November 2000

Pacific Northwest Tree Improvement Research Cooperative

Annual Report 1999-2000

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COLLEGE OF FORESTRY

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Pacific Northwest Tree Improvement Research Cooperative

ANNUAL REPORT

1999-2000

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HIGHLIGHTS OF 1999-2000

- Tom Adams resigned from the PNWTIRC's leadership to become Head of the Forest Science Department at Oregon State University.
- Families varied considerably in their response to summer drought applied in the nursery, in terms of growth, growth increment, xylem cavitation, hydraulic conductivity, and shoot damage. Genetic correlations between drought hardiness traits in the same year were high, and were low to moderate between different years. Seedling growth potential of families under well-watered conditions is uncorrelated with drought hardiness, suggesting that selection for growth traits will not reduce drought hardiness of the selected trees. Moderate drought applied to seedlings during the second growing season greatly reduced their growth increment in the following recovery year.
- Due to their low heritability and difficulty of measurement, there seems to be little practical utility to using drought sensitivity coefficients (DRCs) to assess drought hardiness in older, field-grown trees. The lack of correlation between DBH and DRCs is encouraging because like in seedlings, it suggests that selection for rapid stem growth in older trees will not inadvertently reduce hardiness to summer drought. The relationship between drought hardiness in seedlings and the ability to grow under summer moisture deficits later in the rotation is unclear.
- Additional analysis of data from the Cooperative's Early Testing Study showed unfavorable correlations between growth potential and stem form and branching traits in seedlings, such that selection for growth alone is expected to increase the number of whorls with steep-angle branches and stem sinuosity in older trees. To avoid negative impacts, appropriate stem form and branching traits should be included, along with growth traits in nursery-stage selection. Alternatively, growth potential could be emphasized in initial nursery culling, with stem form and branching traits dealt with later in field selections.
- Microsatellite and minisatellite DNA markers are highly polymorphic. These markers should be very effective for mating studies, including pollen contamination analysis. Four to six microsatellite marker loci should be sufficient for pollen contamination estimation in Douglas-fir seed-orchards.
- A site for the Miniaturized Seed Orchard Study was selected and prepared for planting of rootstocks in winter 2001. An advisory committee of seed orchard managers and other experts was formed to guide the implementation of the study.

A Note to the Cooperative Membership from Tom Adams

This is my last annual report as Leader of the Pacific Northwest Tree Improvement Research Cooperative. Beginning December 1, 2000, I take on new responsibilities as Head of the Forest Science Department at Oregon State University. Thimmappa Anekonda will be interim leader until my replacement is hired, hopefully by spring 2001.

In 1983 I decided to become more involved in tree improvement efforts in the region. With the help of several forest geneticists from industry and public agencies, we launched the PNWTIRC in July of that year.

My involvement with the PNWTIRC and its members has been extremely satisfying and rewarding. The research has always been interesting and I have enjoyed seeing the results of our work take hold and significantly impact the direction of tree improvement efforts in the region.

I want to thank all of you for supporting the PNWTIRC with both your time and financial resources over the years. Some of our projects have required substantial effort on your part and this is deeply appreciated. The enthusiasm you have shown for the Cooperative and its research has been a key to its success and contributed greatly to my satisfaction as your leader.

I look forward to working with all of you in my new capacity as Department Head. I know that the PNWTIRC will remain strong and continue to be an important source of supporting research for tree improvement efforts in the Pacific Northwest.

Logan Norris, current Head of the Department, has always been a strong supporter of our Cooperative. My plan is to keep this tradition alive.

Thanks again for a great 17 years!

Tom Adams

Introduction

A major development this year for tree improvement in the region was the move, in April, of the Northwest Tree Improvement Cooperative (NWTIC) to Oregon State University. Now, both cooperative operational tree improvement programs (NWTIC) and supporting tree improvement research (PNWTIRC) are centered in the Department of Forest Science. Dr. Keith Jayawickrama, recently with Forest Research in Rotorua, New Zealand, was hired as the new Director of NWTIC and began work in July. Other members of his staff are Dan Cress, Technical Coordinator, and Denise Steigerwald, Data Manager. The PNWTIRC has always enjoyed a close working relationship with the NWTIC, much of which was due to the enthusiastic support of our efforts by Dr. Jess Daniels, former Director of the NWTIC. Location of the NWTIC in the Department of Forest Science, however, offers even greatly opportunities for close cooperation and interaction between our cooperatives in the future. We welcome the NWTIC to the Department and look forward to working with Keith and his staff!

No new studies were initiated this year, but much progress was made on four continuing investigations, and a project from the past was re-visited. A great deal was accomplished in analyzing and interpreting data from our two drought hardiness studies. Before leaving for a Ph.D. program in Germany, Christine Lomas (M.S. student in the Cooperative last year), spent many months helping in these analyses. With her help and the assistance of Darek Nalle, Ph.D. student in the Forest Resources Department, analysis of the Field Drought Study was completed. Much progress was also made in analyzing data from the Seedling Drought Physiology Study, but some work remains on this project for next year.

Two major developments occurred in the Miniaturized Seed Orchard Project. The first was selection of an excellent site for the experimental orchards. Land in western Oregon recently purchased by one of our cooperators for their second-generation orchard complex was selected for this purpose. The second development was the formation of the Seed Orchard Advisory Committee, which met in March. This committee, consisting of Mike Albrecht, Jerry Barnes, Don Copes, Jeff DeBell, Randall Greggs, Glen Miller, Jim Reno, and Jim Smith, is a rich resource of seed orchard experience, and will play a key role in guiding implementation of the study.

Results from the Pollen Contamination Study are very encouraging because they reveal a vast amount of variation at the microsatellite loci being developed. Dr. Kostya Krutovskii was greatly aided in this research by visiting Ph.D. students from Turkey, Nuray Kaya, and Spain, Santiago Gonzalez-Martinez. We also collected data on clonal variation in floral phenology and abundance in the seed orchard chosen for this study. These data will be used to evaluate the effects of floral phenology and ramet location (edge versus middle of the orchard) on levels of contamination.

Finally, one of the Cooperative's former students (Ph.D. in 1990), Dr. Jesus Vargas-Hernandez, worked with us this year to further analyze and write-up results from the Early Testing Study. Jesus, a Professor of Forest Genetics in the Post Graduate College at Chapingo, Mexico, was here on a sabbatical leave from his university. The Early Testing Study was the major project of the Cooperative in the late 1980's. Although the major findings from this study were presented in previous annual reports (see PNWTIRC An-

nual Reports 1988-92), we have, with Jesus' help, taken a fresh look at early testing and prepared manuscripts for journal publication.

Summaries of this year's progress on all our studies are presented in the remainder of this report. We also discuss activities planned for the coming year.

Current PNWTIRC Research

Seedling Drought Physiology

The Seedling Drought Physiology Study was initiated in 1996, with the ultimate goal of developing efficient screening techniques for assessing drought hardiness in families of coastal Douglas-fir. It is well known that summer droughts adversely affect Douglas-fir growth in the Pacific Northwest, particularly in southwestern Oregon and rain shadow regions in Washington and British Columbia. On average, genotypes adapted to dry sites burst and set bud earlier, grow slower, and have higher root to shoot ratios than genotypes adapted to moist conditions. These genetic adaptations influence decisions on seed movement for reforestation across moisture regimes. Having genotypes capable of growing well under drier moisture regimes is valuable for reforestation programs. Developing such genotypes, however, requires accurate identification of traits associated with drought hardiness, determination of the genetic control of these traits, and understanding the relationships between drought hardiness and growth potential. Specific objectives of this study were as follows:

1) To identify morphological and physiological traits associated with drought hardiness and recovery from drought injury.
2) To determine genetic control of traits related to growth potential, drought hardiness, and recovery from drought injury.
3) To assess relationships between growth potential and drought hardiness, and between drought hardiness and recovery from drought.

Materials and Methods

In spring 1996, germinated seedlings of 39 full-sib families of coastal Douglas-fir from southwestern British Columbia (Vancouver Island and the coastal mainland) were sown in nursery beds at Oregon State University and allowed to grow under well-watered conditions during the establishment year. The experimental design was a split plot replicated in five blocks. Main plots consisted of alternative watering regimes applied during the second (1997) and third (1998) growing seasons. Each family (sub-plot) was represented by 8 trees in two randomly-located 4-tree row-plots within each main plot, with seedling spacing of 8 cm x 8 cm. At the end of the second growing season, every other seedling in all row plots was harvested, with treatments in the third year applied to only one-half of the original number of seedlings.

In the second growing season, the three watering regimes were well watered or control, mild drought, and moderate drought applied from mid-June through early September. Both the mild and moderate treatments had limited impact on growth

and survival of seedlings (PNWTIRC Annual Report 1998-99). Thus, a severe drought was applied in the third growing season in main plots that had received mild drought in the second year. The three treatments in the third year, therefore, were control (well watered both years), recovery (moderate drought in year two followed by well-watered in year three), and severe drought (mild drought in year two followed by severe drought in year three).

Heights and diameters of all seedlings were measured several times during the second and third growing seasons. Shoot damage due to drought was recorded at the end of the each season (mid September) in the drought treatments by visually estimating intensity of yellowing and browning of foliage in 10% damage classes. Little or no damage was observed in the well-watered treatments both years. Relative height increment in the third growing season was calculated as ((final height in year 3 minus final height in year 2)/final height in year 2)*100. Relative diameter increment in the third year was calculated in the similar manner.

Physiological responses to drought were measured by xylem cavitation and hydraulic conductivity at the end of each growing season. Xylem cavitation is collapse of xylem conduits resulting in impaired movement of water up the stem. Xylem cavitation was assessed using an anatomical method where saffranin dye was allowed to pass through 3 cm-long basal stem segments of freshly harvested seedlings. The portion of stem cross-sectional area that is not stained by the dye represents the proportion of cavitation. We visually estimated this proportion separately for each of the two annual growth rings (R1 and R2) in the second year, and for the three growth rings (R1, R2, and R3) in the third year, using 10% classes. The current year's annual growth ring is more sensitive to cavitation due to drought than earlier growth rings (PNWTIRC Annual Report 1998-99). Thus, only R2 from the second-year and R3 from the third-year are included in the analysis. In the second-year, cavitation was assessed on 10 seedlings per family in all three treatments (3 treatments x 39 families x 10 trees = 1140, with 30 missing trees due to mortality). In the third-year, it was assessed in the severe drought treatment (39 families x 10 trees = 390).

