AN ABSTRACT OF THE THESIS OF

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Douglas-fir (Pseudotsuga menziesii var. menziesii (Mirb.) Franco) Seedlings.

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Abstract approved: _____

Thimmappa Anekonda

Physiology and genetics of drought hardiness were investigated in two-year-
old coastal Douglas-fir seedlings from 39 full-sib families obtained from coastal
British Columbia and grown at Oregon State University, Corvallis, Oregon. The
seedlings were subjected to three drought treatments (control, moderate, and severe
drought) in their second growing season (June through September 1997). Response to
drought stress was assessed by measuring stomatal conductance, hydraulic
conductivity, xylem cavitation in two annual growth rings, and shoot damage. Growth
traits measured were seedling height and basal stem diameter.

In response to severe drought, a general decrease in predawn xylem water
potential, hydraulic conductivity, and stomatal conductance occurred while xylem
cavitation and shoot damage increased significantly ($p < 0.05$). Drought stress also
reduced seedling growth, with diameter being more severely affected by drought than
height. Negative xylem water potential ($\Psi_x = -1.7$ to -2.5 MPa) during the drought
period reduced hydraulic conductivity of the seedlings.

Mean cavitation in the first annual growth ring was significantly ($p < 0.05$) greater in seedlings grown under severe drought than in seedlings grown in the moderate or control treatments. Family variation for cavitation in the first growth ring, however was significant ($p < 0.001$) in all treatments indicating that genetic variation in cavitation in this ring is independent of drought treatment. Cavitation in the second annual growth ring showed significant ($p < 0.05$) differences among all the treatments. Family variation in cavitation in the second growth ring was significant ($p < 0.0001$) only in the severe drought treatment, but not in control or moderate, suggesting that this trait was particularly sensitive to severe, current-season drought.

Family variation was significant ($p < 0.05$) in two growth potential traits (height and diameter) and in cavitation of the second growth ring and shoot damage (damage traits). Despite relatively high ratios of specific combining ability to general combining ability variance for most traits, family mean heritabilities were moderately high ($h_f^2 = 0.41$ to 0.58), suggesting that selection based on family information will be effective for improving drought hardiness and growth potential of seedlings. Cavitation in the second growth ring and shoot damage appear to be largely under control of the same set of genes. Although estimated family heritability was somewhat greater for cavitation (0.57) than for shoot damage (0.41), shoot damage is likely to be the better choice upon which to base selections for seedling drought hardiness because it is a much easier trait to measure. Damage traits are estimated to have a slight, unfavorable genetic correlation with growth potential traits, thus selection for stem growth at the seedling stage is not expected to have a large influence on the drought hardiness of seedlings.

Physiology and Genetics of Drought Hardiness in Coastal Douglas-fir (*Pseudotsuga*

menziesii var. *menziesii* (Mirb.) Franco) Seedlings

by

M. Christine Lomas

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APPROVED:

Signature redacted for privacy.

_____

Major Professor, representing Forest Science

Signature redacted for privacy.

_____

Chair of Department of Forest Science

_____

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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M. Christine Lomas, Author

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DEDICATION

This thesis is dedicated to: my best friend and mother, Marilyn Lawson, for her omnipresent love, guidance, and strength; to my pops, Charles F. Lawson, for his love and support; and, to Dr. Timothy R. Plumb who first inspired my interest in tree improvement.

PHYSIOLOGY AND GENETICS OF DROUGHT HARDINESS IN COASTAL DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII* VAR. *MENZIESII* (MIRB.) FRANCO) SEEDLINGS

CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

This project represents a portion of the Pacific Northwest Tree Improvement Research Cooperative's (PNWTIRC) study entitled "Genetic Variation in Seedling Drought Physiology of Coastal Douglas-fir" and a continuation of research focused on understanding the adaptation of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) to stressful environments. The Pacific Northwest has a broad range of climatic regions where the native flora has adapted to specialized environments. Within this region, coastal Douglas-fir occupies a variety of microclimates, some of which experience frequent summer drought that influences natural regeneration and survival of seedlings.

