

Genetic variation in drought hardiness of coastal Douglas-fir seedlings from British Columbia¹

T.S. Anekonda, M.C. Lomas, W.T. Adams, K.L. Kavanagh, and S.N. Aitken

Abstract: Genetic variation in drought hardiness traits and their genetic correlations with growth potential and recovery traits were investigated in 39 full-sib families of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) from southwestern British Columbia. Seedlings of these families were grown in raised nursery beds and subjected to three moisture regimes each in the second (well-watered or control, mild, and moderate drought) and third (control, severe drought, and recovery from second-year moderate drought) seasons. Traits assessed included drought hardiness (foliage damage, cavitation of xylem tracheids, xylem hydraulic conductivity, and height and diameter growth increment) in the drought treatments, growth potential (total height and diameter) in the control treatment, and height and diameter growth increments in the recovery treatment. Xylem cavitation in the growth ring produced in a particular year was nearly three times greater under the moderate drought and four times greater under the severe drought than in the control treatment. Xylem hydraulic conductivity of seedlings in the severe drought treatment was 40% lower than conductivity of seedlings under the control treatment. Mean foliage damage in seedlings subjected to severe drought (third season) was much greater (33%) than in seedlings subjected to mild or moderate drought (second season). Families differed significantly in most drought hardiness traits, with individual tree heritabilities averaging 0.19. Thus, much potential exists for identifying drought-hardy families at the seedling stage and using this information for deployment or breeding purposes. In addition, most hardiness traits were strongly intercorrelated (genetic correlations often exceeded |0.80|) indicating that these traits are controlled largely by the same set of genes and that selection for hardiness based on one trait will increase hardiness as reflected in the other traits as well. Genetic correlations were only moderate (0.49) between hardiness traits measured in different years, perhaps due to the large difference in severity of the drought applied in the two seasons. Although injury to seedlings, as reflected in foliage damage and xylem cavitation, was relatively low under the moderate drought of the second season, it did result in reduced growth increment the following (recovery) year. Growth potential under favorable moisture regimes was nearly uncorrelated with drought hardiness, suggesting that drought hardiness could be improved in this southwestern British Columbia breeding population without negatively impacting growth potential in favorable moisture conditions.

Résumé : Les auteurs ont étudié la variabilité génétique des caractères de résistance à la sécheresse et leurs corrélations génétiques avec le potentiel de croissance et les caractères de rétablissement chez 39 descendance biparentales de douglas de Menzies typique (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) provenant du Sud-Ouest de la Colombie-Britannique. Les semis de ces familles ont été cultivés dans des planches et soumis à trois régimes hydriques au cours de la deuxième (irrigation adéquate ou témoin, sécheresse légère et sécheresse modérée) et de la troisième saison de croissance (témoin, sécheresse sévère et rétablissement après la sécheresse modérée de la seconde saison). Les caractères étudiés comprenaient la résistance à la sécheresse (les dommages foliaires, la cavitation dans les trachéides du xylème, la conductivité hydraulique du xylème ainsi que la croissance annuelle en hauteur et en diamètre) pour les traitements de sécheresse, le potentiel de croissance (la hauteur totale et le diamètre) pour le traitement témoin, ainsi que la croissance annuelle en hauteur et en diamètre pour le traitement de rétablissement. La cavitation dans les éléments de xylème du cerne annuel d'une année donnée était presque trois fois plus élevée pour le traitement de sécheresse modérée et quatre fois plus élevée pour le traitement de sécheresse sévère que pour le traitement témoin. La conductivité hydraulique du xylème des semis soumis aux conditions de sécheresse sévère était 40 % plus faible que celle des semis dans le traitement témoin. Les dommages foliaires moyens chez les semis soumis à la sécheresse sévère (troisième saison) étaient beaucoup plus importants (33 %) que chez les semis soumis à la sécheresse légère ou modérée (seconde saison). Les descendance démontraient une variation significative pour la plupart des caractères de résistance à la sécheresse, avec des valeurs d'héritabilité individuelle atteignant en moyenne 0,19. Par conséquent, il y

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T.S. Anekonda,² M.C. Lomas, and W.T. Adams. Department of Forest Science, 321 Richardson Hall, Oregon State University, Corvallis, OR 97331-5752, U.S.A.

K.L. Kavanagh. Department of Forest Resources, University of Idaho, Moscow, ID 83844-1133, U.S.A.

S.N. Aitken. Department of Forest Sciences, 3rd Floor, Forest Science Centre, 3041-2424 Main Mall, The University of British Columbia, Vancouver, BC V6T 1ZT, Canada.

¹Paper No. 3542 of the Forest Research Laboratory, Oregon State University, Corvallis, Oreg.

²Corresponding author (e-mail: thimmappa.aneekonda@orst.edu).

a de bonnes possibilités d'identifier, dès le stade de semis, des familles résistantes à la sécheresse et d'utiliser cette information à des fins de déploiement ou d'amélioration. De plus, la plupart des caractères de résistance étaient fortement corrélés entre eux (les corrélations génétiques excédaient souvent |0,80|), impliquant que ces caractères sont contrôlés en grande partie par le même groupe de gènes et que la sélection pour la résistance à partir d'un caractère augmentera la résistance telle qu'exprimée également dans les autres caractères. Les corrélations génétiques n'étaient que modérées (0,49) entre les caractères de résistance mesurés dans différentes années, possiblement en raison de la grande différence de sévérité du traitement de sécheresse appliqué lors des deux saisons. Bien que les dommages aux semis, tels qu'évalués à partir des dommages foliaires et de la cavitation dans le xylème, aient été relativement faibles pour le traitement de sécheresse modérée de la seconde saison, ils ont tout de même entraîné une réduction de croissance l'année suivante (année de rétablissement). Le potentiel de croissance sous des conditions favorables d'humidité n'était presque pas corrélé à la résistance à la sécheresse. Cette observation porte à croire que la résistance à la sécheresse pourrait être améliorée au sein de cette population d'amélioration du Sud-Ouest de la Colombie-Britannique, sans avoir d'impacts négatifs sur le potentiel de croissance sous des conditions favorables d'humidité.

[Traduit par la Rédaction]

Introduction

Successful regeneration on drought-prone sites depends on the inherent ability of genotypes to tolerate moisture stress. During summer months, drought-induced moisture stress not only limits natural regeneration of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) seedlings (Hobbs et al. 1980) but also reduces basal area increment in mature trees (Spittlehouse 1985).

Current knowledge on genetic variation of drought hardiness in coastal Douglas-fir derives primarily from geographic variation studies. This research has shown that genotypes originating from drier sites (e.g., inland mountains, southern latitudes, and south-facing slopes) are more likely to survive summer droughts than those from more mesic sites (e.g., coastal mountains, northern latitudes, and north-facing slopes) (Ferrell and Woodard 1966; Pharis and Ferrell 1966; Joly et al. 1989; White 1987). Natural selection on xeric sites has favored drought hardiness ability, such that genotypes from dry sites have slower growth rates (Aitken et al. 1995; Joly et al. 1989; White 1987), less shoot/root dry mass ratio (Hermann and Lavender 1968; Sorensen 1983), earlier bud set (Sorensen 1983; White 1987), and more resistance to xylem cavitation (Kavanagh et al. 1999) than genotypes from moist habitats.

Xylem cavitation occurs during drought when xylem water potential (Ψ_x) decreases enough to facilitate air entry into water-filled tracheids (Sperry and Tyree 1988, 1990). The air-filled (or embolized) tracheids may result in a reduced stem hydraulic conductance and stomatal conductance (Kavanagh and Zaerr 1997; Borghetti et al. 1989; Tyree and Sperry 1989), foliage or branch loss (Zimmermann 1983), or plant mortality (Tyree and Sperry 1988). Since tolerance to xylem cavitation is related to a variety of physiological mechanisms, which in turn affect the drought survival-ability of trees (Tyree and Evers 1991; Sperry 1995), it is a valuable trait to assess. In addition, the percentage of live needles remaining on seedlings grown under moisture stress has been used to measure drought tolerance and shown to be an effective proxy variable for survival (White 1987). Thus, although many traits respond to drought stress in this study, we investigated the effects of drought stress on cavitation induction, loss of xylem hydraulic conductivity or xylem specific conductivity, damage to needles and shoots, and reduction in stem growth. Henceforth, we refer to these

traits, measured under moisture stress, as drought hardiness traits.

The focus of previously mentioned studies was the response of Douglas-fir populations from contrasting geographic locations to drought stress. However, for tree improvement purposes, genetic variation within breeding populations, rather than among geographic regions, is of primary importance, and currently, little or no information on within-population variation of drought hardiness traits is available for coastal Douglas-fir. In addition, Douglas-fir breeding programs in the Pacific Northwest are in the process of expanding their existing small, numerous breeding zones into fewer, larger breeding zones (Johnson 1998), which ultimately means that improved genotypes will need to be adapted to a broader range of environmental conditions, including variable degrees of summer moisture availability. The efficient development of drought-hardy genotypes not only requires information on the extent of genetic variation in these traits but also on the strength of their inheritance (e.g., heritability) and their interrelationships with stem growth. To address these information needs, the Pacific Northwest Tree Improvement Research Cooperative subjected seedling families of coastal Douglas-fir to different moisture regimes. The overall goals of this project were to identify seedling characteristics associated with response to short-term moisture stress, evaluate the genetic control of these traits, and develop criteria for screening improved families for drought hardiness in breeding programs. More specifically, we address the following objectives in this paper: (i) evaluate the impact of summer moisture stress on seedling growth and drought hardiness of full-sib families from southwestern British Columbia; (ii) determine the genetic control of growth potential, drought hardiness, and recovery from drought injury in these seedlings; and (iii) assess genetic relationships between the above traits and evaluate the potential for improving seedling drought hardiness through tree breeding.

Materials and methods

Parent trees of the 39 full-sib families used in this study originated from southwestern British Columbia (Vancouver Island and the coastal mainland) and northwestern Washington. The British Columbia Ministry of Forests, in their 1978

breeding program, produced several six-parent half-diallel mating sets, with 15 full-sib families within each set (Woods 1993). From these half-diallels, seed was available in storage to create four smaller five-parent half-diallels, each with $5 \times 4/2 = 10$ full-sib families (i.e., total families = $10 \times 4 - 1$ family missing in half-diallel) = 39). The parent trees used in the crossing program were sampled from a range of soil moisture regimes (dry, moderate, and wet), covering a wide range of latitudes ($48^{\circ}05' - 51^{\circ}03'N$), longitudes ($121^{\circ}36' - 126^{\circ}33'W$), and elevations (30–720 m) (M. Stoehr, British Columbia Ministry of Forests, Victoria, B.C., personal communication). Therefore, the parent trees represent a broad breeding population and the full-sib families obtained by crossing these parents were expected to be variable in their response to drought stress conditions.