Hydraulic conductivity (K_h) is the quantity of water transported through a given length of stem under constant pressure. The magnitude of hydraulic conductivity is a function of stem diameter and the proportion of functioning xylem conduits (i.e., lack of xylem cavitation). Hydraulic conductivity was assessed on only a small subsample of seedlings in the second year, but in the third year it was measured in the same trees measured for cavitation.

Growth potential in the absence of drought was assessed by total height and diameter of trees in the control treatment each year. Drought hardiness was assessed by shoot growth, damage, and physiological response traits in the moderate (year 2) and severe (year 3) drought treatments. Effects of the second year drought on growth and initial results from the third year measurements were addressed in PNWTIRC's 1997-98 and 1998-99 annual reports, respectively. Here, we present results on the genetic control of traits associated with severe drought applied in the third growing season, interrelationship of drought hardiness with growth potential, and the impact of second-year moderate drought injury on recovery of growth increment of these seedlings in the following year.

RESULTS

MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS ASSOCIATED WITH DROUGHT HARDINESS AND RECOVERY

By mid July (i.e., Julian day 190), the severe drought treatment applied during the third growing season was highly effective, as indicated by the sharp decline of pre-dawn xylem water potentials ($\psi_{pd} \approx$ -3 MPa) of seedlings grown in this treatment (Figure 1). The intensity of drought during the first 60 days of the treatment period (i.e., 120-180 Julian days) was mild ($\psi_{pd} >$ -1.5 MPa); it reached a moderate level (ψ_{pd} = -1.5 to –3 MPa) rapidly in the next 20 days, and then remained constant and most severe (ψ_{pd}<–3 MPa) for the remainder of the treatment period. In the control and recovery treatments, xylem water potentials remained above –1 MPa throughout the treatment period.

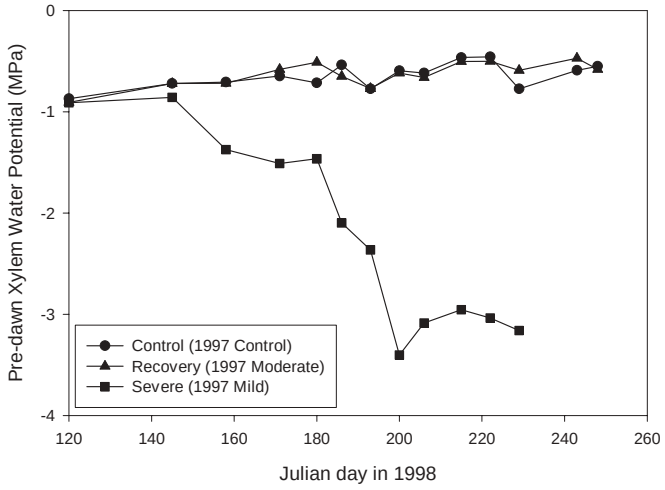

Figure 1. Average weekly pre-dawn xylem water potential measurements under three drought treatments. Soil moisture availability decreases with increasing negative water potential.

Relative to seedling height in the control, third year height in the recovery and severe drought treatments were reduced, on average, by about 10% and 18%, respectively (Figure 2). In all three treatments, total height growth was completed by Julian day 210. Diameter growth of seedlings in the control and recovery treatments, on the other hand, continued well beyond mid-September (i.e., Julian day 260), while stem diameter shrunk slightly in the second-half of the severe treatment period.

Previous analysis showed strongly positive and significant (r_f = 0.65; p<0.01) family mean correlations between moisture regimes for total third-year height and stem diameter (PNWTIRC Annual Report 1998-99). This indicates that total seedling growth is not a sensitive measure of differential family growth response to drought. One might suspect that relative height and diameter increments in the third year would be better assessments of drought hardiness. Much of the height growth in all treatments, however, was completed prior to the drought treatments. Stem diameter, however, continued in the control well beyond the period that drought was most intensive in the severe drought treatments. Therefore, diameter increment under severe drought should be an effective measure of growth response to drought.

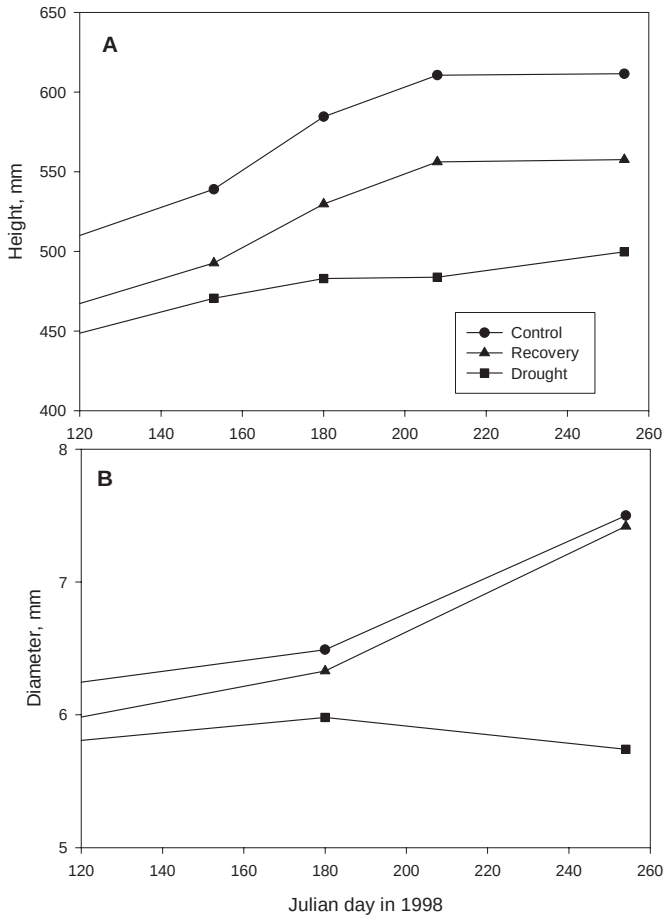

Figure 2. Third-year incremental height and diameter growth of seedlings under three drought treatments.

Genetic control of growth potential, drought hardiness, and drought recovery traits, and genetic interrelationships between drought hardiness traits

In both the second and third year, family means differed significantly in all growth potential, drought hardiness, and recovery traits (Table 1). Coefficients of family variation (CV) for drought hardiness traits were, on average, five times greater than CVs for growth potential, and three times greater than CVs for recovery traits. Thus, there is considerable genetic variation for drought hardiness among the 39 families in this study. Estimated individual tree heritabilities for drought hardiness traits were generally low, but comparable in magnitude to heritabilities for growth potential. Individual tree heritabilities for height and diameter increment in the recovery treatment, however, were very low. Estimated family heritabilities for drought hardiness traits were moderately strong ($h_f^2 \geq 0.36$) suggesting that family selection would be especially effective for improving drought hardiness in breeding programs.

Table 1. Estimated treatment means, ranges and coefficients of family mean variation (CV), and individual (h_i^2) and family (h_f^2) heritabilities for second and third-year growth potential, drought hardiness, and drought recovery traits.

Age	Traits	Mean	Range[a]	CV[b]	h_i^2	h_f^2
Growth potential						
2	Height, mm	354.00	299-438	9	0.27	0.50
2	Diameter, mm	5.50	4.60-6.70	8	0.08	0.16
3	Height, mm	611.00	514-729	10	0.42	0.61
3	Diameter, mm	7.49	6.03-8.93	9	0.21	0.44
Drought hardiness						
2	Ring 2 cavitation, %	18.30	5.0-60.6	64	0.46	0.72
2	Shoot damage, %	17.30	10.0-34.0	34	0.16	0.57
3	Ring 3 cavitation, %	57.00	28.0-92.0	26	0.13	0.36
3	Shoot damage, %	32.00	13.5-60.0	32	0.24	0.55
3	Hydraulic conductivity (Kg-m s^{-1} MPa^{-1})	3.32	0.61-9.63	62	0.14	0.53
3	Diameter increment, %	8.53	0.28-16.7	53	0.09	0.36
Recovery						
3	Height increment, %	68.20	55.1-85.6	12	0.11	0.34
3	Diameter increment, %	49.20	37.1-68.8	15	0.01	0.05

[a]Families differed significantly ($p<0.05$) for all traits.

[b]CV is the standard deviation of family means expressed as a percentage of the treatment mean.

Family mean correlations (average $r_f = |0.66|$) and genetic correlations (average $r_A = |1.00|$) are strong between drought hardiness traits measured in the same year, suggesting that in a given year, drought hardiness traits are largely controlled by the same set of genes (Table 2). Which measure to use in screening seedlings for drought hardiness depends on the cost of assessment of a given trait, magnitude of its heritability, and the degree to which the trait predicts drought hardiness in actual field conditions. Shoot damage and diameter increment under drought are both relatively easy to assess. Heritability of shoot damage appears higher than for diameter increment. In addition, only one measurement is required to assess shoot damage, while diameter increment requires caliper measurements at the beginning and end of the growing season. Thus, shoot damage may be preferred to diameter increment, but shoot damage is assessed visually, so can be influenced by the scorer. Nutrient deficiency or disease may also affect seedling foliage color, confounding the true effects of

Table 2. Estimated family mean correlations (r_f) and additive genetic correlations (r_A) between drought hardiness traits measured in the same year on 39 coastal Douglas-fir families.