Breeding Douglas-fir to withstand summer moisture-stress requires quantification of variation in traits that respond to drought and determination of their genetic control. Because physiological and morphological responses to drought are controlled by a variety of complex pathways, it is important to study several responses simultaneously. With this consideration in mind, the PNWTIRC's Drought Physiology Study has four important goals: (1) to assess the degree and type of genetic

variation in drought hardiness, (2) to identify morphological and physiological characteristics associated with seedling drought hardiness, (3) to determine the degree to which growth and drought hardiness are associated, and (4) to determine the relationships between drought and cold hardiness (PNWTIRC Annual Report 1995-1996).

The project described in this thesis was primarily concerned with physiological responses to drought and their interrelationships with growth traits. The influence of drought on growth (height and diameter) and damage (shoot damage, cavitation of xylem conduits, reduction of hydraulic conductivity, and reduction of stomatal conductance) traits are assessed. Of these traits, only the two growth traits and two of the damage traits (xylem cavitation and shoot damage) were measured on the full-set of 39 families in the experiment. The usefulness of using either xylem cavitation or shoot damage to screen Douglas-fir genotypes for their drought hardiness ability at the seedling stage is discussed.

THESIS ORGANIZATION

The thesis contains this brief introductory chapter (chapter 1), a chapter on the physiological response of Douglas-fir seedlings to drought-stress (chapter 2), a chapter on the quantitative genetics of seedling drought hardiness, growth potential, and their interrelationships in coastal Douglas-fir (chapter 3), and a chapter integrating the overall conclusions from this research (chapter 4). Chapter 2 contains information on the materials and methods for assessing physiological, morphological, and damage

traits; and, interrelationships among these traits and their response to drought. Chapter 3 addresses the genetic control of xylem cavitation, shoot growth and damage, and the genetic interrelationships among these traits. The advantages and disadvantages of using xylem cavitation or shoot damage scores to screen Douglas-fir seedlings for drought hardiness are also discussed in this chapter. Chapter 4 integrates the conclusions drawn from chapters 2 and 3 and offers suggestions for further research in physiology and genetics of drought hardiness. Following chapter 4 are three brief appendices. Appendix A contains a brief experiment in which two of the 39 full-sib families in this study were assessed for their loss in maximum hydraulic conductivity due to increasing tension on the water column as expressed by vulnerability curves. Appendix B provides a sample of the programming code for the statistical analyses of traits in chapter 3. Appendix C lists the variance components for the models in chapter 3 as well as the derivation of the test of significance of general combining ability variance for the traits included in this chapter.

CHAPTER 2

PHYSIOLOGICAL RESPONSE OF DOUGLAS-FIR SEEDLINGS TO DROUGHT-STRESS

ABSTRACT

The main objectives of this study were to determine the influence of drought on physiological, growth, and damage traits and to determine the interrelationships among these traits in two-year-old coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) seedlings. Plant material included seedlings from 39 full-sib families grown in raised nursery beds and subjected to three drought treatments in the second growing season (June-September 1997): control (well-watered), moderate, and severe drought. Response to drought stress was assessed by measuring stomatal conductance, stem hydraulic conductivity, xylem cavitation in two annual growth rings, and shoot damage. Growth traits measured were seedling height and basal diameter.

In response to severe drought, a general decrease in predawn xylem water potential, hydraulic conductivity, and stomatal conductance occurred while xylem cavitation and shoot damage increased significantly ($p < 0.05$). Drought stress also reduced seedling growth, with diameter being more severely affected by drought than height.

