SOAC, in particular, we are well underway toward learning whether miniaturized seed orchards will play a significant role in Douglas-fir tree improvement.

Pollen Contamination Study

Introduction

Pollen contamination[1] can be a serious problem if seed orchard blocks are located near native stands of trees, or near blocks that contain trees from other breeding zones. For example, the proportion of seeds fertilized by non-orchard pollen often exceeds 40% in conventional Douglas-fir orchards (Adams and Burczyk 2000). This 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 called isozymes (Adams 1992). Nonetheless, these markers have some drawbacks. Because they are only moderately variable, it is necessary to measure many isozyme markers (loci) and many offspring to get reasonable estimates of pollen contamination. One goal of the *Pollen Contamination Study* is to develop improved DNA-based genetic markers for estimating pollen contamination and other mating parameters in Douglas-fir seed orchards.

Recent advances in DNA markers suggest that genetic markers called simple sequence repeats (SSRs), or microsatellites, will be much better than traditional isozymes for measuring pollen contamination. SSRs have two desirable characteristics. First, they are highly variable, often having more than 10 alleles per locus (Goldstein and Pollock 1997). Thus, they should be better than isozymes for identifying the parents of seed orchard seed. Second, they are usually codominant, which makes them more useful than dominant markers, such as RAPDs (Randomly Amplified Polymorphic DNA).

SSRs will also facilitate the development of new methods to reduce pollen contamination. SSRs should be useful for comparing alternative methods of supplemental mass pollination. Although SMP can be used to minimize pollen contamination, the proportion of seeds fertilized by SMP is usually less than 30% in conventional orchards. SSRs could also be used to judge the effectiveness of 'bloom delay.' Orchard trees are sometimes cooled by overhead irrigation to delay flowering. By the time the orchard trees flower, pollen from nearby stands or other orchard blocks is no longer available to contaminate the seed crop. In addition, improved markers like SSRs would make it easier to measure pollen contamination for individual ramets or clones. Pollen contamination may be higher for clones that flower either very early, or very late, in the spring (El-Kassaby and Ritland 1986). It would also be good to know whether seed from ramets on the edge of the orchard have more pollen contamination than seed from ramets in the center. If substantial differences in pollen contamination exist, it might be beneficial to subdivide the orchard into pollen contamination classes based on the timing of flowering, location of the ramets within the orchard, or direct estimates of pollen contamination based on SSR data. Different pollen contamination classes could be managed differently to capture the greatest possible genetic gain. Although this study focuses on pollen contamination, ge-

[1]Pollen contamination is measured as the proportion of seeds fertilized by pollen coming from outside of the seed orchard block.

netic markers are also useful for understanding the genetics of natural populations, measuring inbreeding, developing gene conservation plans and mapping tree genomes.

We will develop SSR markers and use them to measure pollen contamination in a conventional Douglas-fir seed orchard. This project has two phases. The objectives of the first phase are to:

- Develop 7-10 SSR marker loci for Douglas-fir.
- Confirm the inheritance of the markers.
- Measure their genetic variability.
- Use the most variable markers to measure pollen contamination in a conventional seed orchard.
- Optimize testing and estimation procedures.

The objectives of the second phase are to use the SSR markers to determine how pollen contamination varies with (1) flowering phenology and (2) location of the ramets within the seed orchard. We will measure pollen contamination in one orchard block of the Plum Creek seed orchard in central Oregon, hereafter referred to as the *Test Block*.

A good SSR marker is genetically variable (e.g., has 8-15 alleles in the test population), has a low frequency of null alleles,[1] and amplifies a single locus in each PCR reaction.[2] A number of key steps are used to develop new SSR markers (Figure 3). First, genomic clones, which contain short pieces of Douglas-fir DNA, are sequenced to find regions that contain SSRs. Second, PCR primers that match the DNA sequences on either side of the SSR are designed. Third, the SSR markers are amplified using these PCR primers, then visualized on gels using electrophoresis.

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., $(AC)_{12}$ = ACACACACACACACACACACAC), 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.

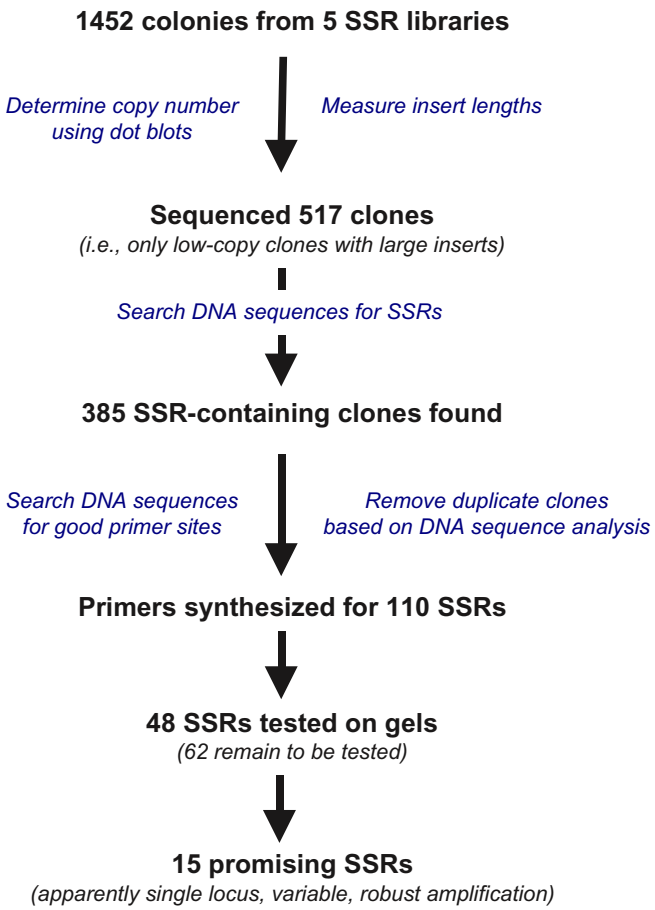


Figure 3. To date, fifteen promising SSR markers have been developed from an initial screen of over 1452 colonies from five SSR libraries. The next step is to confirm the inheritance of these SSRs.