Age	Paired traits		r_f[a]	r_A
2	Ring 2 cavitation	Shoot damage	0.71	1.23
3	Ring 3 cavitation	Hydraulic conductivity	-0.86	-0.97
3	Ring 3 cavitation	Shoot damage	0.85	1.96
3	Ring 3 cavitation	Diameter increment	-0.69	-1.02
3	Hydraulic conductivity	Shoot damage	-0.79	-1.01
3	Hydraulic conductivity	Diameter increment	0.68	0.98
3	Shoot damage	Diameter increment	-0.69	-1.03

[a]All family mean correlations are significantly ($p<0.05$) different from zero.

drought treatments. So, in addition to shoot damage, it is useful to consider other measures of drought hardiness.

Cavitation and hydraulic conductivity (K_h) directly measure functioning xylem vessels in the stem. Cavitation assessment is relatively more labor intensive, time consuming, and more subjective measure of xylem function compared to K_h assessment. It is possible to assess xylem cavitation more quantitatively by electronically scanning images of dyed, stem cross-sections, but this technique requires additional time and labor, increasing the cost of assessment. Hydraulic conductivity, on the other hand, is a quantitative, relatively unbiased, measure of xylem function that could be measured fairly readily on a large number of seedlings. Thus, hydraulic conductivity seems the best candidate to include, along with shoot damage, in assessing seedling drought hardiness.

Thus, hydraulic conductivity seems the best candidate to include, along with shoot damage, in assessing seedling drought hardiness.

Genetic correlations (r_A) between year 2 and year 3 drought hardiness traits are generally low (i.e., 0.19 between shoot damage assessments and 0.46 between R2 and R3 cavitation), suggesting low year-to-year repeatability of these traits. This low repeatability, however, should be partly because failure of the moderate drought applied in the second year to cause sufficient levels of shoot damage and cavitation leading to under expression of these traits.

An interesting alternative to assessing stem cavitation after drought treatments is to apply centrifuge force to create desired levels of cavitation rapidly in the excised stem segments. This method potentially eliminates the need for applying drought treatments in nursery or field environments, and may save considerable time, labor, and money. However, practical utility of this method in handling large number of samples is unknown, and yet to be evaluated.

Table 3. Estimated family mean (r_f) and genetic correlations (r_A) between drought hardiness and growth potential traits.

Age	Drought hardiness	Growth potential	r_f[a]	r_A
2	Ring 2 cavitation	Height	0.06	0.09
2	Shoot damage	Height	0.03	0.05
2	Ring 2 cavitation	Diameter	-0.02	-0.05
2	Shoot damage	Diameter	0.07	0.16
3	Ring 3 cavitation	Height	-0.03	-0.07
3	Hydraulic conductivity	Height	0.18	0.24
3	Shoot damage	Height	-0.08	-0.11
3	Diameter increment	Height	0.30	0.46
3	Ring 3 cavitation	Diameter	-0.14	-0.25
3	Hydraulic conductivity	Diameter	0.35*	0.53
3	Shoot damage	Diameter	-0.16	-0.25
3	Diameter increment	Diameter	0.37*	0.65

[a]A * indicates that the family-mean correlation is significantly ($p<0.05$) different from zero.

Relationship between growth and drought hardiness

Estimated family mean and genetic correlations between drought hardiness and growth potential traits were either near zero or favorable in sign, indicating that improvement for stem growth in breeding programs will not reduce drought hardiness of selected individuals (Table 3). Both hydraulic conductivity and stem diameter of trees grown under severe (age 3) drought had moderately strong, positive genetic correlation with stem diameter in the control treatment. This suggests that families with enhanced diameter growth in well-watered conditions may actually be hardier to severe drought at the seedling stage, than families with slower growth potential.

Impact of drought injury on recovery of growth increment

Genetic correlations between drought hardiness traits at age 2 (i.e., ring 2 cavitation and shoot damage) and recovery traits (height and diameter increment) at age 3

were strong and negative (average r_A = -1.19). Thus, families most injured by the moderate drought in the second year produced the lowest stem-growth increments in the following year. Although mean shoot and cavitation levels were low (17 & 18%, respectively) in the second-year moderate drought, capacity for growth in the following year was substantially impacted.

Drought hardiness measures evaluated in this study mostly involve drought tolerance mechanisms; that is, the ability to withstand and survive damage from severe drought. Developing breeding programs for drought tolerance mechanisms has immediate benefit to those regions in the Pacific Northwest where drought can be relatively severe and frequent (e.g., southwest Oregon). Also, drought tolerant genotypes can serve as insurance against unforeseen drought events in these and other regions. It is, perhaps, just as important to address drought avoidance mechanisms, such as timing of seasonal growth to avoid late summer drought, which we plan to address in the next year's annual report. Understanding both avoidance and tolerance mechanisms should help in developing suitable and effective screening techniques for mild, moderate and severe drought environments. Together these mechanisms should provide alternative screening options for breeding programs interested in improving drought hardiness traits in Douglas-fir.

Understanding both avoidance and tolerance mechanisms should help in developing suitable and effective screening techniques for mild, moderate and severe drought environments.

Field Drought Study—Genetics of Drought Sensitivity in Older Trees

Just as we did previously with our cold hardiness studies, we investigated the genetics of drought hardiness in Douglas-fir in both seedlings in a nursery experiment and in older trees growing in field progeny tests. The goal of the field study was to evaluate whether annual growth rings, and their earlywood and latewood components, can be used to retrospectively assess hardiness of Douglas-fir families to summer drought. This study was designed to occur in two stages. In Stage 1 the objective was to determine the degree to which components of annual rings in pole-size trees are sensitive to growing season soil moisture availability. If growth ring components showed consistent sensitivity to drought, the study was to proceed to Stage 2, in which the objective was to determine the extent to which sensitivity to growing season drought is under genetic control.

Summary of Stage 1 Results

Stage 1 was the subject of Andy Bower's 1998 M.S. thesis. The results of this study were reported in the 1998 and 1999 Annual Reports, so only a brief summary will be provided here. Andy collected breast height increment cores from each of 16 trees in eight progeny test sites on Vancouver Island and the coastal mainland of British Columbia. Each core was subjected to an X-ray densitometry analysis that provided a profile of densities across the core and subdivided the core into annual growth rings. In addition, from the density profile of each ring, four annual ring growth variables (ring width, earlywood width, latewood width, latewood proportion) and three ring density variables (ring density, earlywood density, latewood density) were derived. Data from 10 rings were generally available from each tree.

The eight sites represented a range of growing season moisture availability, which was summarized for each year and site by estimating soil moisture deficit (SMD). SMD estimates were calculated by Dr. David Spittlehouse (Research Branch, British Columbia Ministry of Forests) from inputs of climate, soil type and topography, utilizing a water balance model. The greater the SMD, the greater the growing season drought stress. The mean and standard deviation of SMD were near zero at two of the test sites (i.e., no drought stress was detected at these sites). Thus, two of the eight sites were dropped from further analysis.

The relationships between the annual ring variables of each core and SMD were evaluated in the following manner. Because ring variables in young trees change rapidly and consistently with increasing distance from the pith, quadratic regression equations were first applied to the annual rings of each core to relate the change in each variable to tree age. These "age models" fit the data quite well (PNWTIRC Annual Report 1998-1999). Residuals from the age-model regressions for each core, which represent the annual ring variables after adjusting for the effects of tree age (i.e., age-adjusted ring variables), were then regressed on ln(SMD) using linear regression. The slope of the linear regression, which we call the "Drought Response Coefficient (DRC)", measures the sensitivity of an annual ring variable to increasing SMD. DRC equal to zero for a particular ring variable and increment core indicates that the variable was not sensitive to increasing SMD in the tree analyzed. A positive (negative) DRC, however, suggests that the magnitude of the annual ring variable was sensitive to growing season drought, and that the ring variable increased (decreased) in response to increasing SMD.

With the exception of DRC for latewood width, mean DRCs for all ring variables were significantly different ($p < 0.05$) from zero at one or more of the six test sites, indicating that, on average, ring variables are sensitive to varying levels of growing season soil moisture availability (PNWTIRC Annual Report 1998-1999). At the driest site (site 52, where mean SMD was two to three times greater than any other test location), mean DRC for earlywood width and for overall ring density were negative. These relationships are expected if increasing SMD results in proportionally less latewood to overall ring growth. The mean DRC for latewood density was always positive across the six sites, but the sign of DRCs for other ring variables were not consistent. For example, while DRC for ring width was significantly negative for two sites, it was significantly, but weakly, positive at two others, indicating that at these other sites, ring width increased with increasing SMD. On sites with low average SMD, soil moisture availability in spring may not be low enough to negatively impact stem growth, yet other factors associated with greater SMD, such as sunnier, warmer days, may actually enhance growth. Thus, in Stage 1 we found that ring variables were sensitive to SMD, that DRCs are consistent across sites for latewood density, and that variation in mean DRCs across sites for other ring variables could be reasonably explained. Given these encouraging results, we proceeded to Stage 2 in order to examine genetic variation in DRCs.

Stage 2 Results

Site 59 was chosen for intensive family sampling because: 1) this site contained the same 39 full-sib families included in the Seedling Drought Physiology Study; and,

2) Site 59 had the greatest mean SMD and range of SMD values over years among the four sites sampled in Stage 1 containing these families (PNWTIRC Annual Report 1998–99). One increment core was extracted in fall 1997 from all surviving trees of the 39 families at Site 59. Because limb pruning of the trees on this site (up to 3 m on the bole) in winter of 1994-95 profoundly influenced ring growth in subsequent years, only rings produced prior to 1995 were utilized, leaving a maximum of 10 useful rings per tree (1985-94). Also, trees that did not have a minimum of five rings required for the regression analyses were not used. In total, cores from an average of 11 (7-16) individuals per family were included in the remainder of the study.

Four specific questions were addressed in the Stage 2 analysis:

1) What is the extent of genetic variation and control of DRCs?

2) To what degree are DRCs related to other important economic traits (i.e., stem growth and wood density)?

3) To what extent are DRCs related to drought hardiness assessed in the same families at the seedling stage?