The more negative xylem water potentials of seedlings subjected to drought ($\Psi_x = -1.7$ to -2.5 MPa) treatments compared to those under the control treatment indicated severely reduced stomatal conductance due to drought. A positive

relationship between treatment means of specific hydraulic conductivity and stomatal conductance, and a negative relationship between hydraulic conductivity and shoot damage suggested that xylem tracheids may have been permanently damaged.

Mean cavitation in the first annual growth ring was significantly ($p < 0.05$) greater in seedlings grown under severe drought than in moderate or control treatments. The frequency distribution of percent cavitation in the first annual growth ring is similar among the three treatments, suggesting variation in cavitation in this ring is independent of the drought treatments. The second annual growth ring showed significant ($p < 0.05$) differences among the treatments, with the control being less cavitated (6.4%), the moderate slightly more cavitated (8.7%), and the severe the most cavitated (17.9%). The frequency distributions of cavitation in the second annual growth ring suggest that this trait was particularly sensitive to the imposed drought treatments.

INTRODUCTION

Summer drought is common in many regions of the natural range of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) in the Pacific Northwest. Both intensity and seasonal timing of drought greatly influence natural and artificial regeneration of Douglas-fir and reduce the ability of seedlings to withstand damage from insects, pathogens, and frost injury (Hobbs et al. 1980; Spittlehouse 1985). Because of drought-induced damage, young Douglas-fir seedlings are unable to reach deeper water reserves, prevent wilt and transpirational-water loss, or reach

adequate height to outgrow their competitors. The success of early seedling

establishment on droughty sites, therefore, depends on the extent to which planting

stock is able to tolerate drought stress conditions. In order to improve stock for

planting on drought-prone sites, the variation in physiological responses of Douglas-fir

seedlings to drought conditions needs to be quantified. Understanding how drought

influences the water transport system, growth and health of seedlings, as well as the

interactions of these physiological responses, will help in selection of traits that are

best suited for assessing drought hardiness in Douglas-fir. In this chapter, the extent of

drought injury in Douglas-fir seedlings is assessed in terms of both physiological

responses and reduction in growth.

Influence of drought on stomatal conductance

Stomates are minute pores in conifer needles through which carbon dioxide

enters and water is lost during transpiration. Reduction in cellular water during

drought reduces turgor in stomatal guard cells and prevents them from opening

(Johnson and Ferrell 1982). Therefore, the rate at which water vapor is lost through

stomata (stomatal conductance, g_s) is an important indicator of seedling response to

drought. Stomatal conductance is usually expressed as the quantity of water vapor lost

per unit of leaf area over a specified time period, and is typically given in terms of

$mmol\ m^{-2}\ s^{-1}$. Several studies have reported decreased stomatal conductance in

response to drought. Stomatal conductance of one-year-old western larch (*Larix

occidentalis* Nutt.) seedlings remained constant throughout the growing season in a

well-watered control, but g_s values under the drought treatment reached only 30% of the values measured under well-watered conditions with g_s values under drought reaching ~45 mmol m^{-2} s^{-1} and ~20 mmol m^{-2} s^{-1} in June and September, respectively (Zhang and Marsall 1994). Stomatal conductance of both *Pinus sylvestris* L. and *Picea sitchensis* (Bong.) Carr were reduced to near zero under a drought treatment (Jackson et al. 1995). When stomatal conductance rates are zero, the stomates are presumed closed, therefore, water vapor ceases to be lost and the tension on the water column may be eased depending on the soil water pressure.