[1]Null alleles are scored when the PCR reaction seems to have worked but no SSR bands appear on the gel.

[2]Simultaneous measurement of multiple loci, or 'multiplexing,' can be achieved by combining multiple single-locus primers into the same PCR reaction or by running multiple PCR reactions in a single lane.

Finally, the SSR markers are tested on a sufficient number of seed to see if they give consistent results, vary among individuals, and have a simple pattern of inheritance.

Last year we reported on six promising markers: five nuclear SSR markers and one chloroplast minisatellite marker. Based on follow-up analyses, three of the SSR markers (BCPSMAC5, BCPSMAC8A and BCPSMAC8B) were eliminated because they did not segregate, or because multiple loci were detected on the same gel (i.e., alleles from different loci were too close in size to be distinguished from one another). The two remaining SSR markers (BCPSMAG38, BCPSMAG39) were also problematic—they gave weak signals and inconsistent results. Ultimately, we may be able to use these two problematic markers, but we are now focusing our efforts on developing other higher-quality SSR markers.

The good news is that the chloroplast minisatellite marker still performs well and could be used to analyze pollen contamination (PNWTIRC Annual Report 1999-2000). Chloroplast markers are different from nuclear markers because the chloroplast is haploid (i.e., each individual has only one allele) and the marker is inherited through the pollen. Therefore, the genotype of the maternal parent does not confound the analysis of pollen movement. Despite these advantages, the large sizes of the chloroplast minisatellite bands are not as good for distinguishing among bands (alleles) that are about the same size. Therefore, a sufficient number of high-quality SSR markers are also needed.

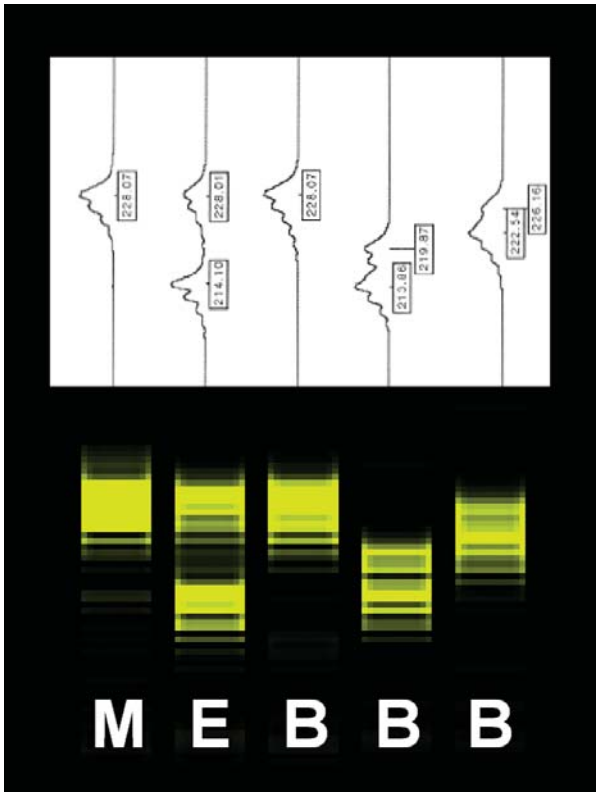


Figure 4. Analysis of one of the promising SSR markers using DNA isolated from megagametophyte (M), embryo (E), and bud (B) tissues. Douglas-fir DNA was PCR-amplified using SSR primers and fluorescently-labeled nucleotides (dNTPs), then visualized using polyacrylamide gel electrophoresis. The bottom of the figure shows the SSR bands as they appear on the polyacrylamide gel, whereas the top of the figure shows the resulting chromatogram. The next steps are to improve the resolution of the bands using fluorescently-labeled primers and verify the inheritance of the putative alleles.

The other encouraging news is that our overall conclusions about SSR markers still seem to hold. Based on our recent work, as well as work in other conifers (Echt et al 1996, Elsik et al 2000, Pfeiffer et al 1997), SSR markers are highly variable and should be better than isozymes for measuring pollen contamination. Nonetheless, these expectations still need to be confirmed for Douglas-fir.

We focused on Phase 1 of the *Pollen Contamination Study* during 2000-2001. During the past year, we isolated new SSR markers that seem to perform well (Figure 4). The key to our recent success has been the use of new set of high-quality SSR libraries (discussed below). Analyses of SSR inheritance and pollen contamination will begin once we complete the development of these new SSR markers.

Accomplishments for 2000-2001

A major addition to the *Pollen Contamination Study* was the arrival of Gancho Slavov in September 2000. Gancho is a Ph.D. student from Bulgaria who will oversee the remainder of this study and publish the results in his Ph.D. dissertation.

Our objectives for this year included (1) further testing of selected SSR markers and (2) development of new SSR markers. Gancho made good progress toward achieving both of these objectives. Although we planned to measure pollen con-

tamination last year, this was not done because we were too optimistic about the time needed to develop high-quality SSR markers. Because of the large and repetitive genomes of conifers, it is difficult to develop SSRs for species like Douglas-fir (Smith and Devey 1994). To overcome these hurdles, we developed new collaborations with experts that have completed large SSR projects on both trees and other crop plants. These new collaborations have been extremely valuable. Gancho's recent work, for example, emphasizes the importance of starting with a large genomic library that is highly enriched for SSRs, selecting against clones that contain highly repetitive sequences, and focusing on SSR-containing clones that have long inserts (discussed below).

…we developed new collaborations with experts that have completed large SSR projects on both trees and other crop plants. These new collaborations have been extremely valuable.

New SSR libraries produce promising SSR markers

The first set of SSR markers had problems because of the particular SSR library we used. Because many of the clones represented highly repetitive sequences, many of the resulting SSR markers detected too many loci. Second, many of the clones had small inserts, with only short stretches of DNA on either side of the SSR. This provided little opportunity for designing high-quality SSR primers.