4) What is the potential of using DRCs to improve drought hardiness in Douglas-fir tree improvement programs?

On average, over all test trees, ring growth traits decreased (i.e., mean DRC was negative) and ring density traits increased (i.e., mean DRC was positive) with increasing SMD (Table 4). These results are expected if increasing SMD results in reduced overall ring growth without altering the proportion of latewood versus earlywood. Significant variation among families in mean DRCs was found for earlywood width, latewood proportion, and ring density. DBH also varied significantly among the 39 families, but not mean (overall) core density. Ranges among family means for DRCs were considerable (Table 4). For example, while earlywood width decreased with increasing SMD (i.e., mean DRC < 0) in two-thirds of the families, it was essentially unaffected by increasing SMD in the remaining families (i.e., mean DRC ≥ 0). Thus, there seems to be potential for increasing drought hardiness by selecting against genotypes whose ring variables are negatively influenced by increasing SMD.

Unfortunately, despite the wide variation in DRCs, estimated individual tree heritabilities for these traits were quite low

Table 4. Estimated test means, ranges among family means (39 full-sib families), and family variances for drought response coefficients (DRC) of annual ring variables, and for diameter at breast height (DBH) and overall core density, for pole-size trees of Douglas-fir growing on a single test site in British Columbia.

Trait	Means			Family variance[b,c]
	Test[a]	Family range		
DRC				
Ring width	-0.0194**	-0.0768	0.0125	3.300^{NS}
Earlywood width	-0.0116**	-0.0718	0.0223	8.802**
Latewood width	-0.0078**	-0.0358	0.0181	2.213^{NS}
Latewood proportion	-0.0036^{NS}	-0.0588	0.0436	8.545*
Ring density	0.0044**	-0.0157	0.0250	6.715*
Earlywood density	0.0024*	-0.0112	0.0153	1.073^{NS}
Latewood density	0.0154**	-0.0144	0.0354	2.144^{NS}
DBH (cm)	10.867**	7.765	15.460	7.248*
Core density (fm/cm^3)	0.576**	0.476	0.6952	5.893^{NS}

[a] ** and *indicate that the test mean for DRC is significantly different from zero at p <0.01 and p < 0.05, respectively; NS means the test mean is not significantly different from zero (p >0.05).

[b] Family within half-diallel variance component. The 39 families consisted of four 5-tree half-diallels [(10 families per half-diallel x 4) – 1 missing family in one half-diallel = 39).

[c] ** and * indicate that the family within half-diallel variance is significantly different from zero at p < 0.01 and p < 0.05, respectively; NS means the variance is not significantly different from zero (p > 0.05).

Table 5. Estimated individual-tree (h_i^2) and full-sib family (h_f^2) narrow-sense heritabilities for three drought response coefficients (DRC) and for DBH.[a]

Trait	h_i^2	h_f^2
DRC		
Earlywood width	0.07	0.16
Ring density	0.07	0.17
Latewood proportion	0.13	0.30
DBH	0.04	0.11

[a] The estimates for full-sib family heritabilities assume that each family consists of 20 individuals assessed on a single test site.

(Table 5). These low individual tree heritabilities reflect the difficulty of separating the influence of moisture availability on ring variables from the myriad of other factors that influence these traits. Even with a family size as high as 20, heritabilities of full-sib family means are estimated to be low (Table 5). Because we measured DRCs for trees from only one test site, both individual and family heritabilities in Table 5 are probably overestimated. Therefore, it appears that DRCs are weakly inherited, at best.

Except for the genetic correlation between DRCs for latewood proportion and ring density, estimated phenotypic and genetic correlations between family means for the other DRC traits were strong (Table 6). This indicates that these traits are largely under the control of the same genes. In no case, however, was the phenotypic correlation between DRC traits and DBH significant ($P < 0.05$) or greater in magnitude than | 0.28| (Figure 3). This suggests that the drought hardiness of families, as reflected by DRCs, is unrelated to their stem growth rate. Finally, estimated phenotypic correlations between family means for DRCs and for seedling drought hardiness traits were low and never significantly different from zero (Table 7), indicating little or no relationship between drought hardiness of families measured at the seedling stage under artificial drought, and sensitivity to drought measured retrospectively in older, field-grown trees.

Table 6. Estimated phenotypic (above diagonal) and genetic (below diagonal) correlations between full-sib family means for drought response coefficients (DRC) of three annual ring variables.[a]

	Earlywood width	Ring density proportion	Latewood
Earlywood width	—	-0.65**	-0.73**
Ring density	-1.00	—	0.74**
Latewood proportion	-0.86	0.29	—

[a] Based on 39 families. ** means the phenotypic correlation coefficient is significantly different from zero at $p < 0.01$.

Table 7. Estimated phenotypic correlations between family means for drought response coefficients (DRC) of annual ring variables in pole-size trees and seedling drought hardiness traits.[a]

Seedling[b]	DRC		
	Earlywood width	Ring density	Latewood proportion
Hydraulic conductivity	0.02	-0.04	-0.04
Shoot damage	-0.13	0.03	0.21
Diameter increment	-0.05	0.18	0.08

[a] Based on 39 full-sib families. In no case is the estimated phenotypic correlation coefficient significantly different from zero ($p < 0.05$).

[b] See description of traits under "Seedling Drought Physiology Study."

Due to their low heritability and difficulty of measurement, there appears to be little practical utility to using DRCs to screen for drought hardiness in Douglas-fir tree improvement programs. The apparent lack of correlation between DBH and DRCs, however, is encouraging because it suggests, like we have observed in seedlings, that selection for rapid stem growth in older trees will not, inadvertently, reduce hardiness to summer drought. It appears that it is best to measure drought hardiness at the seedling stage when trees are most susceptible to damage by drought, and where drought conditions can more readily be controlled in test environments. The relationship between

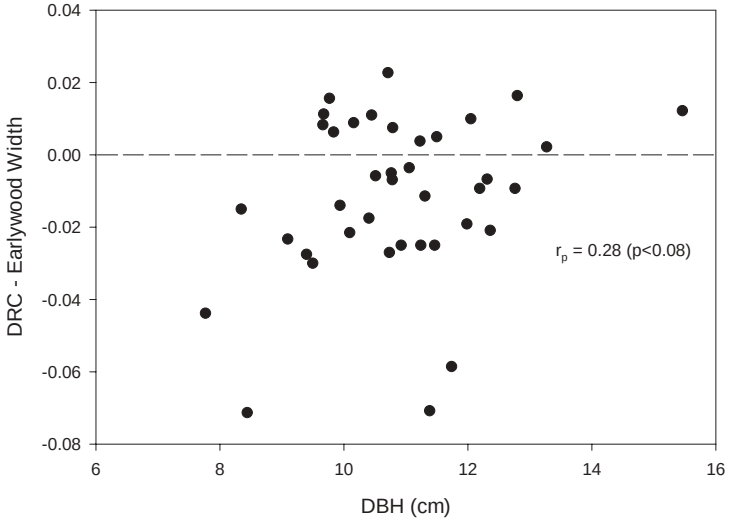

Figure 3. Relationship between full-sib family means for DBH and for drought response coefficient (DRC) of earlywood width. r_p is the estimated phenotypic correlation between the family means.

drought hardiness in seedlings and the ability to grow under summer moisture deficits later in the rotation is not clear.

Early Testing Revisited

Background

The Early Testing Study was started by the PNWTIRC in 1984. The overall objective of this research project was to determine how well family performance for different traits, including stem growth and form, branching habit, shoot phenology, and wood density in 10- to 15-year-old Douglas-fir trees could be predicted from measurements on 1- and 2-year-old seedlings in the nursery. It has been shown that if nursery-field phenotypic correlations between family means (r_{xy}) are at least moderately strong (i.e., $r_{xy} > 0.30$) and consistent across nursery test environments, early testing can be used to cull families which ultimately would have the poorest field performance before outplanting field tests. Thus, early testing is an effective tool for reducing the size and cost of field-testing without limiting overall expected genetic gains.

Sixty seven Open-Pollinated (OP) families from sets 2, 4, and 5 of the Noti Breeding Unit (331 total families) and 66 Full-Sib (FS) families from series 75 and 77 of the Coastal BC Tree Improvement Program (930 total families) were grown for two years in two different nursery regimes, each replicated twice (i.e., a total of four separate nursery trials). In the "bareroot" regime, replicate trials were established in 1986 and 1987 by sowing the families directly into adjacent beds in the same nursery. In the "transplant" regime, replicate trials were established in 1987 by sowing the families in containers in two different greenhouses and transplanting them one year later into beds of two different nurseries.

In previous PNWTIRC Annual Reports (1989-90, 1990-91, and 1991-92) the efficacy of early testing for growth potential was emphasized. The main conclusions obtained from earlier analyses (and confirmed with new ones) are:

a) Family heritability (h^2_f) for 1-year height was consistent across nursery trial replicates for each family type and similar in magnitude to h^2_f estimates for stem volume in field trees. Thus, the genetic control of seedling growth appears to be little influenced by the testing environment.

b) Family-mean correlations between 1-year height and stem volume in 12- to 15-year-old trees were moderately strong and homogeneous across nursery trials for both OP (mean $r_{xy} = 0.33$) and FS families (mean $r_{xy} = 0.40$).

c) Relative efficiency of selection for bole volume at age 15, based on a single stage of selection for height at age 1, was estimated to be 50%. That is, the predicted response (genetic gain) in stem volume of 15-year-old trees (OP families) from selection based solely on first-year height was 50% of response predicted if selection were delayed until trees had been tested for 15 years in the field.

d) Early selection based on 1-year height has particular promise in two-stage selection scenarios. Culling the bottom 25% of the families in the nursery based on 1-year height and making final selections of the remaining families on the basis of stem volume at age 15 was predicted to produce about the same genetic gains expected from selection after testing all families in the field, with savings of about 20% in costs of progeny testing (PNWTIRC Annual Report 1990-91).

With the exception of number of branches, genetic correlations between traits in 12-year-old trees were similar in relative magnitude to those observed in seedlings.