Bond and Kavanagh (1999) modeled results from several studies of four different woody plants and found that stomatal conductance is linked with water potential of the soil as well as with the leaf-to-air vapor pressure gradient. As stomatal conductance increases over the course of the day, and as water becomes less available from the soil, water in the stomatal aperture retreats further into the stomatal pore thereby increasing tension on the water column (i.e., decreased xylem water potential (Ψ_x); Kavanagh 1993). This occurs until transpirational demand is decreased and stomatal pores are closed once again, thus, allowing the tension on the water column to equalize to soil water availability. Bond and Kavanagh's (1999) model for Douglas-fir indicates that, under drought conditions when Ψ_x is -2.0 MPa, the maximum g_s possible will be zero as the pressure gradient needed to move water through the hydraulic pathway exceeds the safety threshold, which may cause the water column to break (cavitation). Borghetti et al. (1998) suggest that stomatal closure in *Pinus halepensis* Mill. may not be specifically related to avoidance of cavitation by hydraulic constraints. Likewise, reduction in diurnal stomatal

conductance has been linked with reduction in vapor pressure deficit only so long as the hydraulic system can deliver sufficient water to meet transpirational demand (Kavanagh and Zaerr 1997). However, if cavitation has occurred, stomatal conductance will not be able to recover to expected control levels even after a period of well-watered conditions (Kavanagh and Zaerr 1997).

Influence of drought on xylem cavitation

Water transport in the xylem occurs under tension (or negative pressure). Drought induced obstruction (embolism) of xylem tracheids (cavitation) occurs when xylem water pressure is low enough to pull air bubbles into the xylem conduits through adjacent pit membranes (Tyree and Sperry 1988). Pit membranes with larger pore diameters require less tension to overcome capillary forces and pull air bubbles relatively easily into the cell, thus, triggering more extensive embolism in xylem tracheids (Zimmermann 1983). Zimmerman's (1983) segmentation hypothesis states that the plant 'sacrifices' lateral branches to preserve the main stem. These lateral branches are presumed lost due to extensive cavitation. Thus, cavitation reduces the leaf area available for photosynthesis when lateral branches are lost, which reduces the growth potential of the seedling and allows site-domination by better-adapted competitors. Therefore, the intensity of cavitation is one measure of drought injury to plants and, inversely, the plant's drought hardiness ability.

Anatomical differences in pore diameter of latewood and earlywood tracheids have been shown to influence the tension required to allow the entry of air bubbles

into the tracheid cell (Gregory and Petty 1973). Thin-walled, large tracheids of earlywood are more vulnerable to cavitation than thick-walled, latewood tracheids (LoGullo and Salleo 1991; LoGullo et al. 1995). In other words, when tension on the water column increases (i.e., when g_s increases and soil water is less available), the occurrence and frequency of cavitated tracheids increases, thus reducing the xylem area available to transport water. In addition, the pressure level at which the water column cavitates from this tension varies from species to species (Cochard 1992) and between populations adapted to dry and wet environments (Kavanagh et al. 1999). Conifer tracheids appear to cavitate across a wide range of xylem water potential (from -0.5 to -5.0 MPa) and are more susceptible to damage from drought-induced cavitation in early spring when most of the annual ring consists of earlywood (Gregory and Petty 1973).

Sperry et al. (1993) showed that, when 80% of the conducting xylem had embolized in *Betula occidentalis* Hook., leaves turned brittle, brown, and died due to dehydration. Total cavitation of mature Douglas-fir branches occurred when Ψ_x reached -5.0 MPa, while 50% cavitation was evident at -3.5 MPa (Cochard 1992). Tognetti et al. (1997) found that in two-year-old *Pinus halepensis* Mill. seedlings exposed to a severe drought (predawn xylem water potential (Ψ_{pd}) of -3.0 MPa), a 50% reduction in hydraulic conductivity occurred due to cavitation.

It has been noted that stomatal closure under drought is necessary to avoid catastrophic blockage of water movement due to cavitation in root xylem of *Acer grandidentatum* Nutt. (Alder et al. 1996). Therefore, stomatal regulation is intimately linked with xylem function in both roots and shoots of plants.