Improved techniques for constructing SSR libraries are now available—techniques that were not available when the first SSR library was constructed. In the past year, we obtained five new SSR libraries that were constructed using these improved techniques (Table 3). We obtained one library from Dr. Keith Edwards at the University of Bristol, UK. The other four libraries were constructed by a commercial laboratory (Genetic Identification Services, Chatsworth, CA). Compared to the first SSR library, the new commercial libraries have larger inserts and are more likely to yield good SSR markers. Gancho carefully analyzed these five libraries before proceeding with SSR development. First, he used dot blots to eliminate clones with highly repetitive DNA—clones that are more likely to detect multiple loci. Second, he eliminated clones with inserts shorter than 400 bp. Finally, he sequenced the clones with an automated ABI 3700 sequencer. Compared to the slab-gel sequencers used in the past, the capillary system of the ABI 3700 is better for obtaining good sequence data from clones that contain long SSRs, and good sequence data should result in better SSR primers. DNA sequence analysis was done in collaboration with Dr. Gerald Tuskan at the Oak Ridge National Laboratory in Oak Ridge, Tennessee.

Table 3. Comparison of SSR development from five SSR-enriched genomic libraries.

	Number of:					
Library enriched for: [1]	Colonies processed *(1)*	Colonies sequenced *(2)*	Colonies with SSRs *(3)*	Unique clones with primers synthesized *(4)*	Efficiency (%) *[(3)/(2)]*100*	Average number of DNA repeats per SSR *(5)*
$(AC)_n$	864	322	292	81	91	36
$(AG)_n$	182	62	58	17	94	34
$(AAT)_n$	96	35	9	0	26	-
$(ATC)_n$	96	50	14	4	28	14
$(AC)_n + (AG)_n$	214	48	12	8	25	15
Total (mean)	**1452**	**517**	**385**	**110**	**(53)**	**(25)**

[1]The first four libraries were constructed by Genetic Identification Services (Chatsworth, CA) using a common source of genomic DNA. The fifth library was provided by Keith Edwards (University of Bristol, UK).

During the past year, Gancho analyzed 1452 colonies from five SSR libraries (Figure 3, Table 3). To date, Gancho obtained 15 promising SSR markers (see bottom of Figure 3). Because we have 62 SSRs remaining to be tested on gels, we expect to have additional promising markers in the future. These new markers seem to be much better than the SSR markers we discussed last year, and our current number of promising markers is above the 7-10 markers that we originally hoped to develop.

The proportion of SSR-containing clones (i.e., library *Efficiency*) varied considerably among the libraries. For example, the *Efficiency* of the commercial libraries ranged from 91-94% for the dinucleotide libraries [$(AC)_n$ and $(AG)_n$] to 26-28% for the trinucleotide libraries [$(AAT)_n$ and $(ATC)_n$] (Table 3). The dinucleotide libraries also contained longer SSR regions (see *DNA repeats per SSR* in Table 3). On the other hand, the resolution of trinucleotide markers is usually better than that of dinucleotide markers.

Plans for 2001-2002

Our plans for this year are to verify the inheritance of 7-10 of the best SSR markers, optimize PCR and multiplexing procedures, then begin genotyping the seed orchard seed. We will study inheritance by analyzing SSR segregation in haploid megagametophytes from open-pollinated seeds. We already collected the materials needed to estimate pollen contamination in the *Test Block* (PNWTIRC Annual Report 1999-2000). These materials include dormant buds from 58 clones in the *Test Block*, 60 clones in adjacent seed orchard blocks and 44 trees in nearby wild stands. DNA has already been isolated from all of the bud samples. We also collected seed from individual ramets in the *Test Block*, control-pollinated seed from the *Test Block*, and bulked seed from the 1999 and 2000 cone crops. Data has already been collected on daily pollen cone abundance, timing of pollen shed, pollen density, and female cone receptivity for the 2000 flowering season (PNWTIRC Annual Report, 1999-2000).

Plans for future years

After Gancho finishes measuring pollen contamination in the *Test Block*, he will use the marker data to compare different analytical techniques for estimating pollen contamination, then determine how pollen contamination varies with flowering phenology and the location of the ramets within the seed orchard.

We hope our new SSR markers and libraries will become valuable tools for forest geneticists, tree breeders, and seed orchard managers. At the end of this project, we expect to have at least 7-10 high-quality SSR markers. These markers will be useful for measuring and managing pollen contamination as well as for other population genetics studies of Douglas-fir. Our SSR libraries will continue to be a source of new SSRs for many years to come. Additional markers may be valuable for clonal identification, genome mapping and other studies and applications that are still on the horizon.

Seedling Drought Physiology Study

Introduction

Summer droughts are a problem for Douglas-fir trees in southwestern Oregon and the rain shadow regions of Washington and British Columbia. Drought leads to poor survival of planted seedlings and poor basal area growth of mature trees (Hobbs et al 1980; Spittlehouse 1985). In addition, transplant shock in planted seedlings is mainly caused by drought stress (Haase and Rose 1993). Because many Douglas-fir breeding zones are being consolidated into a smaller number of larger zones, improved genotypes must be adapted to a broader range of moisture regimes. Therefore, it would be valuable to have genotypes that can grow well under droughty conditions. Breeding for broadly adapted genotypes may also mitigate the effects of climate change. A better understanding of the genetics of drought hardiness will make it easier to breed for better adapted planting stock and improve forest regeneration sucess in the Pacific Northwest.