Germinated seeds from all families were planted into two custom-built, raised nursery beds ($20 \times 1.5 \times 1$ m), at Oregon State University ($44^{\circ}34'N$, $123^{\circ}12'W$; elevation 62 m). Each bed was placed on a surface of coarse gravel that was covered with weed-barrier cloth to prevent future root penetration and was filled with a forest (sandy loam) soil. Plastic barriers were placed between the walled sections of the beds to prevent movement of water between drought treatments. All seedlings in the first year were grown under well-watered conditions. 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. Within each main plot (1.5×2.5 m), each of the 39 families was represented by eight trees in two randomly located, four-tree family row plots with a seedling spacing of 8×8 cm. Experimental seedlings totaled 4680 at planting (5 blocks \times 3 treatments per block \times 39 full-sib families \times 2 row plots per family \times 4 seedlings per family plot). In addition, six auxiliary row plots of seedlings were located randomly in each nursery bed to be used for predawn xylem water potential assessments.

The total survival of seedlings at the onset of the second growing season was 88.3% of the original planting stock. About 10% of the mortality was due to hypocotyl rot disease (*Fusarium oxysporum*) that occurred several months after planting but was brought under control by decreasing the frequency of irrigation, applying fungicides (Captan, Banrot, and Benlate), and covering the nursery beds with 50% shade cloth for about 1 month. At the end of the second growing season, every other seedling was harvested, so that drought treatments in the third year were applied to only half of the original number of seedlings.

Drought treatments

Moisture regimes were imposed by controlled application of irrigation and by protecting the nursery beds from natural rainfall, largely by placing clear plastic covers on PVC hoops over the beds during each rainfall event. The plastic covers were removed when rain ceased. The hoops kept the covers well above the seedlings (0.5–1.5 m) so that the potential for sun-scald damage, heat stress, and interference with atmospheric gas exchange and incoming radiation was minimized. Soil moisture levels were assessed as predawn xylem water potential (Ψ_{pd}) of seedlings in the auxiliary rows by using a pressure chamber (Waring and Cleary 1967). Once a week over the course of the growing season

(May through September), three shoot samples were collected systematically from three of the six different auxiliary rows in each main plot and Ψ_{pd} was assessed. Timing and frequency of irrigation were based on the most recent mean Ψ_{pd} .

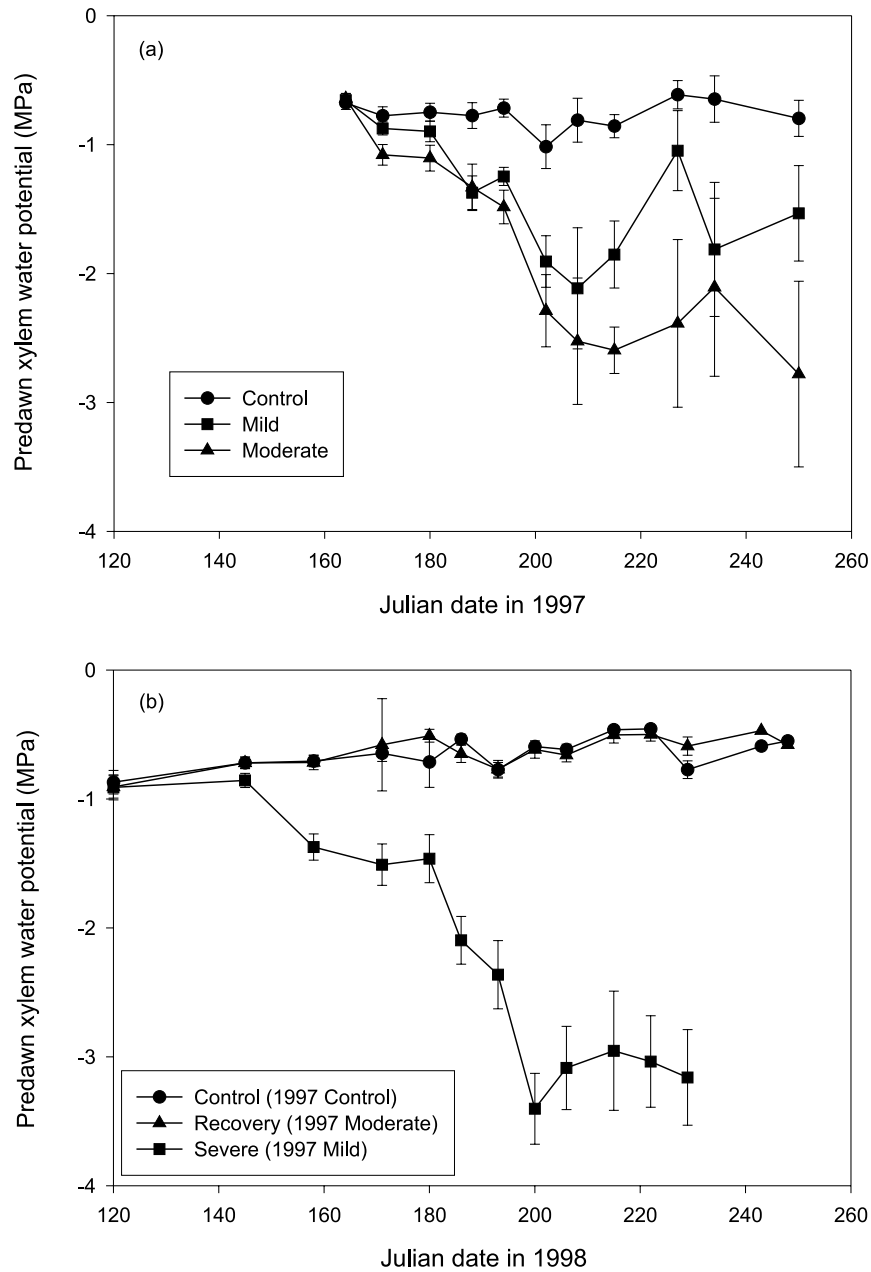
Previous seedling studies suggested that seedlings will respond significantly to drought, including extensive foliage damage and some mortality, if Ψ_{pd} is brought to and subsequently kept below -2.0 MPa during the active stem growth period (O'Neill 1999; White 1987). Therefore, in the second growing season, the three watering regimes were well watered or control ($\Psi_{pd} > -1$ MPa), mild drought ($\Psi_{pd} = -1$ to -2 MPa), and moderate drought ($\Psi_{pd} = -2$ to -3 MPa), applied from mid-June to early September (Fig. 1a). The impact of the drought treatments on drought-hardiness traits were less effective than desired during the second year, so an earlier and more severe drought was applied in the third growing season to main plots that had received mild drought the second year (Fig. 1b). In this case, $\Psi_{pd} = -3$ to -4 MPa were achieved by midsummer. The remaining main plots were well watered in the third growing season. Thus, the three treatments in the third year were control (well watered both years), recovery (moderate drought in year 2 followed by well watered in year 3), and severe drought (mild drought in year 2 followed by severe drought in year 3).

On average, to achieve moisture levels close to field capacity, the control and recovery treatments were watered once a week until midsummer and then were watered twice weekly. When water potential was at the target level for these treatments (i.e., $\Psi_{pd} > -1$ MPa), approximately 109 L was applied to each main plot; this amount was increased by 36–72 L per main plot when water potential fell below the target and reduced by a similar amount when above the target. Following the same procedure, the mild treatment was watered every other week (target $\Psi_{pd} = -1$ to -2 MPa). The moderate treatment was watered only once (~ 109 L per main plot) in early August, and the severe treatment was never watered during the weeks of drought treatment.

Measurement of traits

Table 1 provides a summary of all traits, number of seedlings measured in each treatment, and the type of statistical model (see the next section for details) used for analyzing each trait. Seedling height and diameter of the main stem were measured each year on all seedlings remaining in the study plots. Seedling height was measured five times during the second season, three times during the third season, and after all stem growth had ceased both years. Diameter was measured at the end of the second season and twice during the third season (midway and at the end). Control seedlings were grown under more or less ideal conditions; overall height and diameter at the end of the second and third growing seasons are referred in the rest of this paper as “growth potential” traits. Growth response of seedlings to drought and recovery from drought the previous year were assessed in the third growing season by calculating height and diameter growth increments in the severe drought and recovery treatments, respectively. Height increment was calculated as ((third-year height – second-year height)/second year height) \times 100. Diameter increment was calculated in a similar manner.

Fig. 1. Mean weekly predawn xylem water potential measurements under control, mild, and moderate drought treatments in 1997 (water withheld commencing on Julian date 162) and control, severe drought, and recovery treatments in 1998 (water withheld commencing on Julian date 120). Soil moisture availability decreases with increasing negative water potential. Error bars are the SEs of the treatment means at each measurement date. The last two weekly predawn measurements in the severe treatment were biased and are not shown in Fig. 1b because of high mortality in the auxiliary row plots.



Foliage damage was scored on seedlings in the drought treatments at the end of the growing season (in mid-September) but was not scored in the control or recovery treatments, where little or no damage was found. In the second year, entire foliage of the crown of each seedling was visually scored into five 20% damage classes on the basis of the intensity of yellowing and browning of needle and shoot tissues (dark green, 0–20% damage; yellowish-green, 21–40% damage; yellowish or light-green, 41–60% damage; yellowish to brown, 61–80% damage; brown to brittle,

81–100% damage) in response to moisture stress (Lomas 1999). Similarly, 10 classes were used in the third year.

For assessing xylem cavitation in the second year, we sampled every other seedling (two total) from one randomly selected row plot of each family within each main plot (Table 1). In the third year, cavitation was assessed in the severe drought treatment by randomly sampling one seedling remaining from each family row plot (39 families \times 10 trees = 390). Only a subset of 60 individuals was measured in the control and recovery treatments the third year, by randomly

Table 1. Summary of all traits, number of trees sampled in the moisture regimes applied in different years, and types of statistical models employed in this study.