Influence of drought on hydraulic conductivity

The volume of water transported through a given path length of plant tissue under a given pressure is expressed as hydraulic conductivity (K_h) and is derived mathematically by the following equation:

$$K_h = [F \times (l)] / (p) \quad \text{(g-mm s}^{-1}\text{ MPa}^{-1}\text{)} \qquad \text{Equation 2.1}$$

where, the hydraulic conductivity is expressed as the average flow (F) times the length of segment (l) divided by the amount of pressure applied (p). It serves as both an estimate of the percentage of xylem cells that remain fully functional in water transport (Panek 1996) and as an indication of the porosity of the materials contained in the segment (Kavanagh 1993). A reduction in hydraulic conductivity, relative to the maximum value possible, reflects a reduction in functional xylem tracheids. However, K_h can also be reduced by a change in pressure gradient mediated by stomatal conductance and/or by deposition of solutes as water transport is slowed (Kozlowski and Pallardy 1997). Specific hydraulic conductivity (K_s) takes into account cross-sectional xylem area and allows for comparison of different size samples. Specific hydraulic conductivity is derived from the following equation:

$$K_s = K_h / A \quad \text{(g s}^{-1}\text{ MPa}^{-1}\text{ cm}^{-1}\text{)} \qquad \text{Equation 2.2}$$

where, K_h is the hydraulic conductivity of the segment and A is the cross-sectional xylem area. Furthermore, a vulnerability curve can be derived to reflect the percent loss in hydraulic conductivity (or specific hydraulic conductivity) relative to the

maximum xylem water potential (Kavanagh et al. 1999; Pammenter and Willigen 1998). As populations within a species have been shown to differ in their vulnerability curves (Kavanagh et al. 1999), a side experiment was done in the Fall of 1998, on seedlings of two representative families utilized in this study with the objective of determining within family variation in vulnerability to cavitation. These vulnerability curves are meant to indicate the cavitation initiation point for seedlings utilized in this study as well as the rate at which cavitation is expected to occur with increasing drought stress. The procedures and results from this experiment are presented in Appendix A.

A vulnerability curve indicates the Ψ_x at which cavitation is initiated by relating K_h to xylem water potential. This cavitation initiation point is the xylem water potential at which hydraulic conductivity begins to be impacted (i.e., decreases) with increasing Ψ_x. The steeper the curve after the point of initiation, the more vulnerable a species is to catastrophic loss of functioning tracheids. A recent study in western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) showed greater than 50% conductivity loss when xylem water potential fell below -3.0 MPa (Kavanagh and Zaerr 1997), indicating that western hemlock has a high initiation point (i.e., high value of Ψ_x when initiation occurs) as well as a steep vulnerability curve. The steepness of the curve at the 50% loss point (abscissa of the curve) is used to measure the rate at which conductivity is lost due to xylem pressure induced cavitation. The vulnerability of a population to cavitation is an adaptive trait. For example, Kavanagh et al. (1999) found that among four populations of Douglas-fir, those from coastal sources (i.e., from the most mesic environments) had the steepest curves (i.e., loss of hydraulic

conductivity was most sensitive to increased water potential). While inland populations (i.e., those originating from climates with a higher 15-year maximum temperature and lower rainfall, therefore creating more growing-season water stress), on the other hand, were more resistant to stem cavitation, and had more gradual vulnerability curves, allowing seedlings to maintain stomatal conductance at water potentials lower than populations from coastal locations.

In summary, stomatal conductance, xylem cavitation, and hydraulic conductivity are interrelated. Under ambient conditions, short-term reduction in stomatal conductance prevents Ψ_x from dropping below cavitation inducing tensions. During periods of drought stress when either dynamic (transpirational) or static (Ψ_{soil}) pressures reach a minimum threshold, cavitation is induced. Since cavitation reduces stem conductivity, stomata must close to retard further cavitation (Sperry and Pockman 1993; Borghetti et al. 1989; Kavanagh and Zaerr 1997). Two primary factors cause Ψ_x to go below the threshold for cavitation: (1) dynamic drought stress in a situation where the change in Ψ_x is too low to maintain the rate of volumetric flow (flux) through the hydraulic pathway (as seen by rearranging the K_h equation so that flux is expressed as hydraulic conductivity by change in pressure); or, (2) Ψ_{soil} is so low that cavitation is induced (i.e., by cuticular water loss).