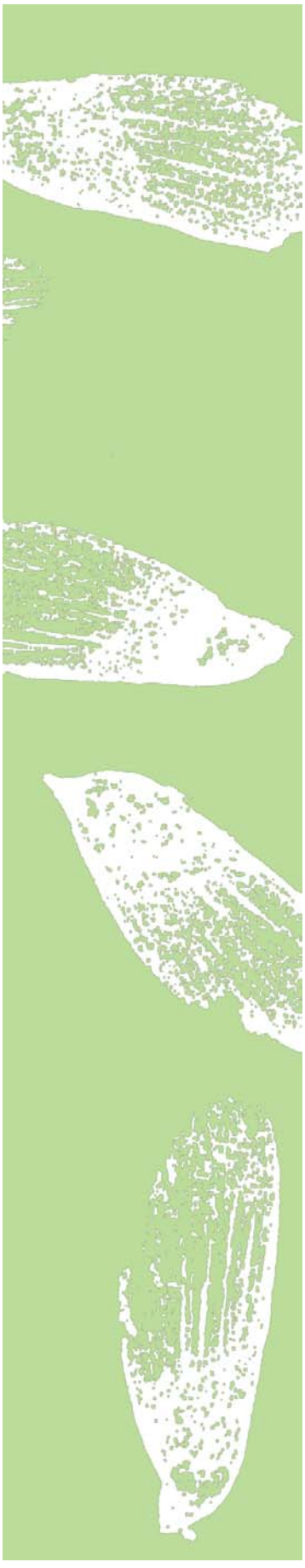
Shoot damage, xylem cavitation and hydraulic conductivity can be used to evaluate the drought hardiness of Douglas-fir families (PNWTIRC Annual Report 1998-1999, 1999-2000). Xylem cavitation, which is the collapse of xylem cells, impairs the movement of water up the stem. We measure xylem cavitation by passing saffranin dye through the stem, then visually estimating the proportion of the stem cross-sectional area that is not stained, presumably because of xylem cavitation. Hydraulic conductivity is the quantity of water transported through a given length of stem under constant pressure. Hydraulic conductivity is a function of stem diameter and the proportion of functioning xylem cells (i.e., non-cavitated xylem conduits).

The *Seedling Drought Physiology Study* was begun in 1996 to develop efficient screening methods for drought hardiness in families of coastal Douglas-fir. We previously showed that drought hardiness traits vary considerably among full-sib families of Douglas-fir (PNWTIRC Annual Report 1998-1999, 1999-2000; Anekonda et al submitted). Significant genetic variation was found for xylem cavitation, hydraulic conductivity and shoot damage. The narrow-sense heritabilities for these traits were low—about same size as typical heritabilities for seedling height and diameter. Therefore, drought hardiness traits should be amenable to genetic improvement. Height and diameter growth under well-watered conditions is genetically uncorrelated with drought hardiness. This indicates that it should be possible to improve both drought hardiness and seedling growth at the same time, and that selection for stem growth is unlikely to affect drought hardiness.

In addition to the drought hardiness and growth traits discussed above, we also measured bud phenology, second flushing and fall cold hardiness in the same experiment. In this report, we examine how these traits are affected by drought and how they are related to the drought hardiness traits discussed above.

The three main objectives of this study are to:

- Determine the impact of drought stress on bud phenology, second flushing and fall cold hardiness.



- Determine how drought affects genetic variation in bud phenology, second flushing and fall cold hardiness, and whether relative family performance for these traits changes across moisture regimes.
- Assess genetic interrelationships among bud phenology, second flushing, fall cold hardiness and drought hardiness traits.

This report focuses on measurements of spring bud burst, bud set, second flushing and fall cold hardiness.

Materials and Methods

Responses to drought were measured in 39 full-sib families of coastal Douglas-fir. The parents of these families represent areas with diverse soil moisture regimes, latitudes (48°05'-51°03'N), longitudes (121°36'-126°33'W) and elevations (30-720 m) in southwestern British Columbia and northwestern Washington (M. Stoehr, pers. comm.).

During the first growing season, the seedlings were grown under well-watered conditions in two custom-built nursery beds in Corvallis, OR. The experimental design was a split-plot design with five blocks. The main plots consisted of three watering regimes applied during the second and third growing seasons (1997 and 1998). Each family sub-plot consisted of two randomly located, four-tree row-plots within each main plot, with a seedling spacing of 8 × 8 cm. In the second growing season, the treatments consisted of a well-watered control (ψ_{pd} > -1 MPa), mild drought (ψ_{pd} = -1 to -2 MPa) and moderate drought (ψ_{pd} = -2 to -3 MPa). These treatments were applied from June 13 to early September 1997 by controlling the amount of water applied to the nursery beds. Because every other seedling was harvested at the end of the second growing season, the treatments in the third year were only applied to one-half of the original number of seedlings.

Because the mild and moderate drought treatments in year two had little impact on seedling survival (PNWTIRC Annual Report 1998-1999), a severe drought treatment was applied during the third growing season to those seedlings that had received the mild drought treatment in year two. Therefore, during the third growing season, the watering regimes consisted of a well-watered control (i.e., well-watered in both years), a 'recovery' treatment (moderate drought in year two followed by well-watered conditions in year three) and a severe drought treatment (mild drought in year two followed by severe drought in year three). The severe drought treatment was begun on April 30, 1998 and had a ψ_{pd} of -3 to -4 MPa by mid summer.

This report focuses on measurements of spring bud burst, bud set, second flushing and fall cold hardiness. During the second growing season, we measured the number of days to bud burst from December 31 (BBDAY), the percentage of trees that had a terminal bud on August 1 (BS%), the total number of shoots that second-flushed on each tree (SF#, counting both the leader and branches), the proportion of trees that second flushed, treating the leaders (SFL%) and branches (SFB%) separately, and seedling height (HT). We measured fall cold hardiness at the end of the second growing season by visually estimating the proportion of damaged tissues in needles (NCD%), buds (BCD%) and stems (SCD%) (Aitken and Adams 1996; Anekonda et al submitted).

During the third growing season, we measured BBDAY, the number of days to bud set from December 31 (BSDAY), SFL% and HT.