Trait	No. of trees per treatment			Model ^a
	Treatment 1	Treatment 2	Treatment 3	
Year 1				
Drought treatment	Control	Control	Control	
Xylem predawn water potential (Ψ_{pd})	na ^b	na ^b	na ^b	—
Height	1382	1380	1373	—
Year 2				
Drought Treatment	Control	Mild	Moderate	
Xylem predawn water potential (Ψ_{pd})	15 ^c	15 ^c	15 ^c	—
Height	774 ^{d,e}	773 ^d	761 ^d	F + G
Diameter	775 ^{d,e}	774 ^d	762 ^d	F + G
Foliage damage	None	774	761 ^e	F + G
Ring 1 cavitation (R1)	348	348	348	MF
Ring 2 cavitation (R2)	348	348	348 ^e	MF + MG
Xylem hydraulic conductivity (K_h)	20	20	20	MF
Xylem specific conductivity (K_s)	20	20	20	MF
Year 3				
Drought treatment	Control	Severe	Recovery	
Xylem predawn water potential (Ψ_{pd})	15 ^c	15 ^c	15 ^c	—
Height	776 ^e	747	773	F + G
Diameter	776 ^e	748	772	F + G
Foliage damage	None	772 ^e	None	G
Xylem hydraulic conductivity (K_h)	60	390 ^e	60	MF + MG
Xylem specific conductivity (K_s)	60	390 ^e	60	MF + MG
Ring 1 cavitation (R1)	60	390	60	MF
Ring 2 cavitation (R2)	60	390	60	MF
Ring 3 cavitation (R3)	60	390 ^e	60	MF + MG
Year 2 and 3 comparisons				
Drought treatment comparison	Control–control	Mild–severe	Moderate–recovery	
Height increment	767	746 ^e	773 ^e	F + G
Diameter increment	769	747 ^e	772 ^e	F + G

Note: Detailed explanations of statistical models are presented in the Statistical analyses section.

^aFull model (F) is shown in eq. 2, in which main plots or drought treatments and all other components are included (see Statistical analyses section for details). The modified full (MF) model also includes the main plots, but some components of the eq. 2 are missing because of unequal number of samples between treatments or because of fewer seedlings sampled within a treatment, or because of a combination of both. The genetic (G) model is shown in eq. 3. In the modified genetic (MG) model, some components are further missing based on whether one or two seedlings per plot were sampled within a block.

^bNot assessed; well-watered conditions.

^cOn average, 15 trees per week were assessed for predawn xylem water potential.

^dThe number of trees left after thinning; trees were thinned after the end of second growing season.

^eThese trees were used for genetic analyses; results are given in Tables 2–4.

sampling 12 trees per block. Each tree was harvested at ground level, placed into a plastic bag containing moist towels, and immediately taken to an adjacent laboratory for analysis. Xylem cavitation was assessed by passing safranin stain (filtered to 0.2 μm) by gravitational flow through the length of a 5 cm long stem segment cut from the seedling base, followed by examining a cross section of the segment for proportion of area stained by the dye (i.e., cross-sectional area containing functioning, noncavitated tracheids) (Ewers 1985; Kavanagh 1993; Kavanagh and Zaerr 1997; LoGullo and Salleo 1991). The proportion of cross-sectional area that was stained was visually estimated into 10% classes for each

year's growth ring (R) (i.e., R1 (1996, closest to the pith) and R2 (1997) in year 2 and R1, R2, and R3 (1998) in year 3).

Xylem hydraulic conductivity (K_h) and xylem specific conductivity (K_s) were assessed in the second year on 20 random trees from each drought treatment and on the same 510 (390 + 60 + 60) trees measured for cavitation in the third year. Sample preparation for K_h assessment was similar to the procedure presented for the cavitation method; however, instead of safranin dye, a weak solution of oxalic acid in deionized water (1.26 g/L) was allowed to pass through the stem segments (Kavanagh et al. 1999; Sperry et al.

1993). The solution conducted through the stem was absorbed into laboratory tissue and weighed after 1 min of collection. Three or four such measurements were taken for each sample, and a mean flow was calculated. Xylem hydraulic conductivity (in $\text{mmol}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$) was then estimated using

$$[1] \quad K_h = F \left(\frac{\Delta L}{\Delta P} \right)$$

where F is the rate of flow of water ($\text{mmol}\cdot\text{s}^{-1}$), L is the length of stem segment (m), and P is the pressure (MPa). Xylem specific conductivity (K_s , $\text{mmol}\cdot\text{m}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$), a measure of stem porosity, was calculated by dividing K_h by stem cross-sectional area.

Statistical analyses

To evaluate the impact of drought treatments on seedling growth and drought hardiness traits (objective 1), treatment means were estimated using the MEANS statement of the general linear model (GLM) procedure, and differences among means were tested with Duncan's multiple range test (SAS Institute Inc. 1990). All tests of significance were carried out at the 0.05 probability level. The GLM procedure used the following linear model for a split-plot design:

$$[2] \quad Y_{ijklm} = \mu + m_i + b_j + mb_{ij} + f_k + fb_{jk} + fm_{ik} \\ + fbm_{ijk} + p_{ijkl} + w_{ijklm}$$

where Y_{ijklm} is the individual seedling value; μ is the overall mean; m_i is the fixed effect of the i th drought treatment; b_j is the random effect of the j th block; mb_{ij} is the random interaction effect of drought treatment with block; f_k is the random effect of the k th family; fb_{jk} is the random interaction effect of family with block; fm_{ik} is the random interaction effect of family with drought treatment; fbm_{ijk} is the random interaction effect of family with block and drought treatment; p_{ijkl} is the within-block, among-family plot error; and w_{ijklm} is individuals within-family plot error. This model was applied to analyze the height and diameter of seedlings in both years, their increments in year 3, as well as the second-year foliage damage in the mild and moderate drought treatments (Table 1, full model (F)). However, it was necessary to modify the model (MF) for the remaining traits according to the components included. The plot component (p_{ijkl}) was dropped from year-2 and -3 cavitation traits and year-3 xylem hydraulic conductivity and xylem specific conductivity. The block (b_j) and the family (f_k) components were dropped from the model for year-2 xylem hydraulic conductivity and xylem specific conductivity, because only 20 trees were randomly sampled across blocks from each treatment. There was considerable imbalance in the sample sizes among treatments for year-3 cavitation, xylem hydraulic conductivity, and specific conductivity, as we sampled all 390 trees in the severe drought but only 60 trees each in the control and recovery treatments (Table 1).

To evaluate the nature and extent of genetic variation in growth potential, drought hardiness, and recovery traits (objective 2), eq. 2 was modified in two ways: (i) the drought treatment (m) component was removed from the model, because only one treatment was examined at a time, and (ii) the effect of diallel sets (d) was added to the model.

Therefore, in addition to the other components, diallel (d_i) and family within-diallel (f_{ik}) components were estimated using the following linear model (model G):

$$[3] \quad Y_{ijklm} = \mu + d_i + b_j + f_{ik} + db_{ij} + fb_{jk} + p_{ijkl} \\ + w_{ijklm}$$

where d_i is the random effect of i th diallel, $E(d_i) = 0$, $\text{var}(d_i) = \sigma_{d_i}^2$; f_{ik} is the random effect of k th family within the i th diallel, $E(f_{ik}) = 0$, $\text{var}(f_{ik}) = \sigma_{f(d)}^2$; db_{ij} is the random interaction effect of the i th diallel with the j th block, $E(db_{ij}) = 0$, $\text{var}(db_{ij}) = \sigma_{db}^2$; and fb_{jk} is the random interaction effect of the k th family with the j th block, $E(fb_{jk}) = 0$, $\text{var}(fb_{jk}) = \sigma_{bf(d)}^2$.

The interaction of block with diallel (db_{ij}) was excluded in the final analyses, because it was nonsignificant in all cases, and the sums of squares associated with this component were pooled with the interaction of family with block (fb_{jk}) component. The genetic model was further modified for cavitation, xylem hydraulic conductivity, and xylem specific conductivity traits (Table 1, model MG). Variance components of the modified linear model were estimated using the restricted maximum likelihood (REML) method of the SAS VARCOMP procedure (SAS Institute Inc. 1990). Mean squares were also estimated from the corresponding GLM procedure to perform F tests of various variance components (see below). Because type I and type III sums of squares were nearly identical, all tests were based on the type I estimates.

In preliminary analyses, residuals for all traits, except foliage damage and R2 in year 2, and R3, K_h and K_s in year 3, were normally distributed. A square-root transformation of K_h and K_s was applied before final analysis of these variables, but no transformation (square root, log, arcsine, or other variations) successfully normalized the distributions of R2, R3, or second-year foliage damage, so analyses on these traits were performed on nontransformed data. When the normality assumption is violated, true levels of significance are usually, but not always, slightly greater than the assumed significance. For example, family variances that were declared significant at the 5% level may actually be significant only at 7–8% level of probability (Steel and Torrie 1980). All traits were standardized (e.g., mean = 0 and standard deviation = 10) prior to estimation of variance component analyses to minimize scale effects.

Both general combining ability (GCA) and specific combining ability (SCA) effects contribute to family variation within diallel sets (Griffing 1956). GCA measures the mean effect of each parent involved in a cross, whereas SCA quantifies the deviation of the mean of a specific cross from the mean GCA of the two parents. The main difficulty with diallel mating designs is apportioning the family into GCA and SCA variance components. In the past, estimation of GCA (σ_{GCA}^2) and SCA (σ_{SCA}^2) variances has been facilitated by special computer programs, such as DIALL (Schaffer and Usanis 1969). These programs, however, lack the ability to handle large data sets (e.g., high numbers of replications or sites). Recently, Johnson and King (1998) proposed a relatively simple method of estimating σ_{GCA}^2 and σ_{SCA}^2 using two variance component analyses (VARCOMP 1 and 2; SAS Institute Inc. 1990).

In the VARCOMP 1 analysis of each trait in this study, a REML estimate of the family-within-diallel variance ($\sigma_{f(d)}^2$) is equal to one-third of the additive variance (σ_A^2) + one-quarter of the dominance variance (σ_D^2) (Johnson and King 1998). To separately estimate σ_A^2 and σ_D^2 , a second VARCOMP procedure (VARCOMP 2) is required. In VARCOMP 2, dummy variables are used to account for the GCA effects of the parents in the diallels, such that $\sigma_{f(d)}^2$ in VARCOMP 2 equals $0.25\sigma_D^2$. Additive variance is then calculated as $\sigma_A^2 = 3 \times ((\sigma_{f(d)}^2 \text{ in VARCOMP 1}) - (\sigma_{f(d)}^2 \text{ in VARCOMP 2}))$ (Johnson and King 1998); $\sigma_{GCA}^2 = 0.25\sigma_A^2$ and $\sigma_{SCA}^2 = 0.25\sigma_D^2$.