Early Testing for Stem Form and Branching Traits

Results from previous analyses of stem form and branching traits, using data from the OP families in the bareroot regime, were presented in the PNWTIRC Annual Report 1988-89. This year we did additional analyses that included both types of families (OP and FS) grown in all four of the nursery trials. In these analyses we focused on: 1) understanding the genetic control of stem form and branching traits in two-year-old seedlings and the interrelationships of these traits with stem growth; 2) comparing the genetic control of stem form and branching traits in seedlings with their control in older trees; and 3) evaluating the effectiveness of early testing for these traits.

For 2-year-old seedlings, we included information on height, stem diameter, top weight, total number of branches, branch length, branch angle, and stem sinuosity (crookedness or waviness of stems). For the field trees, we used data from OP families (sets 2 and 4) only, measured at ages 12 and 24 years. Age-12 data came from the Measurement Study (PNWTIRC Annual Reports 1985-89) and included information on height, DBH, stem volume, branching traits (number, length, diameter and angle) in the whorl closest to breast height, and stem sinuosity (measured in the second interwhorl from the top of the tree). Age-24 data was obtained from the data set used by Fatih Temel in his M.S. Thesis (PNWTIRC Annual Report 1997-98), and included stem sinuosity (visually assessed for the length of the bole) and number of whorls with steep-angled branches (WSABs), which included forks and ramicorns.

Moderate levels of genetic control for stem form and branching traits were observed in 2-year-old seedlings, with h^2_f estimates similar to those observed for growth traits (Table 8). On average, family heritabilities were slightly higher in the bareroot regime than in the transplant regime, but the differences were not large. Since survival in the transplant regime was very high, the lower precision of this regime is probably due to additional environmental variation introduced by the transplanting process. Relative magnitudes of heritability among traits were consistent across testing regimes and types of family (Table 8). For instance, heritability estimates for stem sinuosity were always the lowest. In addition, family

Table 8. Family heritability (h^2_f) estimates for growth, stem form, and branching traits of two-year-old seedlings grown in two nursery regimes (bareroot and transplant) for OP and FS families.

Seedling	OP families		FS families	
trait	Bareroot	Transplant	Bareroot	Transplant
Height	0.81	0.80	0.90	0.83
Diameter	0.73	0.41	0.85	0.71
Top weight	0.73	0.58	0.86	0.80
No. of branches	0.78	0.75	0.94	0.90
Branch length	0.76	0.70	0.86	0.76
Branch angle	0.77	0.73	0.88	0.75
Stem sinuosity	0.59	0.32	0.78	0.36
Mean:	**0.74**	**0.67**	**0.87**	**0.73**

rankings were relatively stable across nursery environments in both OP and FS families. Type-B genetic correlations between same seedling traits in different nursery trials (a measure of repeatability of family performance for a given trait across nursery environments) were typically above 0.90 when replicates of the same testing regime were compared and above 0.50 between testing regimes.

Genetic relationships among growth, stem form and branching traits measured in seedlings were similar across nursery testing regimes and types of family, so only the estimates obtained for the OP families in the bareroot regime (Table 9, above the diagonal) are described and compared with the genetic correlation structure of similar traits in 12-year-old trees (Table 9, below the diagonal). As expected, growth traits in seedlings are strongly and positively interrelated, indicating they are largely controlled by the same genes. Branching traits and stem sinuosity are also positively interrelated among themselves and with growth traits in seedlings; the only exception being branch angle, which appears to be nearly independent of all traits in terms of genetic control. Thus, at the nursery stage, taller seedlings tend to have more and longer branches and more sinuous stems. With the exception of number of branches, genetic correlations between traits in 12-year-old trees were similar in relative magnitude to those observed in seedlings. While number of branches was strongly and positively related to growth in seedlings, it was unrelated to growth in the older trees. In seedlings, all branches were counted, and obviously this number is strongly associated with growth potential at this age. Only branches at one whorl (near breast height) were counted in older trees.

Table 9. Estimated genetic correlations between growth, stem form, and branching traits in two-year-old seedlings in the bareroot regime (above the diagonal) and in 12-year-old trees (below the diagonal).

Trait	Height	Diameter	Top weight[a]	No. of branches	Branch length	Branch angle	Stem sinuosity
Height	—	0.77	0.88	0.62	0.78	0.22	0.41
Diameter	0.61	—	0.95	0.56	0.67	0.09	0.33
Top weight[†]	0.84	0.95	—	0.73	0.75	0.18	0.45
No. of branches	-0.13	-0.03	-0.04	—	0.53	0.16	0.47
Branch length	0.52	0.72	0.68	-0.07	—	0.19	0.51
Branch angle	0.19	-0.07	0.08	0.33	-0.04	—	0.27
Stem sinuosity	0.15	0.02	0.09	-0.35	0.34	0.26	—

[a]In 12-year-old trees, bole volume was measured instead of top weight.

Family-mean correlations (i.e., r_{xy} estimates) for comparable traits in seedlings and older trees were consistent across nursery testing regimes, but only some of these estimates were strong enough to be useful for early testing purposes (Table 10). Branch length (mean $r_{xy} = 0.38$) and branch angle (mean $r_{xy} = 0.37$) were positively correlated between seedlings and saplings. In addition, branch diameter in saplings was positively, but weakly, associated with branch length in seedlings, while WSAB was moderately, but negatively, associated with branch angle in seedlings (neither branch diameter nor WSAB were measured in seedlings). This

Table 10. Family-mean correlations between seedlings and saplings (r_{xy}) and relative efficiency (RE) of early selection (age 2) for stem form and branching traits of OP families grown in two nursery regimes (bareroot and transplant).

Trait		r_{xy}		RE (%)	
Field	Nursery	Bareroot	Transplant	Bareroot	Transplant
Age 24					
Stem sinuosity	Stem sinuosity	-0.05	-0.21	10.2	42.9
WSAB	Branch angle	-0.28	-0.33	55.1	64.7
Age 12					
No. of branches	No. of branches	0.11	-0.03	26.9	7.3
Branch diameter	Branch length	0.17	0.35	40.5	83.3
Branch length	Branch length	0.33	0.43	67.3	87.8
Branch angle	Branch angle	0.39	0.36	51.3	47.4
Stem sinuosity	Stem sinuosity	0.31	0.09	86.1	25.0

suggests that families with longer branches in seedlings have thicker branches at older ages, and families with flatter-angled branches at the seedling stage tend to produce fewer whorls with steep-angled branches later on. Given these nursery-field relationships, there is potential for early selection of branch length, branch angle, WSABs, and perhaps, branch diameter, using similar traits in 2-year-old seedlings. Efficiencies of early, single-stage, selection in seedlings, relative to direct selection for these traits in the field, were estimated to be nearly 50% or greater (Table 10).

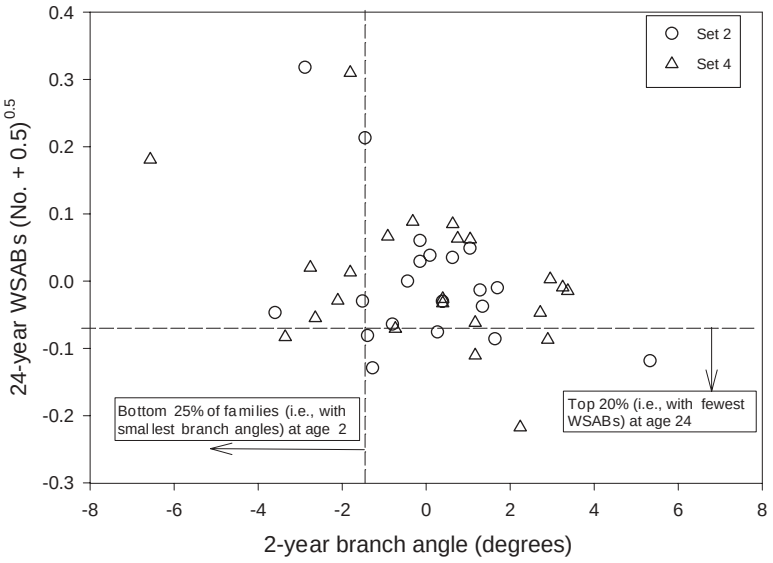

Figure 4. Scatter plot of family means (expressed as deviations from the respective set mean) for branch angle in two-year-old seedlings versus number of whorls with steep-angled branches (WSABs) in 24-year-old trees.

The value of early testing in two-stage selection is illustrated in Figure 4. Assume that we wish to select the 20% of the OP families in sets 2 and 4 that have the lowest number of WSABs at age 24. If the 11 eleven families with the smallest (i.e., steepest) mean branch angle were culled prior to establishing the field tests at age 2 (stage-1 selection), field testing would be reduced by 25%. The remaining families would then be grown in a field test to age 10-15 when families with the lowest mean WSAB would be selected (stage-2 selection). Because only one of the 11 culled families is among the 20% with the lowest WSABs at age 24, culling at age 2 would have little or no impact on genetic gains in WSAB.

Due to unfavorable correlations between growth and some stem form and branching traits both in seedlings and in older trees, unwanted correlated responses in stem form and branching habit will occur if selection is based on stem growth alone (Table 11). For example, selection of the top 20% of the families for height at age 12 is expected to result in small (~ 2%) increases in branch length and stem sinuosity at age 12; but probably more important, an increase of about 10% in the number of WSABs at age 24. A single-stage of early selection for growth potential (using height at age 2), on the other hand, is expected to result in even larger negative impacts on stem form and branching traits, particularly WSABs at age 24.

Table 11. Predicted correlated response (CR%) in stem form and branching traits in the field from selection on height in the field (at age 12) or in the nursery (at age 2).