We also measured drought hardiness traits during the second and third growing seasons (PNWTIRC Annual Report 1998-1999, 1999-2000). At the end of each growing season, we visually scored the percentage of the shoot (i.e., needles plus stem) that was damaged by drought. Damage was scored in 10% damage classes based on the amount yellowing and browning of the needles and stem. At the end of each growing season, we scored xylem cavitation in the current year's annual growth ring. Cavitation was scored in 10% classes for about nine trees per family in all treatments in year two. In year three, we measured 60 trees in the control and recovery treatments and 390 trees in the severe drought treatment. We also measured stem hydraulic conductivity on a small sub-sample of the seedlings in year two, and on the same trees for which cavitation was measured in year three.

Results and Discussion

Impact of drought stress on bud phenology, second flushing and fall cold hardiness.

Our mild and moderate drought treatments inhibited bud set and increased cold damage in the second growing season but had no effect on second flushing (Figure 5). Eighty-one percent of the trees in the control treatment had a terminal bud on August 1, compared to only 36 to 39% of the trees in the mild and moderate drought treatments (Figure 5, A). These results indicate that the drought treatments inhibited bud set in the second growing season (i.e., the trees in the drought treatments were less likely to have a terminal bud on August 1). None of the second flushing traits differed among the treatments (e.g., Figure 5, B). The trees in the mild and moderate drought treatments had significantly more cold damage to the needles, buds and stems than did the trees in the well-watered control. Treatment means for stem cold damage (SCD), for example, are shown in Figure 5, C. Differences in bud burst among the drought treatments were small (1 d) and statistically non-significant (data not shown). This was true for bud burst measured in the spring of the second growing season, before the drought treatments were begun, and in the spring of the third growing season after one season of drought stress.

In the third growing season, the trees in the well-watered control treatment set bud later and were more prone to second-flush than were the trees in either the recovery or severe drought treatments (Figure 6). The proportion of trees that second flushed under severe drought was only 13%. Although the moisture regimes in the recovery and control treatments were identical in the third growing season, BSDAY and SFL% were significantly different between these treatments. These results sug-

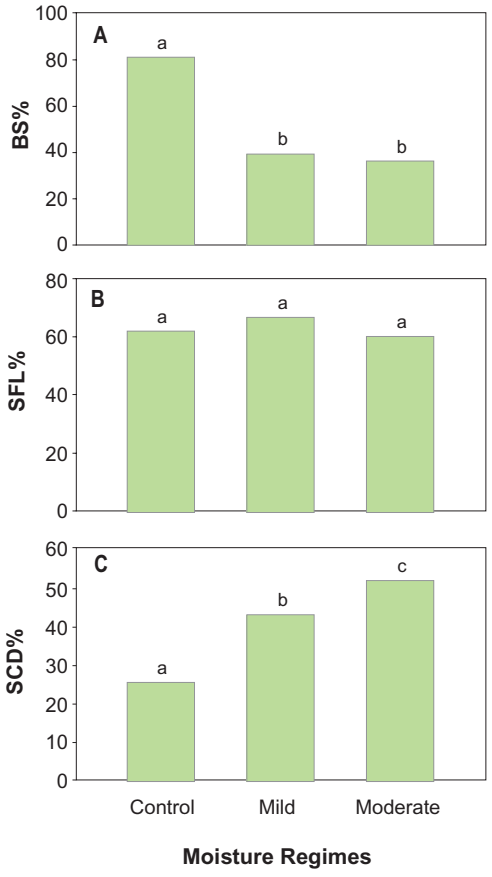


Figure 5. *Average bud set (BS%), leader second flushing (SFL%) and stem cold damage (SCD%) of Douglas-fir seedlings in the second growing season under three drought treatments (control, mild, and moderate). When letters over bars differ, mean values differed significantly between treatments ($p < 0.05$).*

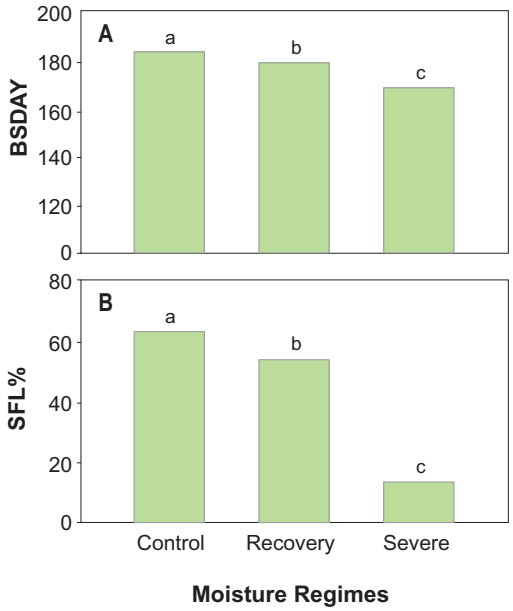


Figure 6. Average bud set (BSDAY) and leader second flushing (SFL%) of Douglas-fir seedlings in the third growing season under three drought treatments (control, recovery, and severe). When letters over bars differ, mean values differed significantly between treatments ($p < 0.05$).

gest that the moderate drought treatment in year two had a carry-over effect that affected these traits the following year (i.e., in the recovery treatment). Cold hardiness was not measured at the end of the third growing season.

The effect of drought stress on bud set and fall cold hardiness depends on the timing of the drought, drought intensity, daylength and the amount of second flushing (Timmis and Tanaka 1976; Blake et al 1979). Bud set occurs early under very mild drought and late under moderate to severe drought conditions (Timmis and Tanaka 1976; Blake et al 1979). Under short days, very mild drought stress (i.e. -0.50 to -1 MPa) induced cold hardiness in coastal Douglas-fir (Lavender et al 1968). In contrast, mild to severe drought stress (i.e., -1 to -1.5 MPa) inhibited cold hardiness in other studies (Timmis and Tanaka 1976; Blake and Ferrell 1977; Blake et al 1979). Second flushing reduces cold hardiness (Anekonda et al 1998), presumably because actively growing tissues are more sensitive to cold injury (Sakai and Larcher 1987).