An F statistic to test the significance of GCA variance was calculated as the ratio of mean squares for GCA to mean squares for SCA estimated from the appropriate Type I sums of squares in the corresponding GLM procedures of the VARCOMP analyses (Griffing 1956). The significance of the F statistic was based on 16 numerator degrees of freedom ($d(n-1)$) and 19 denominator degrees of freedom ($d(n(n-3)/2) - 1$ (missing family in one of the diallel sets)), where d is the number of diallels and n is the number of parents per diallel set. The significance of SCA variance was tested by dividing the mean squares for families within diallels by the mean square for family \times block interaction, both from GLM 2 (Griffing 1956).

Following the analytical procedures described by Johnson and King (1998) and equations provided by King et al. (1998), the individual tree, narrow sense heritability of each trait was estimated as follows:

$$[4] \quad h_i^2 = \frac{\sigma_A^2}{[\sigma_d^2 + (2 \times \sigma_{GCA}^2) + \sigma_{SCA}^2 + \sigma_{bf(d)}^2 + \sigma_p^2 + \sigma_w^2]}$$

where σ_p^2 is the plot error and σ_w^2 is the individual tree (within-plot) error variance. This estimate of heritability is appropriate to making selections of individuals regardless of the diallel of origin because the variance associated with the diallel set is included in the denominator (phenotypic variance).

The narrow-sense family heritability (h_f^2) of each trait was estimated as (Falconer and Mackay 1996):

$$[5] \quad h_f^2 = \frac{1 + (n-1)r}{1 + (n-1)t} h_i^2$$

where n is the harmonic number of individuals in the families (9.62 for R2, R3, K_h , and K_s and 19.77 for the remaining nine traits), r is the coefficient of relatedness for full-sib families in a five-parent half-diallel set (equals one-third; Johnson and King 1998), and t is the intraclass correlation of phenotypic values of members within the families ($t = \sigma_{f(d)}^2 / (\sigma_d^2 + \sigma_{f(d)}^2 + \sigma_{bf(d)}^2 + \sigma_p^2 + \sigma_w^2)$). Standard errors of individual and family mean heritabilities were estimated following Dickerson (1969) and Becker (1992), respectively.

The extent to which pairs of traits are under the same genetic control was estimated by family mean and genetic correlations (the third objective). Family mean correlations were estimated using the PROC CORR procedure in SAS (SAS Institute Inc. 1990). Type A genetic correlations are used when two traits are measured on the same tree, and

type B genetic correlations are for cases when different traits are measured on different trees.

Type A genetic correlations (r_A) were calculated for drought hardiness traits measured on the same seedling in the same year using (Falconer and Mackay 1996):

$$[6] \quad r_A = \frac{\text{Cov}_{1,2}}{\sqrt{\sigma_1^2 \times \sigma_2^2}}$$

where $\text{Cov}_{1,2}$ is the estimated family within-diallel covariance between traits 1 and 2, and σ_1^2 and σ_2^2 are the estimated family within-diallel variances of the respective traits derived from VARCOMP 1. Following Johnson and King's (1998) procedure, σ_1^2 and σ_2^2 were estimated for the two traits and for the sum of two traits (σ_{1+2}^2). Then, $\text{Cov}_{1,2}$ was estimated as

$$[7] \quad \text{Cov}_{1,2} = \frac{\sigma_{1+2}^2 - \sigma_1^2 - \sigma_2^2}{2}$$

To evaluate genetic associations between growth potential traits in the control treatment and drought hardiness traits in either the moderate or severe drought treatments, or between second-year drought hardiness traits and third-year recovery traits, type B genetic correlations (r_B) were estimated (Burdon 1977):

$$[8] \quad r_B = \frac{\text{Cov}_{\bar{F}1,2}}{\sqrt{\sigma_1^2 \times \sigma_2^2}}$$

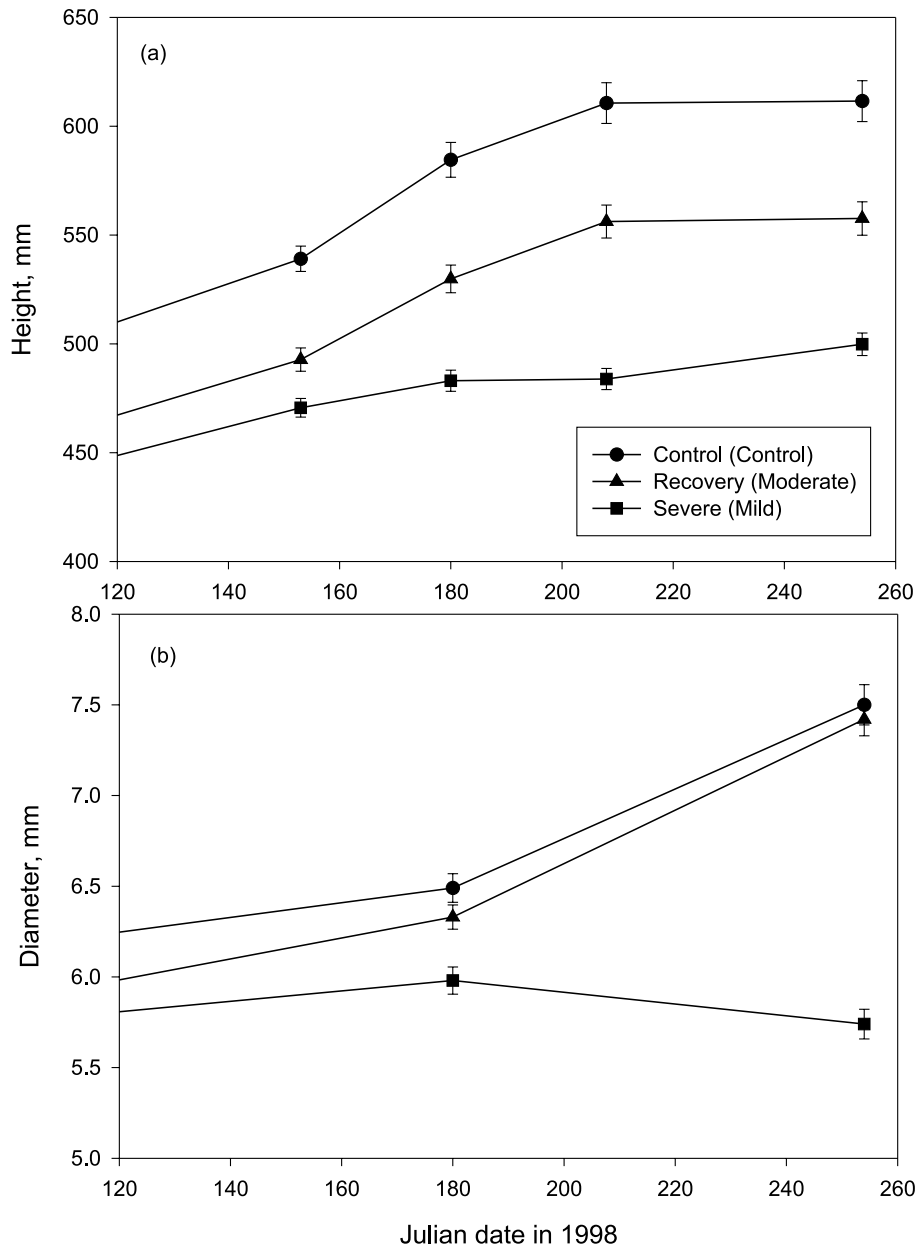
where $\text{Cov}_{\bar{F}1,2}$ is the family mean within-diallel covariance between the traits from VARCOMP 1 for the respective traits. In this study, both type A and type B correlations represent total genetic correlations between traits rather than additive genetic correlations, because the family-within-diallel variance component in VARCOMP 1 is a combination of both additive and nonadditive variances.

Results

Effects of drought treatments on foliage damage, stem growth, cavitation, and xylem hydraulic conductivity

Drought treatments had an almost immediate impact on soil water availability, as evidenced by the predawn xylem water potential (Ψ_{pd}) readings in the treatments (Figs. 1a and 1b). Ψ_{pd} in the drought treatments was always lower than in the control, with the following means taken after mid-July (i.e., after Julian date 200): -1.56 MPa in the mild treatment (year 2), -2.47 MPa in the moderate treatment (year 2), -3.13 MPa in the severe treatment (year 3), and -0.65 MPa in the control treatments (years 2 and 3). In the recovery treatment, Ψ_{pd} was comparable with the control values (Fig. 1b). By mid-July, the severe drought treatment applied during the third growing season had a strong influence on Ψ_{pd} , although the intensity of drought during the first 60 days of the period was mild ($\Psi_{pd} > -1.5$ MPa), reached a moderate level ($\Psi_{pd} = -1.5$ to -3 MPa) rapidly in the next 20 days, and then stayed constant and severe ($\Psi_{pd} < -3$ MPa) for the remainder of the treatment period (Fig. 1b). Relative to no visible damage in the control (both years), severe drought applied during the third growing season caused greater foliage injury (33%) than did the mild (12%) or

Fig. 2. Third-year increment height (*a*) and diameter (*b*) growth of seedlings under control, recovery, and severe drought treatments. The corresponding second-year treatments are shown in parentheses. Error bars are the SEs of the treatment means at each measurement date. The symbols on Julian date 120 are not shown, because the lines connecting symbols simply extend back to the last measurements of the previous season.