Trait	Selection at age 12 (CR%)[a]	Selection at age 2 (CR%)[a]	
		Bareroot	Transplant
Age 24			
Stem sinuosity	-1.17	2.32	3.14
WSAB	9.49	20.71	16.41
Age 12			
No. of branches	-0.59	2.39	1.70
Branch diameter	0.14	1.47	2.27
Branch length	1.83	1.82	2.93
Branch angle	0.79	0.74	0.96
Stem sinuosity	2.50	6.42	13.26

[a]Percentage of change with respect to mean in original population, assuming 20% of the families are selected.

In summary, stem form and branching traits in seedlings showed at least moderate levels of genetic control, with relative magnitudes of heritability among traits consistent across testing regimes and family types. Family performance for these traits was also relatively stable among nursery regimes in both OP and FS families, and genetic relationships between traits in seedlings were similar to those observed in 12-year-old trees, with a few exceptions. More important, seedling-sapling correlations between comparable traits for WSABs, branch length, and branch angle were consistent among nursery regimes and strong enough to be effectively used for early

testing of these traits in either single or two-stage selection scenarios. Because of the unfavorable correlations between stem growth and some stem form and branching traits, early selection for growth alone is expected to produce undesirable correlated responses, particularly on WSABs at older ages. To avoid or minimize these unwanted responses, stem form and branching traits should be included as selection criteria in early testing. Another approach is to base early testing on growth potential (e.g., seedling height) alone and deal with stem form and branching traits at the final stage of selection in the field. The relative impacts of these alternative approaches in terms of genetic responses and overall costs of testing need to be carefully considered.

Miniaturized Orchard Study

Uncontrolled mating, pollen contamination, and large tree size pose serious biological and management problems in conventional seed orchards (see Micro-orchard Study Plan; 1989-99 PNWTIRC's Annual Report). With the goal of eliminating these problems, three alternative orchard types, macro (416 stems/ha), micro (1,250 stems/ha), and mini (3,333 stems/ha), will be established and compared for quantity of flower and seed production, ease and efficiency of management, and ramet health and seed quality. All three orchards will be intensively managed for crown control and flower stimulation. Genetic quality of seeds and seed yield in these orchards are expected to be high due to early flower stimulation, high planting density, ability to control mating, and elimination of pollen contamination. Labor needs, management costs, and seed yields will be recorded and evaluated so that the economic efficiencies of these orchards can be determined.

Genetic quality of seeds and seed yield in these orchards are expected to be high due to early flower stimulation, high planting density, ability to control mating, and elimination of pollen contamination.

Although this study is still in its initial stage, in this section we present a brief summary of important decisions and progress made during the last year. First, a seed orchard site, Site S, was selected as the location for the miniaturized orchard research, and a Seed Orchard Advisory Committee (SOAC) was formed to guide implementation of this study. The members of SOAC have great experience in seed orchard management; thus, they will be a valuable resource for guiding the miniaturized orchard research. The SOAC met on March 27, 2000, at Site S, and suggested the following modifications to our original study plan: a) leave enough space between orchard types to prevent shading of smaller trees in the two other orchards by crowns of taller trees in the macro-orchard, and to allow easy movement of equipment, b) arrange clonal rows in north-south orientation to allow maximum crown exposure to the sunlight, c) include a 4m-wide alleyway lengthwise down the middle of mini-orchard main plots so that mechanical equipment can be moved in this very dense for equipment use, and d) increase the number of ramets/clone from 5 to 6 in the mini-orchard to increase management flexibility in the future (e.g., to be able to thin every other ramet in each clonal row after a couple of initial seed crops, if deemed necessary).

The members of SOAC also agreed that availability of irrigation is essential for miniaturized orchards and irrigation should have three important functions: a) to maximize tree survival, tree growth, and seed yield, b) to control frost, and c) to delay flowering (bloom delay). With these considerations in mind, the Seed Orchard Man-

ager at Site S has been working on potential options with irrigation system experts, and a suitable system will be installed.

Following a recommendation by SOAC, we collected 15 soil samples from the study site and had the samples analyzed at the Central Analytical Laboratory, Oregon State University. Dr. Jim Boyle, a soil scientist in the Department of Forest Resources assisted us in the sampling and evaluation. The soil analyses showed that this site is fertile and has good drainage. In addition, both soil physical characteristics (i.e., proportion of coarse particles, sand, silt, and clay) and nutritional properties (pH, N, P, K, Ca, Mg, B, Zn, Fe, Cu, nitrate nitrogen, and carbon nitrogen ratio) were found to be fairly uniform across the site. The site has been already plowed thoroughly, and the laying of orchard blocks will be completed in early fall 2000.

The soil analyses showed that this site is fertile and has good drainage.

Two batches of rootstock, year 1999 sowing (plug-1 stock) and year 2000 sowing (1-0 plugs, sown and managed for an accelerated growth), will be used for planting in spring 2001. A second batch of rootstock was required because some mortality experienced in the first batch. The question is how to combine these two batches in an efficient way. Fortunately, most of our needs can be met with the 1999 stock, but we will need to fill in with the 2000 stock. After a thorough and careful evaluation of operational feasibility, chances for maximum graft survival, and future trends in seed orchard development, the advisory committee came to several decisions on the use of rootstock and the kind of scion material to be grafted (Field grafting is planned for years 2002 and 2003). These decisions are as follows:

a) Distribute the 2000 rootstock uniformly by including one individual of this rootstock in each clonal row plot across all orchard types.

b) Graft 20 extra ramets/clone, in addition to the rootstock used for planting, and hold these extras in a nearby holding clone bank for use in the event of graft mortality in the orchard.

c) Graft unions should be located at least 18-24 inches above the ground level for easy application of flower stimulation (girdling and GA application) and other treatments.

d) If leaders achieve a growth of about 15" in height, the majority of grafting could be done the year after planting (2002). However, it is expected that at least two years will be required to complete grafting. In addition, grafting should be done when the grafts are ready, but not when it is convenient. The rootstock will be surveyed at the end of the 2001 growing season to assess readiness for grafting in 2002. If the stock is too tall at this time, it will be cut back to a suitable height.

e) The study plan calls for using 16 clones for scion material. The committee decided to use eight clones from forward selections (younger ortets) and eight from backward selections (older ortets). If the information is available, clones with exceptionally early or late flowering will be avoided. In addition, clones showing high frequency of graft incompatibility will not be used.

Finally, a preliminary study on auxin treatment and flower stimulation will be initiated in spring 2001, with treatments applied only to a few selected clones in an already established, young orchard at another site

Pollen Contamination Study

One of the advantages of miniaturized seed orchards is the ability to control mating and eliminate pollen contamination. Pollen contamination is a major concern in conventional seed orchards where pollen is dispersed by wind. Although reported for relatively few Douglas-fir orchards, estimates of proportions of seeds fertilized by non-orchard (background) sources typically exceed 40%. This level of contamination will reduce expected genetic gains from orchard seed by 20% or more.

Levels of contamination are best estimated with genetic markers, whereby paternity of orchard offspring can be assigned or inferred based on genetic analysis. Most contamination studies in the past utilized isozymes (variant forms of enzymes) as genetic markers. Although isozyme genetic markers are quite useful, they are limited in variation, such that large numbers of marker loci and very large offspring sample sizes are necessary to obtain precise estimates of contamination.

The purpose of this study is to develop a set of molecular markers, based on DNA containing microsatellites or simple sequence repeats (SSRs), that can be used for estimating pollen contamination and other mating parameters (e.g., mating success of individual clones, efficacy of supplemental mass pollination) in Douglas-fir seed orchards. The advantage of SSRs is that they can be extremely polymorphic (or "hypervariable"), and as such, are very effective for accurately identifying the parents of offspring.

The purpose of this study is to develop a set of molecular markers, based on DNA containing microsatellites or simple sequence repeats (SSRs), that can be used for estimating pollen contamination and other mating parameters

The steps in the study are:

1) Identify a set (7-10) hypervariable marker loci.
2) Develop efficient methods of estimating pollen contamination using these markers by: a) maximizing the efficiency of collecting marker data; and, b) evaluating alternative methods of analyzing the data.
3) Apply the estimation methods developed in step 2 to evaluating pollen contamination in a conventional Douglas-fir orchard, by: a) estimating the overall levels of pollen contamination in two crop years; and, b) assessing the degree to which pollen contamination varies with floral phenology of clones and location of stems in the orchard.

The orchard chosen for this study is one block (Block X) in a western Oregon first-generation seed orchard complex. Materials sampled so far include:

A) Dormant buds from 1-3 ramets of each of the 58 clones in Block X (155 ramets in total), from a sample of 60 clones (1 ramet each) in adjacent orchard blocks, and from 44 trees in nearby wild stands (total number in the background population sample is 104).

B) Seed from individual ramets in Block X.

C) Seed from various controlled crosses of clones in Block X.

D) Bulked seed samples from the 1999 and 2000 cone crops in Block X.

DNA extracted from A) was used in developing the markers and for evaluating levels of polymorphism that can be expected in seed orchard and background populations. These were the primarily activities this year. The remaining materials will be used in the future to verify the inheritance of the markers and to apply them to estimating contamination. Another activity this year was to collect information on flowering in Block X and in the background population that is needed in step 3b, above.

Highly Polymorphic DNA Markers and Observed Levels of Polymorphism

We used a highly polymorphic minisatellite chloroplast marker based on a hypervariable (so called "hot-spot") region found and characterized earlier in Dr. Steve Strauss' laboratory (Hipkins et al. 1995, Current Genetics 27: 572-579) and several highly polymorphic microsatellite nuclear DNA markers, also called simple sequence repeats (SSR). The nature and origin of these markers, as well description of the basic molecular genetic techniques for their analysis, such as the polymerase chain reaction (PCR) and primer design, were described in last year's annual report (PNWTIRC Annual Report 1998-99).

The objectives of this year were to:

(1) Further test selected PCR primers designed to amplify SSR markers;

(2) Improve PCR conditions to increase the amount and quality of amplified SSR fragments;

(3) Optimize multiplexing conditions allowing us to mix and analyze simultaneously the products of different PCR reactions using automatic laser detection and data collection based on fluorescent dyes;

(4) Develop new primers using sequences of SSR regions from a microsatellite enriched clonal library of Douglas-fir;

(5) Use seeds from controlled crosses to verify inheritance and confirm fidelity of the SSR markers;

(6) Choose a set of markers and genotype all clones in Block X, the sample of clones surrounding Block X, and individual trees in the natural stands outside the seed orchard; and

(7) Calculate allele frequencies, and evaluate the efficiency of these DNA markers for detecting pollen contamination.