In a previous PNWTIRC study, drought stress increased fall cold hardiness of 80 open-pollinated families of Douglas-fir (O'Neill et al 2001). Why do our current results differ? First, even our mild drought treatment was more severe than the very mild treatment used by O'Neill et al (2001), resulting in an inhibition of bud set and cold hardiness as discussed above. Second, the populations used in these two experiments may respond differently to drought. We used parents from British Columbia and Washington, whereas O'Neill et al (2001) used parents from Oregon. Nonetheless, O'Neill et al observed a drought-induced increase in cold hardiness in two distinctly different populations of Douglas-fir (Coast Range and Cascades). Third, the timing of the cold hardiness tests may have contributed to these differences. In our study, cold hardiness was tested about 45 days after all of the trees were re-watered, but O'Neill et al tested cold hardiness about one month after the trees were re-watered. Re-watering of the trees in our mild and moderate drought treatments probably enhanced second flushing (Figure 5, B). Although we avoided testing the second-flushed stems, trees that second-flush are generally more sensitive to cold injury (Anekonda et al 1998). Finally, because the drought treatments in these two studies were applied in different years, the seedlings may have been exposed to different temperature regimes prior to the cold hardiness tests.

Although our experiment was designed to measure drought hardiness under more severe conditions than usual, our results shed light on the practice of using mild drought to promote growth cessation and increase cold hardiness in the fall. If drought is used as a management tool, nursery managers should consider that (1) growth cessation and cold hardiness may not be enhanced if the drought stress is too severe, (2) different populations may respond differently to drought and (3) the impact of the drought treatments may be modified by other environmental conditions.

Selection for cold hardiness will probably be slightly more effective under well-watered conditions than under drought stress.

For each trait, separate analyses were conducted by year and by drought treatment and family means and individual-tree heritabilities were calculated (Table 4). Family means were significantly different ($p<0.05$) for nearly all traits. The individual-tree heritabilities were moderately high for BBDAY (0.45-0.59), heritabilities were low to moderate for second flushing traits (0.10-0.50) and were low for bud set (BS% = 0.11-0.17; BSDAY = 0.17-0.39). These results are consistent with earlier reports (O'Neill et al 2001).

Table 4. Treatment means, family ranges (over 39 full-sib families), individual-tree heritabilities (h_i^2), and percentages of total family variances due to general (GCA) and specific (SCA) combining abilities for bud burst (BBDAY), bud set (BS%, BSDAY), second flushing (SFL%, SFB%, SF#), and cold injury (NCD%, BCD%, SCD%) in the *Seedling Drought Physiology Study*.

Trait	Treatment	Mean	Family range[1]	h_i^2	GCA (%)	Sign. GCA[2]	SCA (%)	Sign. SCA[2]
Year 2								
BBDAY	Control	106	99–114	0.59	99	**	1	ns
	Mild	106	99–115	0.59	96	**	4	ns
	Moderate	105	99–114	0.49	100	**	0	ns
BS%	Control	0.81	0.47–1.00	0.14	100	**	0	ns
	Mild	0.39	0.08–0.74	0.17	100	**	0	ns
	Moderate	0.36	0–0.84	0.11	26	**	74	**
SF#	Control	3.10	0.2–6.0	0.21	34	**	66	ns
	Mild	3.90	0.3–6.2	0.31	75	**	25	ns
	Moderate	3.00	0.1–5.7	0.20	32	**	68	**
SFB%	Control	0.67	0.30–1.0	0.10	32	**	68	ns
	Mild	0.79	0.47–1.0	0.11	56	**	44	ns
	Moderate	0.71	0.20–1.0	0.12	21	*	79	**
SFL%	Control	0.62	0.20–1.0	0.39	89	**	11	ns
	Mild	0.67	0.32–1.0	0.30	79	**	21	ns
	Moderate	0.60	0.10–1.0	0.36	58	**	42	*
NCD%	Control	0.368	0.14–0.69	0.46	94	**	6	ns
	Mild	0.537	0.33–0.81	0.30	100	**	0	ns
	Moderate	0.614	0.41–0.82	0.19	100	**	0	ns
BCD%	Control	0.216	0.08–0.49	0.23	100	**	0	ns
	Mild	0.330	0.13–0.68	0.28	75	**	25	ns
	Moderate	0.411	0.19–0.74	0.22	98	**	2	ns
SCD%	Control	0.260	0.14–0.46	0.31	90	**	10	ns
	Mild	0.432	0.28–0.64	0.20	81	*	19	ns
	Moderate	0.524	0.36–0.73	0.04	100	*	0	ns
Year 3								
BBDAY	Control	108	97–117	0.45	100	**	0	ns
	Recovery	108	99–118	0.50	93	**	7	ns
	Severe	108	97–117	0.51	90	**	10	ns
BSDAY	Control	183	167–197	0.23	100	**	0	ns
	Recovery	179	168–190	0.39	100	**	0	ns
	Severe	169	164–190	0.17	54	*	46	*
SFL%	Control	0.64	0.15–1.0	0.32	100	**	0	ns
	Recovery	0.54	0.11–1.0	0.50	88	**	12	ns
	Severe	0.13	0.0–0.63	0.24	75	**	25	ns

[1]Family differences were significant ($p<0.05$) for all traits except for stem cold damage (SCD%) under moderate drought.

[2]Significance of GCA and SCA. ns = not significant; * = $p < 0.05$; ** = $p < 0.01$.

The heritabilities for the cold hardiness traits were generally low to moderate (0.04-0.46) and gradually decreased as drought stress increased. For example, the heritabilities for SCD% were 0.31, 0.20 and 0.04 in the control, mild drought and moderate drought treatments, respectively. Overall, the magnitudes of the heritabilities for fall cold hardiness are similar to those reported by O'Neill et al (2001). These results suggest that genetic selection for cold hardiness will be slightly more effective under well-watered conditions than under drought stress.

General combining ability was high for most traits, but SCA was also important for second flushing.