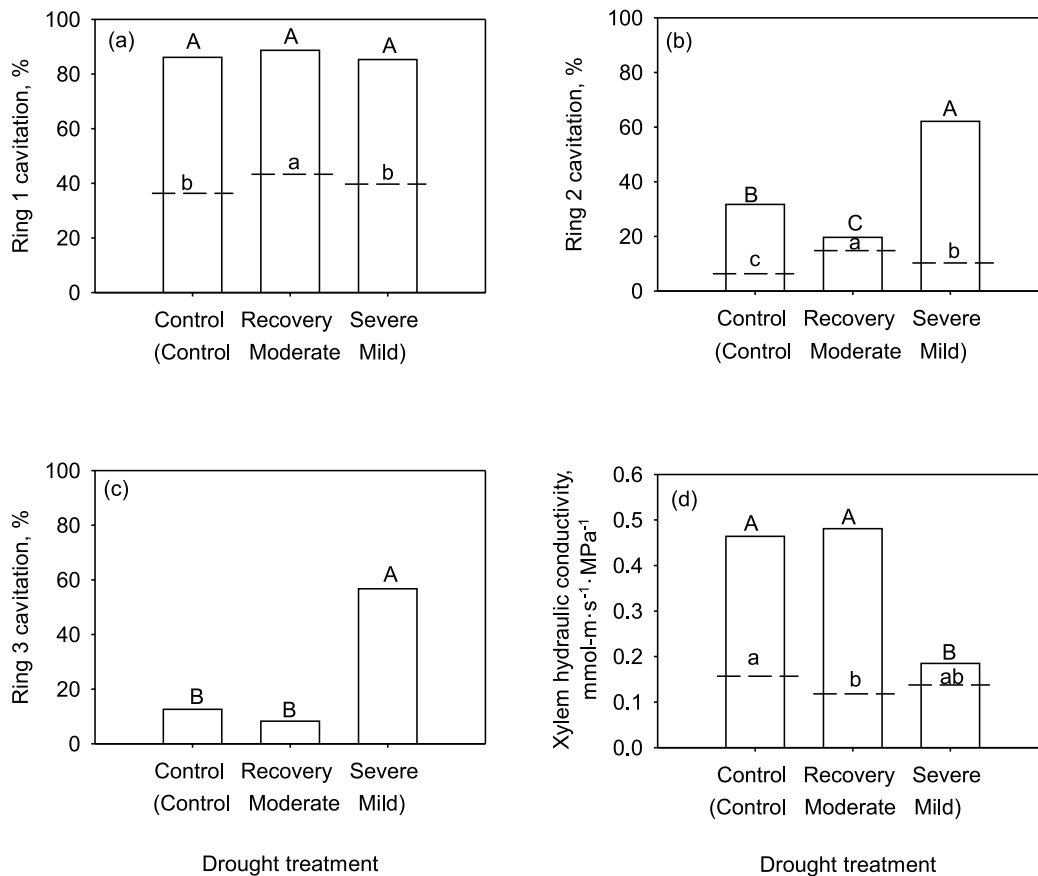


moderate (17%) drought treatments in the second growing season. The severe drought significantly ($p < 0.05$) reduced total height (Fig. 2a) and diameter (Fig. 2b) of the seedlings relative to the control by a mean of 27%. In year 2, the mild and moderate drought treatments reduced growth only by 6.2 and 10.8%, respectively, which indicates that the imposed drought stress in the third year was more effective than the either drought treatment the second year. After a well-watered third growing season, seedlings that received moderate drought in year 2 (recovery treatment in year 3) had nearly the same diameter as the control trees, which were well watered both seasons (Fig. 2b). The mean height

of the seedlings in the recovery treatment, however, was significantly less than that of the control seedlings (Fig. 2a).

For all three treatments in year 3, total height growth was essentially completed by Julian date 210. The diameter of seedlings in the control and recovery treatments, in contrast, continued to increase well beyond mid-September, while total diameter shrunk slightly in the severe treatment, presumably because of loss of water in the stem tissues (Fig. 2b). Because much of the growth increment occurred during the periods of drought treatments, we used height and diameter increments in drought and recovery treatments as measures of growth responses to drought or recovery from drought.

Fig. 3. The bars are the mean xylem cavitation and hydraulic conductivity of Douglas-fir seedlings in the third growing season under control, recovery, and severe drought treatments. The corresponding second-year treatments are shown in parentheses. The broken lines are the mean percent cavitation observed in rings 1 and 2, and mean xylem hydraulic conductivity of the stem segments at the end of the second growing season. Different letters over bars (uppercase, year 3) or over broken lines (lowercase, year 2) show significant differences among treatment means ($p < 0.05$).



Mean height and diameter increment responses to mild or moderate droughts in year 2, however, were not significantly different from mean increments in the control treatment. Therefore, year 2 growth increments were not included in this study.

Cavitation showed some interesting patterns among growth rings. The severe drought in year 3 had variable effects on the magnitude of cavitation in the three annual growth rings with 80, 60, and 57% mean cavitation in rings 1, 2, and 3, respectively (Figs. 3a, 3b, and 3c). By the end of the third growing season, nearly the entire first ring was cavitated in all treatments, with mean cavitation in ring 1 double that observed in the previous year (compare the heights of bars with the heights of the broken lines in Fig. 3a). In the second growth ring, percentage cavitation in the control and severe drought treatments, but not in the recovery treatment, increased substantially in relation to the previous year with mean cavitation greatest in the severe drought treatment. Cavitation in the second- and third-year growth rings was significantly ($p < 0.05$) greater for seedlings under severe drought than for seedlings in either the control or recovery treatments. There was no evidence of refilling of previously cavitated tracheids. Even with ample rainfall and watering in fall, winter, and spring, the level of

cavitation increased in all growth rings and all treatments from growing season two to three.

Mean xylem hydraulic conductivity (K_h) of seedlings in the moderate drought in year 2 (recovery in year 3) was significantly ($p < 0.05$) less than the conductivity of seedlings in the control treatment (compare the heights of the broken lines in Fig. 3d). The K_h in the severe drought treatment was only about one-third that in the control and recovery treatments in year 3 (compare the bars in Fig. 3d). The results also show that xylem specific conductivity (K_s) in the control and recovery treatments was much higher and differed significantly ($p < 0.05$) from that in the severe drought treatment in year 3. This finding suggests that severe drought considerably reduced the water movement up the stem and that this reduction is not simply due to decreased stem diameter size but also due to greater xylem cavitation in the severe treatment (Figs. 3b and 3c).

Genetic variation and control of drought hardiness traits

In preliminary analyses, although families differed significantly ($p < 0.001$) for ring 1 percent cavitation (R1) in all treatments in year 2, the frequency distributions of R1 among seedlings were similar across the moisture treatments

Table 2. Estimated overall means, ranges among family means, and coefficients of family variation; individual (h_i^2) and family (h_f^2) heritabilities; and, relative proportions of GCA and SCA variance components for traits related to growth potential under well-watered conditions (control treatments in years 2 and 3), drought hardiness (years 2 and 3), and recovery from drought (year 3 only).

Year	Traits	Mean	Range	CV ^a	$P > F_{f(d)}$ ^b	h_i^2 ^c	h_f^2 ^c	σ_{GCA}^2 ^d	σ_{SCA}^2 ^d
Growth potential									
2	Height, mm	354.00	299–438	9	0.0001	0.26	0.49	53*	47*
2	Diameter, mm	5.50	4.60–6.70	8	0.0001	0.07	0.16	16+	84*
3	Height, mm	611.00	514–729	10	0.0001	0.39	0.61	65*	35*
3	Diameter, mm	7.49	6.03–8.93	9	0.0001	0.17	0.42	48*	52*
Drought hardiness									
2	Ring 2 cavitation, %	18.30	5.0–60.6	64	0.0002	0.46	0.72	100*	0
2	Foliage damage, %	17.30	10.0–34.0	34	0.0372	0.12	0.49	100*	0
3	Ring 3 cavitation, %	57.00	28.0–92.0	26	0.1010	0.13	0.36	100*	0
3	Xylem hydraulic conductivity, mmol·m·s ⁻¹ ·MPa ⁻¹	0.185	0.034–0.542	62	0.0074	0.25	0.56	100*	0
3	Foliage damage, %	32.00	13.5–60.0	32	0.0299	0.12	0.48	100*	0
3	Height increment, %	46.8	34.4–53.9	11	0.1941	0.04	0.25	100	0
3	Diameter increment, %	8.53	0.28–16.7	53	0.0101	0.09	0.34	61*	39
Recovery									
3	Height increment, %	68.20	55.1–85.6	12	0.0062	0.08	0.29	45	55*
3	Diameter increment, %	49.20	37.1–68.8	15	0.0020	0.01	0.04	6	94†

^aCV is the standard deviation of family means expressed as a percentage of the treatment mean.

^bSignificance of difference among families within diallels from the VARCOMP 1 procedure.

^cEstimated standard errors of h_i^2 and h_f^2 averaged 0.08 (range 0.02–0.14) and 0.02 (range 0.01–0.08), respectively.

^d σ_{GCA}^2 and σ_{SCA}^2 are presented as the percentage of the total genetic (GCA + SCA) variation with their statistical significance (†, $p < 0.10$; *, $p < 0.05$).

(control, mild, moderate), and estimated family mean correlations among treatments were moderately strong (mean $r_f = 0.61$, $p < 0.01$), suggesting that cavitation in this ring may be caused by conditions external to the imposition of drought in the second growing season (Lomas 1999). The frequency distributions of cavitation in ring 2, in contrast, were progressively skewed toward higher levels of cavitation with increasing drought. In addition, families differed significantly ($p < 0.05$) for R2 only in the moderate drought treatment, and mean family mean correlations between treatments were low (mean $r_f = |0.17|$). Thus, for the purpose of assessing genetic response to drought, it is best to use percent cavitation in the growth ring formed during the drought period (i.e., R2 in year 2) (Lomas 1999). Similarly, ring 3 from the third year showed predominantly drought-induced response. Therefore, R2 assessed under moderate drought and R3 assessed under the severe drought were subjected to genetic analyses (Table 1).

In this study, K_s and K_h measured on the same seedling in the severe drought treatment had an estimated genetic correlation of unity suggesting that these traits are under the control of the same set of genes and should be equally effective as drought hardiness measures. In addition, the genetic control of K_h is nearly twice as strong as K_s . Perhaps estimates of K_s are more sensitive to small errors associated with seedling diameter measurements. Thus, for the purpose of further presentation and discussion, we used the easily measurable and relatively more accurate K_h .

In both the second and third year, family means differed significantly in all measures of growth potential, five of the seven drought hardiness traits, and in both recovery traits (Table 2). 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 (Table 2). Thus, there is considerable genetic variation for drought hardiness among the 39 families in this study.

Although family differences were not significant for R3 and height increments in year 3, general combining ability among families was significant in R3 ($p < 0.05$). Estimated individual-tree heritabilities for drought hardiness traits were generally low but comparable in magnitude to heritabilities for growth potential (Table 2). Estimated family heritabilities for drought hardiness (mean $h_f^2 = 0.46$) were similar in magnitude to those for growth potential (mean $h_f^2 = 0.42$), and two to three times greater than h_i^2 . Individual-tree and family heritabilities for height and diameter increment in the recovery treatment were low (≤ 0.29).

Relative proportion of total genetic variance due to GCA and SCA effects averaged 46 and 54%, respectively, for the growth potential traits, 94 and 6% for the drought hardiness traits, respectively, and 26 and 74% for the recovery traits, respectively (Table 2).