To find nuclear SSR markers we tested, in the first year of the project, 49 primer pairs prepared by Drs John E. Carson (Pennsylvania State University) and John Hobbs (University of British Columbia, Canada). However, in December 1999, Dr. Carson provided us additionally with the original sequences of the SSR regions from his microsatellite enriched clonal library of Douglas-fir. This allowed us to choose better primers for testing and to develop our own primers to amplify specific nuclear SSR regions in Douglas-fir.

DNA was isolated from buds and seeds using a standard protocol developed in Dr. S. Strauss' laboratory (see http://www.fsl.orst.edu/tgerc/protocol.htm). Primers were designed using GeneRunner software. DNA fragments containing SSR regions were amplified using the MJR PCT-100 ThermoCycler. We used both high resolution MetaPhor agarose and ABI-377 polyacrylamide sequencing gels to separate PCR amplified SSR fragments. Gels were analyzed using the ABI GeneScan and GenoTyper software and scored using GenoGrapher software. The most promising primers were labeled by different fluorescent dyes for the use in automatic laser detection systems such as the ABI-377 sequencer.

So far, we have applied 5 nuclear SSR markers and the chloroplast minisatellite marker to tentatively genotype the 58 Block X clones and 104 clones and trees in the background. The number of marker variants (alleles) detected at each locus is remarkable (Table 12). The chloroplast minisatellite marker produced a total (overall) of 23 alleles in a broad range of easily recognizable sizes (from 574 to 1109 base pairs, Fig. 5). This very high level of variation is similar to that observed earlier in a British Columbia seed orchard with the same marker (Stoehr et al. 1998, Canadian Journal of Forest Research 28: 187-195). The chloroplast marker is particularly useful for mating studies because the chloroplast is haploid (i.e., contains only one allele at the marker locus) and is inherited through the pollen; thus, marker alleles in pollen gametes can be scored directly in the DNA of orchard offspring.

Table 12. Genetic diversity observed at five nuclear SSR loci and one chloroplast minisatellite locus among 58 clones in Block X, and 104 individuals in background stands surrounding Block X.

	Number of different alleles per locus			Expected heterozygosity [a]		
Marker locus	Block X	Background	Overall	Block X	Background	Overall
Chloroplast minisatellite locus						
CPPSMHS1	14 (1[b])	21 (8[c])	23	0.81	0.89	0.89
Nuclear SSR loci						
BCPSMAC5	25 (3)	25 (3)	28	0.94	0.94	0.94
BCPSMAC8A	8 (1)	12 (5)	13	0.70	0.66	0.68
BCPSMAC8B	17 (1)	21 (6)	23	0.88	0.89	0.90
BCPSMAG38	19 (6)	21 (8)	27	0.86	0.86	0.87
BCPSMAG39	28 (3)	28 (3)	32	0.95	0.93	0.95
Mean	18.5	21.3	24.2	0.86	0.86	0.87

[a] Estimated probability that any two alleles drawn from the population will be different.
[b] Number of alleles unique to Block X (i.e., those that occur in Block X but not in background stands).
[c] Number of alleles unique to background stands (i.e., those that occur in background stands but not in Block X).

Because nuclear SSR markers are inherited bi-parentally and are diploid, both male- and female-derived marker alleles are observed in offspring embryos and offspring. The genotype of the pollen gamete in offspring embryos, however, can be determined by comparing the embryo genotype with that of the haploid genotype of the seed's megagametophyte, which is equivalent to the genotype of the egg gamete. This comparison, however, requires two DNA samples per offspring (embryo + megagametophyte), where only the embryo is needed to determine the pollen gamete genotype when the chloroplast marker is used.

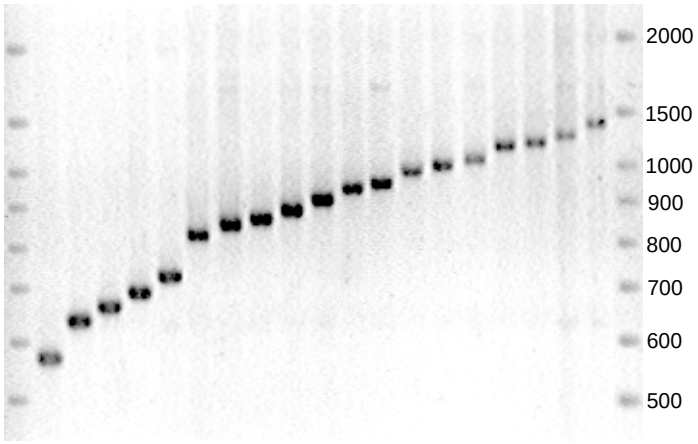

Figure 5. Agarose gel with PCR amplified chloroplast minisatellite "hot-spot" DNA fragments (CPPSMHS1) stained by ethidium bromide (reversed "positive" image) representing 19 of the 23 alleles found in the Douglas-fir seed orchard and surrounding trees. Multiple bands on both sides of the gel are DNA molecular weight size standards. The length of each DNA size standard, in terms of number of nucleotide base pairs, is shown on the right side.

Table 13. Estimated paternity exclusion probabilities[a] for six DNA markers in Block X depending on whether diploid embryo genotypes or haploid pollen genotypes are assayed in orchard offspring[b].

Marker locus	Paternity exclusion probability			
	Embryos		Pollen gametes	
	single	cumulative	single	cumulative
Chloroplast minisatellite locus[c]				
CPPSMHS1	0.8082	0.8082	0.8082	0.8082
Nuclear SSR loci				
BCPSMAC5	0.8767	0.9764	0.8826	0.9775
BCPSMAC8A	0.4761	0.9876	0.5176	0.9891
BCPSMAC8B	0.7639	0.9971	0.7807	0.9976
BCPSMAG38	0.7177	0.9992	0.7389	0.9994
BCPSMAG39	0.8991	0.9999	0.9033	0.9999
Mean	**0.7570**		**0.7719**	

[a] Proportion of potential pollen parents that can be excluded as the true father of a particular diploid offspring based on genotypes alone.

[b] When nuclear markers are used, haploid pollen gamete genotypes can be inferred by comparing the DNA extracted from embryo tissue (diploid genotype derived from pollen and egg genotypes) with DNA extracted from megagametophyte tissue (egg genotype only) of seeds.

[c] The chloroplast is haploid and inherited through the male parent. Thus, when chloroplast DNA in embryos is assayed, the minisatellite haploid genotype of the pollen gamete is assessed directly.

Genetic variation for the nuclear SSR markers is also considerable, with the number of alleles detected, overall, per locus, ranging from 13 to 32 (mean = 24.6, Table 12). The high levels of diversity observed at the marker loci means they will be very effective in discriminating among pollen parents in mating analyses. The proportion of potential males in a population that can be excluded as the true pollen parent of any single offspring on the basis of genotype data alone is known as the paternity exclusion probability (PEP). Estimates of PEP for each marker locus individually, and cumulatively over all loci, for Block X, assuming all mating is among the 58 clones in the block (i.e., no pollen contamination), are presented in Table 13. The estimates assume either only the embryos of each seed offspring are sampled (i.e., diploid genotypes for the nuclear markers), or both the embryo and megagametophyte tissues are assayed so that pollen genotypes can be determined. Estimated PEP is high for the minisatellite marker because it has a very large number of alleles and because it is haploid. PEPs for any single locus are always higher when pollen gametes are assayed than when only embryos are utilized. Nevertheless, exclusion probabilities rapidly approach 1.0 when additional loci are included in the analysis, regardless of whether embryos or pollen gametes are analyzed.

The high levels of diversity observed at the marker loci means they will be very effective in discriminating among pollen parents in mating analyses.

For the purposes of using genetic markers in pollen contamination analysis, we are more interested in the probability of detecting offspring sired by background (i.e., non-orchard) pollen (called the detection probability), than in being able to discriminate among potential males within the orchard block. The detection probability (d) is the probability that an orchard offspring resulting from pollen contamination will have a genotype different from what could be produced by mating among clones within the orchard. This probability increases with increasing allele frequency differences between the orchard block and the background population. It also increases with decreasing number of clones in the orchard block, because with fewer orchard clones, fewer gamete genotypes can be produced within the orchard, relatively to those possible among a much larger number of genotypes in the background population.

Despite low single locus values the expected cumulative detection probability using 5-6 markers is very high in Block X (d > 0.99, Table 14). With isozyme genetic markers at 11 and 14 loci, cumulative d was estimated to be only 0.19 and 0.38, respectively, in another Douglas-fir seed orchard (Adams et al. 1997, Canadian Journal of Forest Research 27: 131-134). In Table 14, we estimated detection probabilities assuming genotypes of pollen gametes are inferred (i.e., both embryo and megagametophyte tissues are assayed). We expect cumulative d's to be somewhat lower if

only embryos are assayed, but not greatly so. Thus, it is clear that hypervariable molecular DNA markers can be used to greatly improve the efficiency of pollen contamination estimation over that previously experienced with isozymes.

At this point our population analysis of genetic variation based on the SSR and minisatellite DNA markers, and estimates of paternity exclusion and detection probabilities must be considered tentative. The reason is that we still need to do more rigorous validation of the markers through inheritance testing. Problems arise in two ways. Ironically, the first problem is because there are so many different alleles at each locus, some variants are of similar size (i.e., similar number of nucleotide base pairs) are difficult to tell apart. Second, for a number of reasons, sometimes no marker band appears on the gel. We need to understand how often this problem occurs, whether there is a genetic explanation (i.e., whether there are so called null alleles), and how best to accommodate this issue in our analysis. Regardless of these problems, the large amount of variation we have observed in these markers indicates that they will be extremely useful for mating studies in Douglas-fir. In addition, it appears that only 4-6 markers would be necessary for highly efficient pollen contamination analysis.