For each trait, we partitioned the family variance into variance due to general combining ability (GCA) and variance due to specific combining ability (SCA). GCA is the average effect of each parent involved in a cross, whereas SCA is the deviation of the mean of a specific cross from the average general combining ability of the two parents. The proportion of family variance due to GCA was generally high for bud burst (BBDAY), bud set (BS% and BBDAY) and the cold hardiness traits (NCD%, BCD% and SCD%) (Table 4). Thus, we should be able to get good genetic gains for these traits via random mating among genetically superior parents in seed orchards.

Table 5. Statistical significance of main effects and interactions, plus the genetic correlations between drought treatments for traits measured in the *Seedling Drought Physiology Study*.

			Genetic correlations between paired treatments (r_g)		
Trait	Drought treatments[1]	Family x tmt. interaction[1]	Control vs Mild	Control vs moderate	Mild vs moderate
Year 2					
Bud burst (BBDAY)	ns	ns	1.00	1.00	1.00
Bud set percentage (BS%)	***	*	1.00	0.55	1.00
Second flushing leader (SFL%)	ns	ns	1.00	1.00	1.00
Second flushing branch (SFB%)	ns	ns	1.00	1.00	0.86
No. of second flushed shoots (SF#)	ns	ns	0.94	0.95	0.92
Needle cold damage (NCD%)	***	ns	1.00	1.00	1.00
Stem cold damage (SCD%)	***	ns	1.00	1.00	1.00
Bud cold damage (BCD%)	***	ns	1.00	0.97	1.00

Trait	Drought treatments[1]	Family x tmt. interaction[1]	Control vs severe	Control vs recovery	Severe vs recovery
Year 3					
Bud burst (BBDAY)	ns	ns	1.00	0.96	0.98
Bud set day (BSDAY)	***	*	0.75	0.84	1.00
Second flushing leader (SFL%)	***	**	1.00	1.00	1.00

[1]ns = non-significant, whereas *, **, *** = significant at the 5, 1, and 0.1% levels of probability, respectively.

Both GCA and SCA seem to be important for the second flushing traits (SF#, SFB% and SFL%) (Table 4). Therefore, making and deploying specific crosses should enhance genetic gains for these traits. Effecient methods of vegetative propagation would help in this process.

Family rankings were relatively stable across drought treatments.

It is important to know the impact of test environment on family rankings. For example, selections made under well-watered conditions may differ from selections made under drought stress, particularly for traits such as bud set, second flushing, cold hardiness and height growth—traits known to be influenced by drought. We examined treatment x family interactions and genetic correlations among the moisture regime treatments for these traits in both the second and third growing seasons. In year two, the treatment x family interaction was non-significant for all traits except BS% (Table 5). For this trait, the lowest genetic correlation (0.55) was found between the control and moderate drought treatments (Table 5). In year 3, there was a significant treatment x family interaction for BSDAY and SFL% (Table 5). The genetic correlation for BSDAY was lowest (0.75) between the control and severe drought treatments. For SFL%, the genetic correlation between each of the treatments was 1.0. Therefore, there is little change in family rankings across drought treatments, even for traits that are apparently affected by drought stress. This is good news for breeders because families that show superior cold hardiness or bud phenology under well-watered conditions should also rank highly for these same traits under moistures stress conditions.

Genetic correlations among bud phenology, second flushing, cold hardiness and drought hardiness traits.

Genetic correlations can be used to judge whether selection for one trait will adversely affect another. Tree breeders should proceed cautiously when two traits have a strong adverse genetic correlation. In this study, genetic correlations (r_g) include both GCA and SCA effects. Our main aim was to determine whether selection for bud set can be good surrogate for fall cold hardiness and whether increased drought hardiness would adversely affect other important traits like bud phenology, second flushing, cold hardiness and growth. Because the cold hardiness traits are highly correlated with one another (r_g = 0.66-1.00), we only report results for SCD% (i.e., genetic correlations for NCD% and BCD% are not included in Table 6). Because the second flushing traits are highly correlated with one another (r_g = 0.83-1.00), we only report results for SFL% (i.e., genetic correlations for SFB% and SF# are not included in Table 6).

On a family mean basis, none of the drought hardiness traits (i.e., cavitation, hydraulic conductivity and shoot damage) were significantly correlated with any of the other traits (BBDAY, BS%, BSDAY, SFL% and SCD%). Therefore, genetic correlations involving the drought hardiness traits are not presented. These results suggest that selec-

Table 6. Genetic correlations (r_g) among bud burst (BBDAY), bud set (BS% or BSDAY), second flushing (SFL%), cold injury (SCD%) and seedling height (HT). Correlations were calculated separately for each treatment and year combination, then averaged across treatments within each year.

	Genetic correlation (r_g)	
Trait	Year 2	Year 3
BBDAY vs Bud set[1]	-0.18	0.11
BBDAY vs SFL%	0.44	0.02
BBDAY vs SCD%	0.62	-[2]
BBDAY vs HT	0.58	0.15
Bud set[1] vs SFL%	-0.89	0.98
Bud set[1] vs SCD%	-0.75	-[2]
Bud set[1] vs HT	-0.72	0.75
SF% vs SCD%	0.70	-[2]
SF% vs HT	0.60	0.73
SCD% vs HT	0.58	-[2]

[1]Bud set was scored as BS% in year 2 and as BSDAY in year 3.

[2]SCD% was not measured in year 3.

tion for increased drought hardiness will have little impact on the other traits. Conversely, selection for bud phenology, second flushing and cold hardiness should not dramatically change drought hardiness.