Correlations between traits

Genetic correlations were used to assess genetic interrelationships between traits measured on the same tree (r_A) and to evaluate consistencies of family ranks for traits measured on different trees (r_B) across the moisture regimes.

Except for correlation estimates associated with height increment, family mean correlations (mean $r_f = |0.75|$) and genetic correlations (mean $r_A = |1.00|$) were strong between drought hardiness traits measured on the same seedling and are in the expected direction (Table 3). For example, ring 3 cavitation is positively correlated with foliage damage and negatively correlated with xylem hydraulic conductivity (greater the cavitation, lower the ability to conduct water up the stem and more damage to foliage). Height increment was

Table 3. Estimated family mean correlations (r_f) and type A genetic correlations (r_A) between drought hardiness traits measured on the same seedling growing under moderate (year 2) and severe (year 3) drought treatments.

Year	Paired traits	r_f^a	r_A
2	Ring 2 cavitation and foliage damage	0.71*	1.00 ^b
3	Ring 3 cavitation and xylem hydraulic conductivity	-0.86*	-0.96
3	Ring 3 cavitation and foliage damage	0.85*	1.00 ^b
3	Ring 3 cavitation and height increment	-0.43*	-0.29
3	Ring 3 cavitation and diameter increment	-0.69*	-1.00
3	Xylem hydraulic conductivity and foliage damage	-0.79*	-0.84
3	Xylem hydraulic conductivity and height increment	0.19	0.22
3	Xylem hydraulic conductivity and diameter increment	0.68*	0.98
3	Foliage damage and height increment	-0.33*	-0.34
3	Foliage damage and diameter increment	-0.69*	-1.00 ^b
3	Height increment and diameter increment	0.06	0.15

^aFamily mean correlations with asterisks are significantly ($p < 0.05$) different from zero.

^bGenetic correlation estimate exceeded 1.00.

poorly correlated with diameter increment and xylem hydraulic conductivity, because much of the height increment in the severe drought treatment occurred prior to the period of the most intense moisture stress.

Both cavitation and foliage damage were measured in the moderate (year 2) and severe (year 3) drought treatments. Estimated family mean and genetic correlations for the same trait in the two years were positive but low (cavitation $r_f = 0.33$ and $r_B = 0.54$; foliage damage $r_f = 0.15$ and $r_B = 0.44$). Similarly, estimated family mean and genetic correlations between drought hardiness and growth potential traits were also generally quite low (mean $r_f = |0.11|$; mean $r_B = |0.34|$; Table 4).

Although mean foliage damage and cavitation levels were low (17 and 18%, respectively) after the moderate drought applied in year 2 (Table 2), the families most affected by the drought recovered the least in terms of growth the following year. Estimated correlations between drought hardiness traits at age 2 (i.e., ring 2 cavitation and foliage damage) and height and diameter increment of seedlings at age 3 in the recovery treatment were both negative and relatively strong (mean $r_f = -0.45$, mean $r_B = -0.84$).

Discussion

Impact of summer moisture stress on seedling growth and stem hydraulics

Our data show that the severe drought treatment dramatically increased xylem cavitation and reduced xylem hydraulic

Table 4. Estimated family mean correlations (r_f) and type B genetic correlations (r_B) between drought hardiness and growth potential traits (height and diameter in the control treatments) measured on different seedlings.

Year	Drought hardiness	Growth potential	r_f	r_B
2	Ring 2 cavitation	Height	0.06	-0.17
2	Foliage damage	Height	0.03	-0.10
2	Ring 2 cavitation	Diameter	-0.02	0.01
2	Foliage damage	Diameter	0.07	0.26
3	Ring 3 cavitation	Height	-0.03	-0.47
3	Xylem hydraulic conductivity	Height	0.18	0.36
3	Foliage damage	Height	-0.08	-0.27
3	Ring 3 cavitation	Diameter	-0.14	-0.68
3	Xylem hydraulic conductivity	Diameter	0.35*	0.67
3	Foliage damage	Diameter	-0.16	-0.37

Note: Family mean correlation with an asterisk is significantly ($p < 0.05$) different from zero.

conductivity of seedlings (Figs. 3a–3d), which is consistent with the findings of previous studies (Hargrove et al. 1994; Jackson et al. 1995; Jarbeau et al. 1994; Pena and Grace 1986; Pockman et al. 1995; Salleo and LoGullo 1986; Tyree and Dixon 1986; Tyree and Sperry 1988, 1989). Although the general impact of drought on stem hydraulic characteristics is well known, specific knowledge about the induction of cavitation in individual growth rings is limited (Sperry and Tyree 1988; Sperry et al. 1991). We report several significant findings related to the impact of drought on the occurrence of cavitation in the xylem tracheids of different ages in coastal Douglas-fir.

The large amount of cavitation in the first annual growth ring, even in the control seedlings, was unexpected. There are three possible explanations for the high amount of cavitation in this growth ring: (i) the pith at the seedling stage has a spongy texture and is full of large intercellular air spaces (Taiz and Zeiger 1998) that may have been largely responsible for the nucleation of air bubbles within tracheids of the adjacent, first annual growth ring; (ii) the presence of hypocotyl rot disease in the first growing season may have created a confounding effect on the degree of xylem cavitation in the first annual growth ring, as fungal disease is known to cause cavitation, preventing water uptake by the plant, regardless of soil moisture (Joseph et al. 1998); and (iii) overwinter freezing between the first and second seasons (winter 1996–1997) may have induced cavitation by freezing the xylem sap and forcing air out of solution, which would nucleate cavitation in the subsequent thaw (Ewers 1985; Sperry et al. 1994; Tyree and Sperry 1990). The winter of 1996–1997, however, was generally mild suggesting that winter freezing contributed little to cavitation in the first growth ring.

Subsequent cavitation in the second annual growth ring under the strong negative xylem tension could be linked to the availability of air emboli within the adjacent xylem tracheids of the first annual growth ring. This trend in air seeding, from the pith outward across the growth rings, appears to have occurred in the seedlings of this study. Because cavi-

tation increased from the second to the third year in both rings 1 and 2 of control seedlings, in spite of well-watered conditions, the hypothesis that cavitation occurs because of intrinsic (characteristics of the pith) or environmental (hypocotyl rot, winter freeze, etc.) factors is supported, although it was not tested explicitly in this study. This increase in cavitation is also notable in that there was no evidence of recovery or refilling of cavitated tracheids on a seasonal basis. Following a prolonged period of high soil moisture and low vapor pressure deficit (i.e., fall, winter, and spring), there was a consistent increase in the level of cavitation in ring 1 and 2 from year 2 to 3 for all treatments (Figs. 3a–3c). However, since we only sampled for cavitation at the end of the growing season it is possible the tracheids refilled over the winter and recavitated during the summer. Several studies have reported refilling of cavitated conifer xylem in situ (Panek and Waring 1995; Zwienecki and Holbrook 1998) and on detached branch segments (Sobrado et al. 1992; Borghetti et al. 1991, 1998). While the level of drought stress in the severe drought treatment may have exceeded the xylem tensions required to reinitiate cavitation in Douglas-fir seedlings (Kavanagh et al. 1999), in the well-watered control and recovery treatments the level of xylem water potential would not have been expected to exceed the level causing cavitation. To be certain no refilling had occurred, this experiment would have to be repeated and samples assessed for percent cavitation in spring before the onset of water stress.

It is notable that mean year 3 cavitation in ring 2 was significantly lower in the recovery than in the control treatment (Fig. 3b), despite the fact that seedlings were well watered in both treatments. While mean percent cavitation in the second annual growth ring of the recovery seedlings increased from 18 to 20% during the third growing season, it increased from 5 to 30% in the control treatment. Perhaps the moderate drought in year 2 preconditioned the xylem tracheids produced in that year (e.g., by producing smaller cells or thicker cell walls) so that they were less susceptible to non-drought-induced cavitation the following recovery year. Consistent with our findings, previous studies have shown that thick-walled tracheids with small lumens in the latewood, formed under moisture stress during late summer, are less vulnerable to cavitation than are earlywood, which consists of large, thin-walled tracheids (LoGullo and Salleo 1991; Zimmermann 1983). A recent investigation of several deciduous species, however, demonstrated that some *Populus* species suffer from "cavitation fatigue", and following a cycle of cavitation and refilling, they exhibited a sharp increase in vulnerability to cavitation. (Hacke et al. 2001).

Although we did not investigate the cause for progressively increased cavitation as xylem tracheids age (in ring 1 and ring 2 of all treatments), a previous study in *Populus tremuloides* Michx. suggests that the breakdown of middle lamella between cellulose fibrils by pectinases may be responsible for increased permeability of the pit membranes in aged xylem vessels (Sperry et al. 1991). It is not certain if these same processes occur in conifer tracheids. Similarly, the role of oxalic acid secretion has been implicated in causing massive cavitation and permeability of ageing xylem in other tree species (Sperry and Tyree 1988, 1990).

In both the moderate and severe drought treatments, cavitation was greatest in the ring produced in the growing season when drought was applied. Thus, cavitation in the current season's ring appears to be the most useful for measuring hardness to moisture stress.

Reduction of xylem hydraulic conductivity in droughted seedlings may be attributed to four causes: (i) loss of xylem function due to cavitation; (ii) reduced stem diameter (Kavanagh et al. 1999); (iii) deposition of solutes (Kozłowski and Pallardy 1997); and (iv) a change in the ratio of earlywood to latewood. Under drought conditions, newly formed cells are predominately latewood cells, creating a change in the ratio of earlywood to latewood (Emmingham 1977). Although any or all of the four causes can reduce xylem hydraulic conductivity (Sperry et al. 1996), the high negative correlation (i.e., $r_f = -0.86$; Table 3) between cavitation in the current season's ring and hydraulic conductivity indicates that the occurrence of xylem cavitation is a major cause of limiting water flow through the stem.

Genetic control of growth potential, drought hardness, and drought recovery traits

Drought hardness traits had narrow-sense heritabilities roughly equivalent to those of growth potential traits, being fairly weak (mean h^2) at the individual tree level but moderate at the family level (Table 2). Thus, drought hardness should be readily amenable to improvement by selection and breeding, especially when family selection is employed. Traits measuring growth (height and diameter increments) recovery in the year following drought, however, were only weakly inherited and, thus, may not respond well to selection.