Table 14. Estimated detection probabilities[a] for six DNA markers in Block X based on observed diploid genotypes in Block X and allele frequencies in background.

	Detection probability	
Marker locus	single	cumulative
Chloroplast minisatellite locus[c]		
CPPSMHS1	0.2431	0.2431
Nuclear SSR loci		
BCPSMAC5	0.0547	0.7287
BCPSMAC8A	0.0471	0.8144
BCPSMAC8B	0.0330	0.9435
BCPSMAG38	0.1113	0.9870
BCPSMAG39	0.0256	0.9989
Mean	**0.0858**	

[a] Probability that a background pollen gamete will have a genotype different from those produced by any of the orchard clones.

In the coming year, we expect to sort out the above problems and also plan to evaluate a few additional marker loci that were not included above. Inheritance and expression of all markers will be verified by assaying open-pollinated seeds from individual orchard clones and seeds from controlled crosses. Once we have settled on the best set of markers, seeds from Block X crops will be sampled in order to estimate pollen contamination and to assess alternative estimation procedures. A Ph.D. student from Bulgaria, Gancho Slavov, arrived early in September 2000. The remainder of this project will be Gancho's Ph.D. dissertation research.

Orchard Flowering and Pollen Dispersal

The following data were collected in and around Block X during the 2000 flowering season:

1) Pollen traps (i.e., glass slides with sticky tape) on 1 m-high poles were placed at a total of nine locations in Block X, in adjacent orchard blocks, and in nearby open fields outside the orchard complex. The slides were replaced daily. Pollen density counts from the slides will be used to estimate Block X and background pollen loads each day during the flowering period.

2) All ramets in the central 1/3 of Block X (i.e., ramets stimulated to flower in 2000), and a sample of 8 ramets from each adjacent block, were scored for timing of pollen shed and female conelet receptivity.

3) Pollen conelet abundance was scored on the ramets sampled in 2) at the end of the flowering season.

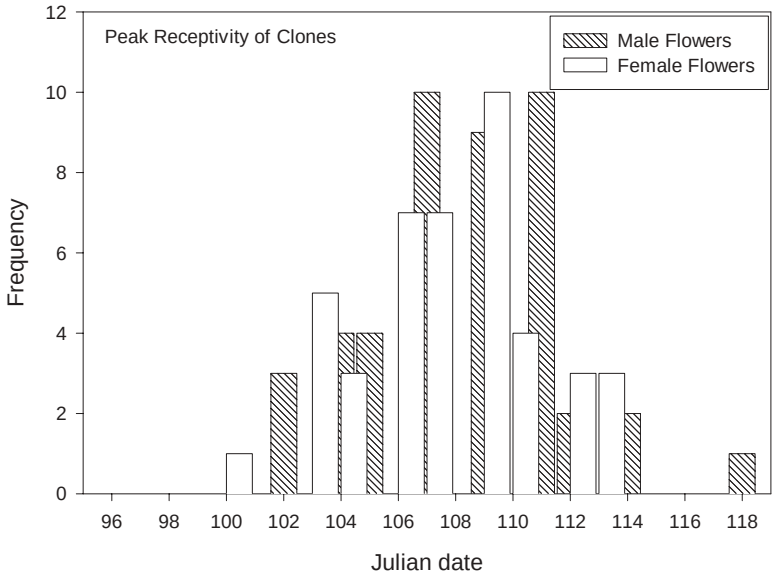

Figure 6. Frequency distribution of timing of peak male and female flowering of ramets in Block X.

The timing of estimated "peak" male and female flowering (i.e., the Julian dates when half of all male conelets had shed pollen and when half the female conelets were receptive) ranged about two weeks among the sampled Block X ramets (Figure 6). The ramets with the earliest (~Julian date 100) and latest (~Julian date 113) female conelet peak receptivity are presumably the most susceptible to pollen contamination because few ramets within Block X were shedding pollen when these trees were receptive. To assess the influence of female phenology on pollen contamination, seed was collected in summer 2000 from four ramets having early-, late- and mid-season timing of female receptivity in Block X. In addition, seed was collected from four ramets on two edges of Block X, and in the middle of the block, so that the influence of ramet location on contamination can be investigated.

Activities Planned for 2000-2001

An extremely important task this year will be to conduct a search for and hire a new leader for the Cooperative. A major research effort will be to complete the remainder of data analysis in the Seedling Drought Physiology Study, with a goal of understanding the nature of the relationships between drought hardiness and cold hardiness, and between drought hardiness and bud phenology. Main activities of the Miniaturized Orchard Study include preparation of the study site, field layout of the orchard and supplemental blocks, planting the rootstock, and initiating preliminary studies on flower stimulation and auxin treatments. In the Pollen Contamination Study, the main objectives will be to test additional SSR markers, to understand completely the inheritance and expression of all the markers, and hopefully, to apply these markers to estimating pollen contamination in block X. A goal the past couple of years has been to develop a plan to test the validity of our cold and drought hardiness screening procedures in the filed, and to assess the ability to predict adaptability to stress environments of genetically improved Douglas-fir families. We hope to make a good start on this plan during the year, but progress will, in part, be determined by how rapidly we can find a new leader.

APPENDIX 1

PUBLICATIONS AND ABSTRACTS BY PNWTIRC PERSONNEL: 1999-00

ADAMS, T. and T. ANEKONDA. 2000. Pacific Northwest Tree Improvement Research Coop. Western Forester, 45: 13.

ADAMS, W.T. and J. BURCZYK. 2000. Magnitude and implications of gene flow in gene conservation reserves. Pp 215-224 In:(A. Young, D. Boshier and T. Boyle, eds.) Forest Conservation Genetics: Principles and Practices. CSIRO Publishing, Collingwood, Victoria, Australia.

ANEKONDA, T.S. and W.T. ADAMS. 2000. Genetics of dark respiration and its relationship with drought hardiness in coastal Douglas-fir. Thermochimica acta 349: 69-77.

ANEKONDA, T.S., W.T. ADAMS, and S.N. AITKEN. 2000. Cold hardiness testing for Douglas-fir tree improvement programs: Guidelines for a simple, robust, and inexpensive screening method. Western Journal of Applied Forestry, 15: 129-136.

ANEKONDA, T.S., W.T. ADAMS, S.N. AITKEN, D.B. NEALE, K.D. JERMSTAD, and N.C. WHEELER. 2000. Genetics of cold hardiness in a cloned full-sib family of coastal Douglas-fir. Canadian Journal of Forest Research, 30: 837-840.

ANEKONDA, T.S., R.S. CRIDDLE, and L.D. HANSEN. 2000. Response to Letter from M. Adams. Functional Ecology, 14: In press.

ANEKONDA, T.S., M.C. LOMAS, and W.T. ADAMS. 2000. Genetic variation in drought hardiness of coastal Douglas-fir (Pseudotsuga menziesii var. menziesii) seedlings. Joint meeting of the North American Forest Biology Workshop and Western Forest Genetics Association, Merida, Mexico, July 17-20, 2000. (Abstract).

CRIDDLE, R.S., T.S. ANEKONDA, H. TONG, J. C. CHURCH, F.T. LEDIG, and L.D. HANSEN. 2000. Respiration parameters determine growth traits in Eucalyptus: Effects of growth climate. Australian Journal of Plant Physiology, 27: 435-443.

JERMSTAD, K.D., D.L. BASSONI, N.C. WHEELER, T.S. ANEKONDA, S.N. AITKEN, W.T. ADAMS, and D.B. NEALE. 2000. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir: II. Spring and fall cold hardiness. Theoretical and Applied Genetics, In press.

O'NEILL, G.A., W.T. ADAMS, and S.N. AITKEN. 2000. Quantitative genetics of spring and fall cold hardiness in seedlings from two Oregon populations of coastal Douglas-fir. Forest Ecology and Management, In press.

O'NEILL, G.A., S.N. AITKEN, and W.T. ADAMS. 2000. Genetic selection for cold hardiness in coastal Douglas-fir seedlings and saplings. Canadian Journal of Forest Research, In press.

TEMEL, F. and W.T. ADAMS. Persistence and age-age genetic correlations of stem defects in coastal Douglas-fir (Pseudotsuga menziesii var. menziesii (Mirb.) Franco). Forest Genetics, 7: In press.

APPENDIX 2

PNWTIRC FINANCIAL SUPPORT FOR FISCAL YEAR 1999-00

Regular members	$96,000[a]
Associate members	8,000
Contracts	8,000
Forest Research Laboratory, Oregon State University	105,288[b]
Total	**217,288**

[a] Each regular member contributed $8,000 and each Associate member $4,000. These figures do not include in-kind contributions of labor, equipment, etc.

[b] This figure includes salaries, use of facilities and administrative support.

The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) was formed in 1983 in response to the need for genetics research in support of operational tree improvement programs in the Pacific Northwest. There are several types of memberships in the Cooperative. Regular members contribute directly to the Cooperative through both an annual membership fee and by supplying in-kind support including land, labor and equipment for research projects. Associate members, landowners with less than 100,000 acres, pay a smaller annual fee and do not necessarily participate in research projects to the same degree as Regular Members. Liaison members contribute to discussions on key matters but have no voting rights.

The Policy/Technical Committee guides the activities of the cooperative. It is responsible for making decisions on overall program strategy and support, identifying research problems, establishing priorities, and assisting in the planning, implementation and evaluation of studies. This committee is comprised of representatives of each cooperator.

The PNWTIRC is housed in the Department of Forest Science at Oregon State University. Tom Adams was the Leader of the Cooperative from year 1983 to 2000. Thimmappa Anekonda is the Interim Leader. Gancho Slavov is a graduate student funded partially by the Cooperative.

This report was written by Tom Adams, Thimmappa Anekonda, Jesus Vargas-Hernandez, and Konstantin Krutovskii.