There were significant correlations among the bud phenology, second flushing, cold hardiness and growth traits. In year two, the genetic correlation between BBDAY and the other traits ranged from -0.18, for the correlation with BS%, to 0.62, for the correlation with SCD%. In year three, the correlations were lower, ranging from 0.11 to 0.15. Bud set showed a strong genetic correlation with second flushing ($|r_g|$ = 0.89-0.98) and moderately high correlations with both cold injury (r_g = -0.75) and seedling height ($|r_g|$ = 0.72-0.75). Trees that set bud later had more second flushing, greater cold damage and greater height growth. In year two, the correlations are negative because we measured the proportion of trees with a terminal bud (i.e., greater values indicate earlier bud set). In year three, the correlations are positive because we measured the number of days until bud set (i.e., greater values indicate later bud set). These results suggest that bud set in seedlings can be used as a surrogate for predicting second flushing, cold injury and seedling height.

HT was positively associated with both SFL% and SCD (r_g = 0.58-0.73). The moderate genetic correlation between seedling height and cold damage (r_g = 0.58) suggests that selection based on height growth alone will result in a corresponding increase in cold damage. Earlier studies also found moderate to strong genetic correlations between bud set and cold hardiness (O'Neill et al 2001), second flushing and height growth (Adams and Bastien 1994) and second flushing and cold injury (Anekonda et al 1998). Genetic correlations between cold injury and seedling height growth, however, have been mostly low ($-0.22 \leq r_g \leq 0.22$) in two Oregon and two Washington populations (Aitken and Adams 1995, 1996), suggesting that each population should be treated on a case-by-case basis.

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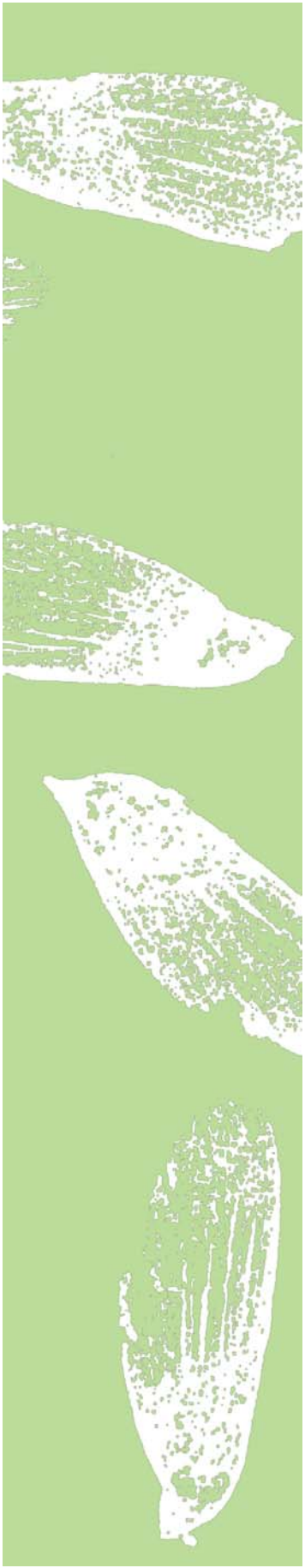

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Activities Planned for 2001-2002

- A major goal for this year will be to develop a new long-term research plan that will guide PNWTIRC research until 2005. Once this planning process is complete, we will prepare a study plan for at least one of our high-priority research topics.

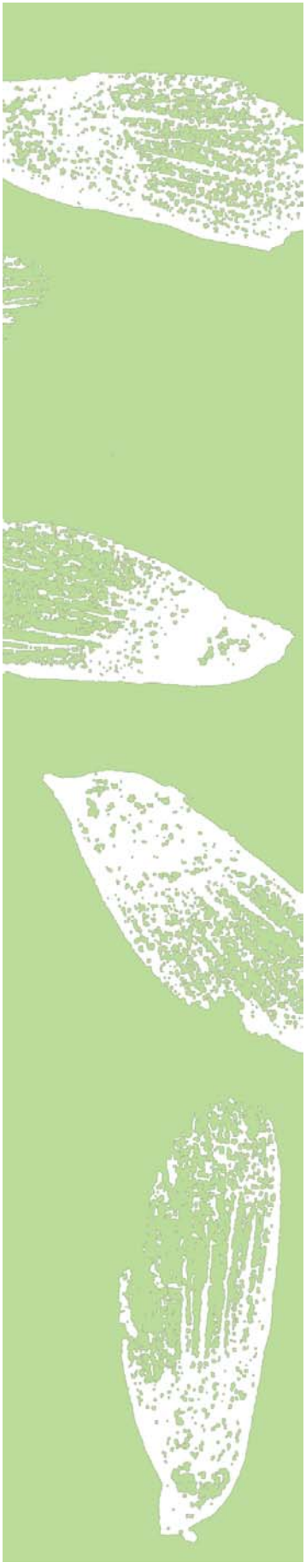
- We will organize at least one technology transfer workshop for PNWTIRC members. This workshop will be used to present an overview of one aspect of PNWTIRC research in a form that can be easily used by our members.

- We will prepare a second drought physiology paper and submit it for publication. This paper will report the results of bud phenology, second flushing and cold hardiness measurements taken on full-sib families growing under three different moisture regimes.

- For the *Early Flowering Study*, we will measure flowering, cone production and seed yields on the grafts that we stimulated in the spring of 2001. New crown control treatments and additional flower stimulation treatments will be applied in the spring of 2002.

- Scions of 16 clones will be grafted onto the rootstock in the *Miniaturized Seed Orchard Study*. Maintenance of the seed orchard blocks will be carried out as needed.

- For the *Pollen Contamination Study*, we will verify the inheritance of the best 10-15 SSR markers, then begin to estimate pollen contamination in the test seed orchard.



Appendix 1

Publications and Abstracts by PNWTIRC Personnel: 2000-2001

Adams, W.T., Aitken, S.N., Joyce, D.G., Howe, G.T. and Vargas-Hernandez, J. 2002. Evaluating efficiency of early testing for stem growth in coastal Douglas-fir. Silvae Genetica (in press).

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Appendix 2

PNWTIRC Financial Support for Fiscal Year 2000-2001

Regular members[1]	$96,000
Associate members[1]	8,000
Contracts	8,000
Forest Research Laboratory, Oregon State University[2]	91,670
Total	**203,670**

[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.