The proportion of total variance due to SCA effects was large for growth potential traits in the control treatments and growth increment traits in the recovery treatment (Table 2), suggesting that dominance effects are important for these traits. Dominance effects include nonadditive gene action, as well as nongenetic and maternal effects (Falconer and Mackay 1996). Although the number of families tested in this study was limited, genetic parameters were estimated with small standard errors (<0.08). The high amount of SCA variance observed in the growth potential and recovery traits indicates that much of the genetic variation in these traits will remain unexploited in breeding programs that rely entirely on additive variance. Yeh and Heaman (1987) found that the σ_{GCA}^2 of height in 7-year-old Douglas-fir trees was six times greater than σ_{SCA}^2 based on field progeny tests in British Columbia. Similarly, Yanchuk (1996) reported that σ_{GCA}^2 for height was three times greater than σ_{SCA}^2 in 7- to 12-year-old Douglas-fir trees. In a series of studies conducted in the western Washington and Oregon timberlands of the Weyerhaeuser Company, σ_{GCA}^2 for height in 8- to 12-year-old coastal Douglas-fir trees was two times greater than σ_{SCA}^2 (Stonecypher et al. 1996). A possible explanation is that σ_{SCA}^2 for growth traits declines with age, as has been observed in *Pinus radiata* Donn. ex D. Don for both height and diameter from age 2 to 7 years (King et al. 1998).

Drought hardness traits were mainly under the control of additive effects (i.e., most of genetic variation was due to GCA; Table 2), suggesting that drought hardness in

Douglas-fir seedlings can be readily improved using conventional selection and breeding methods. Because much of the mortality in reforestation efforts occurs in the first few years after planting (Blake 1983; Rietveld 1989), and because early mortality is associated mainly with transplant shock induced by moisture stress (Smith and Walters 1963; Haase and Rose 1993; Kavanagh and Zaerr 1997), Douglas-fir seedlings with genetic improvement in their drought hardiness may increase the success of reforestation programs for this species. However, this study did not include transplant shock-related moisture-stress factors. We also don't know if the effect of transplant shock induced drought is similar to artificially applied moisture stress. A well-designed experiment could address this issue fairly easily.

Genetic correlations between traits and their implications for screening Douglas-fir seedlings for drought hardiness

Strong family mean correlations and genetic correlations between different drought hardiness traits measured in the same year (Table 3) suggests that these traits are largely controlled by the same set of genes, consistent with findings in a previous study (Joly et al. 1989). Strong genetic correlations between drought hardiness traits have a practical relevance to tree breeding programs. For example, improvement of one drought hardiness trait should improve other traits simultaneously without extra cost or effort. Deciding which trait to use in screening seedlings for drought hardiness depends on the cost of assessment of a given trait, the magnitude of its heritability, and the degree to which the trait predicts drought hardiness in actual field conditions. Foliage damage and diameter increment under drought are both relatively easy to assess. The heritability of foliage damage appears to be higher than that for diameter increment (Table 2). In addition, only one measurement is required to assess foliage damage, whereas diameter increment requires caliper measurements at both the beginning and the end of the growing season. Thus, foliage damage may be preferred to diameter increment, but foliage damage is assessed visually and can be influenced by the scorer. Nutrient deficiency or disease also may affect seedling foliage color, confounding the true effects of drought treatments. Therefore, in addition to foliage damage, it is useful to consider other measures of drought hardiness such as stem hydraulic traits. Both cavitation and K_h directly measure the functioning xylem tracheids in the stem and both require destructive sampling. However, cavitation is a relatively more labor intensive, time consuming, and subjective measure (cavitated cross-sectional area was scored visually) of xylem function than is K_h assessment. It is possible to assess xylem cavitation more quantitatively by electronically scanning images of dyed stem cross sections or by observing the stained sections under a sensitive microscope, but these techniques require additional time and labor, thereby increasing the cost of assessment. Xylem hydraulic conductivity, in contrast, is a quantitative, relatively unbiased assessment of xylem function that could be measured fairly readily for a large number of seedlings. Thus, xylem hydraulic conductivity seems the best candidate to include with foliage damage in assessing seedling drought hardiness.

Estimated family mean and genetic correlations were low for comparable drought hardiness traits measured in different years. These low correlations are, at least, partly due to the failure of the moderate drought applied in the second year to cause sufficient levels of foliage damage and cavitation, which led to an underexpression of these traits.

Even though families represented a range of sites and a large geographic area, estimated family mean and genetic correlations between drought hardiness and growth potential traits were low in both years, suggesting that the improvement in stem growth in coastal Douglas-fir breeding programs can be made without sacrificing drought hardiness in the selected population (Table 4). This is good news for applied tree breeding programs whose main focus is to select for fast stem growth, because the lack of strong genetic correlations between stem growth and drought hardiness will permit simultaneous genetic improvement in both traits. The strongly positive and significant ($p < 0.05$) genetic correlation between xylem hydraulic conductivity and stem diameter increment (Table 3) suggest that families with enhanced diameter growth may be less susceptible to severe drought at the seedling stage than are families with less growth potential, perhaps because larger-diameter trees tend to have more extensive root systems and can extract moisture from a deeper root zone. These results partly support the findings of other investigations, in which fast growing progenies of black spruce under drought stress showed greater drought tolerance than did slow growing progenies (Tan et al. 1992a, 1992b). The idea that fast growing genotypes are more drought tolerant also has been noted among other conifer tree species (Abrams et al. 1990; Blake and Yeatman 1989; Sands et al. 1984). Nevertheless, it is important to note that tree growth and drought tolerance may be negatively correlated in Douglas-fir populations including native environments with relatively severe and frequent droughts, such as southwestern Oregon. In southwestern Oregon, seedlings from dry sites tend to respond to cues of oncoming moisture stress by setting buds earlier, and thus grow less, but have less winter injury than seedlings from more mesic sites (Sorensen 1983; White 1987).

Measuring drought hardiness under controlled drought conditions is difficult, as illustrated in this study by the failure to generate sufficient moisture stress the second year, and by the low h^2 of drought hardiness traits. Because resistance to cavitation appears to be a major factor in drought hardiness, an interesting alternative to drought testing, per se, is to apply negative water potential artificially using a centrifuge system. Centrifugal force can create desired levels of cavitation rapidly in excised stem segments (Alder et al. 1997; Holbrook et al. 1995; Pockman et al. 1995). This method may also be useful for assessing drought hardiness in older, field grown trees using severed shoots, assuming xylem cavitation in shoots is strongly correlated with xylem in the main stem. However, we have not tested the practical utility of this method for genetic screening.

The drought hardiness measures evaluated in this study mostly involve drought tolerance mechanisms, that is, the ability to withstand and survive damage caused by moderate to severe drought. Although it is not clear if the methods applied to our British Columbia population are readily trans-

ferable to other populations, developing breeding programs for drought tolerance mechanisms could have immediate benefit to those regions in the Pacific Northwest, where drought can be relatively severe and frequent. Also, drought-tolerant genotypes can serve as insurance against unforeseen drought events in British Columbia 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. Understanding both avoidance and tolerance mechanisms should assist with the development of suitable and effective screening techniques for Douglas-fir genotypes hardy to mild, moderate, and severe drought environments.

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References

- Abrams, M.D., Kubiske, M.E., and Steiner, K.C. 1990. Drought adaptations and responses in five genotypes of *Fraxinus pennsylvanica* Marsh.: photosynthesis, water relations, and leaf morphology. *Tree Physiol.* **6**: 305–315.
- Aitken, S.N., Kavanagh, K.L., and Yoder, B.J. 1995. Genetic variation in seedling water-use efficiency as estimated by carbon isotope ratios and its relationship to sapling growth in Douglas-fir. *For. Genet.* **2**: 199–206.
- Alder, N.N., Pockman, W.T., Sperry, J.S., and Nuismer, S. 1997. Use of centrifuge force in the study of xylem cavitation. *J. Exp. Bot.* **48**: 665–674.
- Becker, W.A. 1992. *Manual of quantitative genetics*. 5th ed. Academic Enterprises, Pullman, Wash.
- Blake, T.J. 1983. Transplanting shock in white spruce: effect of cold-storage and root pruning on water relations and stomatal conditioning. *Physiol. Plant.* **57**: 210–216.
- Blake, T.J., and Yeatman, C.W. 1989. Water relations, gas exchange, and early growth rates of outcrossed and selfed *Pinus banksiana* (Lamb.) families. *Can. J. Bot.* **67**: 1618–1623.
- Borghetti, M., Raschi, A., and Grace, J. 1989. Ultrasound emission after cycles of water stress in *Picea abies*. *Tree Physiol.* **5**: 229–237.
- Borghetti, M., Edwards, W.R.N., Grace, J., Jarvis, P.G., and Raschi, A. 1991. The refilling of embolized xylem in *Pinus sylvestris* L. *Plant Cell Environ.* **14**: 357–369.
- Borghetti, M., Cinnirella, S., Magnani, F., and Saracino, A. 1998. Impact of long-term drought on xylem embolism and growth in *Pinus halepensis* Mill. *Trees*, **12**: 187–195.
- Burdon, R.D. 1977. Genetic correlation as a concept for studying genotype–environment interaction in forest tree breeding. *Silvae Genet.* **26**: 168–175.
- Dickerson, C.E. 1969. Techniques for research in quantitative and animal genetics. In *Techniques and procedures in animal science research*. American Society for Animal Research, Albany, N.Y. pp. 36–79.
- Emmingham, W.H. 1977. Comparison of selected Douglas-fir seed sources for cambial and leader growth patterns in four western Oregon environments. *Can. J. For. Res.* **7**: 154–164.
- Ewers, F.W. 1985. Xylem structure and water conduction in conifer trees, dicot trees, and lianas. *Int. Assoc. Wood Anat. (IAWA) Bull.* **6**: 309–317.
- Falconer, D.S., and Mackay, T.F.C. 1996. *Introduction to quantitative genetics*. 4th ed. Addison Wesley Longman Ltd., Harlow, U.K.
- Ferrell, W.K., and Woodard, E.S. 1966. Effect of seed origin on drought resistance of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Ecology*, **47**: 499–503.
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Bio. Sci.* **9**: 463–493.
- Haase, D.L., and Rose, R. 1993. Soil moisture stress induced transplant shock in stored and unstored 2+0 Douglas-fir seedlings of varying root volumes. *For. Sci.* **39**: 275–294.
- Hacke, U.G., Stiller, V., Sperry, J.S., Pittermann, J., and McCulloh K.A. 2001. Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance in xylem. *Plant Physiol.* **125**: 779–786.
- Hargrove, K.R., Kolb, K.J., Ewers, F.W., and Davis, S.D. 1994. Conduit diameter and drought-induced embolism in *Salvia mellifera* Greene (Labiatae). *New Phytol.* **126**: 695–705.
- Hermann, R.K., and Lavender, D.P. 1968. Early growth of Douglas-fir from various altitudes and aspects in southern Oregon. *Silvae Genet.* **4**: 143–151.
- Hobbs, S.D., Byars, R.H., Henneman, D.C., and Frost, C.R. 1980. First-year performance of 1–0 containerized Douglas-fir seedlings on droughty sites in southwestern Oregon. *Forest Research Laboratory, Oregon State University, Corvallis, Ore. Res. Pap.* **42**.
- Holbrook, N.M., Burns, M.J., and Field, C.B. 1995. Negative xylem pressures in plants: a test of the balancing pressure technique. *Science (Washington D.C.)*, **270**: 1193–1194.
- Jackson, G.E., Irvine, J., and Grace, J. 1995. Xylem cavitation in Scots pine and Sitka spruce saplings during water stress. *Tree Physiol.* **15**: 783–790.
- Jarbeau, J.A., Ewers, F.W., and Davis, S.D. 1994. The mechanism of water stress-induced embolism in two species of chaparral shrubs. *Plant Cell Environ.* **17**: 695–705.
- Johnson, G.R. 1998. Breeding design considerations for coastal Douglas-fir. *USDA For. Serv. Gen. Tech. Rep. PNW-GTR-411*.
- Johnson, G.R., and King, J.N. 1998. Analysis of half diallel mating designs: I — A practical analysis procedure for ANOVA approximation. *Silvae Genet.* **47**: 74–79.
- Joly, R.J., Adams, W.T., and Stafford, S.G. 1989. Phenological and morphological responses of mesic and dry site sources of coastal Douglas-fir to water deficit. *For. Sci.* **35**: 987–1005.
- Joseph, G., Kelsey, R.G., and Thies, W.G. 1998. Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptoglyphium wagneri*) or annosus (*Heterobasidion annosum*) root disease. *Tree Physiol.* **18**: 333–339.
- Kavanagh, K. 1993. Xylem cavitation in newly planted western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings. Ph.D. thesis, Forest Sciences Department, Oregon State University, Corvallis, Ore.
- Kavanagh, K.L., and Zaerr, J.B. 1997. Xylem cavitation and loss of hydraulic conductance in western hemlock following planting. *Tree Physiol.* **17**: 59–63.
- Kavanagh, K.L., Bond, B.J., Aitken, S.N., Gartner, B.L., and Knowe, S. 1999. Shoot and root vulnerability to xylem cavita-

- tion in four populations of Douglas-fir seedlings. *Tree Physiol.* **19**: 31–37.
- King, J.N., Carson, M.J., and Johnson, G.R. 1998. Analysis of disconnected diallel mating designs II: results from a third generation progeny test of the New Zealand Radiata Tree Improvement Programme. *Silvae Genet.* **47**: 80–87.
- Kozlowski, T.T., and Pallardy, S.G. 1997. *Physiology of woody plants*. 2nd ed. Academic Press, San Diego, Calif.
- LoGullo, M.A., and Salleo, S. 1991. Three different methods for measuring xylem cavitation and embolism: a comparison. *Ann. Bot. (London)*, **67**: 417–424.
- Lomas, M.C. 1999. *Physiology and genetics of drought hardiness in coastal Douglas-fir seedlings*. M.S. thesis, Oregon State University, Corvallis, Oreg.
- O'Neill, G.A. 1999. *Genetics of fall, winter, and spring cold hardiness in coastal Douglas-fir seedlings*. Ph.D. thesis, Forest Science Department, Oregon State University, Corvallis, Oreg.
- Panek, J.A., and Waring, R.H. 1995. Carbon isotope variation in Douglas-fir foliage: improving the delta ^{13}C – climate relationship. *Tree Physiol.* **15**: 657–663.
- Pena, J., and Grace, J. 1986. Water relations and ultrasound emissions of *Pinus sylvestris* L. before, during and after a period of water stress. *New Phytol.* **103**: 515–524.
- Pharis, R.P., and Ferrell, W.K. 1966. Differences in drought resistance between coastal and inland sources of Douglas-fir. *Can. J. Bot.* **44**: 1651–1659.
- Pockman, W.T., Sperry, J.S., and O'Leary, J.W. 1995. Sustained and significant water pressure in xylem. *Nature (London)*, **378**: 715–716.
- Rietveld, R.J. 1989. Transplanting stress in bareroot conifer seedlings: its development and progression to establishment. *North. J. Appl. For.* **6**: 99–107.
- Salleo, S., and LoGullo, M.A. 1986. Xylem cavitation in nodes and internodes of whole *Chorisia insignis* H. B. et K. plants subjected to water stress: relations between xylem conduit size and cavitation. *Ann. Bot. (London)*, **58**: 431–441.
- Sands, R., Kriedmann, P.E., and Cotterill, P.P. 1984. Water relations and photosynthesis in three families of radiata pine seedlings known to differ in their response to weed control. *For. Ecol. Manage.* **9**: 173–184.
- SAS Institute Inc. 1990. *SAS/STAT user's guide*, version 6. 4th ed. Vol. 2. SAS Institute Inc., Cary, N.C.
- Schaffer, H.G., and Usanis, R.A. 1969. General least squares analysis of diallel experiments. A computer program — DIALL. Genetics Department, North Carolina State University, Raleigh, N.C. Res. Rep. 1.
- Smith, J.H.G., and Walters, J. 1963. Planting check (reduction in height growth) of planted Douglas-fir seedlings. University of British Columbia, Vancouver, B.C. For. Fac. Res. Note 42.
- Sobrado, M.A., Grace, J., and Jarvis, P.G. 1992. The limits to xylem embolism recovery in *Pinus sylvestris* L. *J. Exp. Bot.* **43**: 831–836.
- Sorensen, F.C. 1983. Geographic variation in seedling Douglas-fir (*Pseudotsuga menziesii*) from the western Siskiyou Mountains of Oregon. *Ecology*, **64**: 696–702.
- Sperry, J.S. 1995. Limitations on stem water transport and their consequences. *In Plant stems: physiology and functional morphology*. Edited by B.L. Gartner. Academic Press, San Diego, Calif. pp. 105–124.
- Sperry, J.S., and Tyree, M.T. 1988. Mechanism of water-stress induced xylem embolism. *Plant Physiol.* **88**: 581–587.
- Sperry, J.S., and Tyree, M.T. 1990. Water-stress-induced xylem embolism in three species of conifers. *Plant Cell Environ.* **13**: 427–436.
- Sperry, J.S., Perry, A.H., and Sullivan, J.E.M. 1991. Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. *J. Exp. Bot.* **42**: 1399–1406.
- Sperry, J.S., Alder, N.N., and Eastlack, S.E. 1993. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J. Exp. Bot.* **44**: 1075–1082.
- Sperry, J.S., Nicholas, K.L., Sullivan, J.E.M., and Eastlack, S.E. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology*, **75**: 1736–1752.
- Sperry, J.S., Saliendra, N.Z., Pockman, W.T., Cochard, H., Cruziat, P., Davis, S.D., Ewers, F.W., and Tyree, M.T. 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant Cell Environ.* **19**: 427–436.
- Spittlehouse, D.L. 1985. Determination of the year-to-year variation in growing season water use of a Douglas-fir stand. *In The forest-atmosphere interaction*. Edited by B.A. Hutchison and B.B. Hicks. D. Reidel Publishing Co., Dordrecht, the Netherlands. pp. 235–254.
- Steel, R.G.D., and Torrie, J.H. 1980. *Principles and procedures of statistics: a biometric approach*. 2nd ed. McGraw Hill, New York.
- Stoneypher, R.W., Piesch, R.F., Helland, G.G., Chapman, J.G., and Reno, H.J. 1996. Results from genetic tests of selected parents of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in an applied tree improvement program. *For. Sci. Monogr.* **32**: 1–35.
- Taiz, L., and Zeiger, E. 1998. *Plant physiology*. 2nd ed. Sinauer Associates, Inc., Sunderland, Mass.
- Tan, W., Blake, T.J., and Boyle, T.J.B. 1992a. Drought tolerance in faster- and slower-growing black spruce (*Pinus mariana*) progenies: I. Stomatal and gas exchange responses to osmotic stress. *Physiol. Plant.* **85**: 639–644.
- Tan, W., Blake, T.J., and Boyle, T.J.B. 1992b. Drought tolerance in faster- and slower-growing black spruce (*Pinus mariana*) progenies: II. Osmotic adjustment and changes of soluble carbohydrates and amino acids under osmotic stress. *Physiol. Plant.* **85**: 645–651.
- Tyree, M.T., and Dixon, M.A. 1986. Water stress induced cavitation and embolism in some woody plants. *Physiol. Plant.* **66**: 397–405.
- Tyree, M.T., and Evers, F.W. 1991. The hydraulic architecture of trees and other woody plants. *New Phytol.* **119**: 345–360.
- Tyree, M.T., and Sperry, J.S. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiol.* **88**: 574–580.
- Tyree, M.T., and Sperry, J.S. 1989. Vulnerability of xylem cavitation and embolism. *Annu. Rev. Plant Physiol. Mol. Biol.* **40**: 19–38.
- Tyree, M.T., and Sperry, J.S. 1990. Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol. Mol. Biol.* **40**: 19–38.
- Waring, R.H., and Cleary, B.D. 1967. Plant moisture stress: evaluation by pressure bomb. *Science (Washington D.C.)*, **155**: 1248–1254.
- White, T.L. 1987. Drought tolerance of southwestern Oregon Douglas-fir. *For. Sci.* **33**: 283–293.
- Woods, J.H. 1993. Breeding programs and strategies for Douglas-fir in North America. *In Papers presented at the Workshop on Breeding Strategies of Important Tree Species in Canada*, 18 Aug. 1993, Fredericton, N.B. Compiled by Y.S. Park and G.W. Adams. Nat. Resour. Can. Can. For. Serv. Maritimes For. Cent. Inf. Rep. M-X-186E.

- Yanchuk, A.D. 1996. General and specific combining ability from disconnected partial diallels of coastal Douglas-fir. *Silvae Genet.* **45**: 37–45.
- Yeh, F.C., and Heaman, J.C. 1987. Estimating genetic parameters of height growth in seven-year old coastal Douglas-fir from disconnected diallels. *For. Sci.* **33**: 946–957.
- Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag, Berlin.
- Zwienecki, M.A., and Holbrook, N.M. 1998. Diurnal variation in xylem hydraulic conductivity in with ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.). *Plant Cell Environ.* **21**: 1173–1180.