Influence of drought on foliage health (shoot damage)

According to the segmentation hypothesis, during periods of severe drought stress, plants limit damage in the main stem by allowing cavitation to occur in lateral branches (Zimmerman 1983; Sperry and Ikeda 1997). Indeed, the percentage of live needles and branches remaining on a seedling under severe drought has been used to quantify the degree of drought tolerance in Douglas-fir (White 1987).

Influence of drought on seedling growth

Limited water availability during the course of the growing seasons may affect seedling growth in one of two primary ways. First, cell elongation may be reduced, which limits seedling height; and second, xylem tissues tend to show a higher ratio of latewood cells, that are typically smaller and, therefore, limit diameter size. Timmis and Tanaka (1976) found that, by imposing water stress when height growth is most active, seedling growth is rapidly reduced in Douglas-fir. In addition, sources from drier environments or south-facing slopes have been shown to be adapted to shorter growing seasons with trees usually showing shorter stature (Hermann and Lavender 1968). Likewise, exposure to drought conditions ($\Psi_x = -1.2$ MPa) also reduced diameter growth in Douglas-fir seedlings (Timmis and Tanaka 1976).

Objectives

The study described in this chapter addressed the physiological responses of Douglas-fir seedlings to drought stress. Seedlings from 39 full-sib families originating from parent trees in coastal British Columbia were subjected to three levels of soil moisture in their second growing season. Specific objectives of this research were:

1. To determine the influence of drought on stomatal conductance, xylem cavitation in annual growth rings, hydraulic conductivity, seedling growth, and shoot damage; and,

2. To understand the nature of interrelationships among these physiological responses to drought.

MATERIALS AND METHODS

The main study

This study represents a portion of a larger study on drought hardiness in Douglas-fir seedlings ("Genetic Variation in Seedling Drought Physiology of Coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco)") undertaken by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC). Seeds of the 39 full-sib families used in this study were obtained from the British Columbia Ministry of Forestry. The families came from four, six-parent half-diallel mating sets ((6×5)/2 = 15 families per diallel) in series #6, which were originally produced in 1978. From

these six-parent half-diallels, seed was available in storage to make up four smaller

five-parent, half-diallels ((5 x 4)/2 = 10 families per diallel) with one family in one

diallel missing, resulting in the total of 39 families. Parent trees used in the crossing

program came from southwestern British Columbia (Vancouver Island and the coastal

mainland), sampling areas of high, medium, and low summer moisture availability.

Therefore, the full-sib families obtained by crossing these parents are expected to

show variation in their response to drought-stress conditions.

Seed from all families was stratified for 35 days. Germinants were planted

from April 29 - May 1, 1996, into two custom-built raised nursery beds each 20 m

long, 1.5 m wide, and 1 m deep, with the long axis oriented in an east-west direction.

Planting spots were spaced at 8 cm x 8 cm. The beds were filled with a sandy loam

soil to facilitate drainage, and lined at the bottom with landscape cloth to prevent root

penetration beyond the beds. Plastic barriers were placed between the walled sections

of the beds to prevent movement of water between drought treatments.

The experimental design was a split-plot replicated in five blocks. Main plots

consisted of three watering regimes, or treatments, that were applied in summer 1997

(June – September, second growing season): well-watered (control; late-season weekly

average Ψ_{pd} of -0.8 MPa), moderate drought (average Ψ_{pd} of -1.7 MPa), and severe

(average Ψ_{pd} of -2.5 MPa) drought. Within each main plot (1.5 m x 2.5 m), each of

the 39 families was represented by 8 trees in two randomly-located, 4-tree family row-

plots. Main plots were surrounded by two rows of buffer seedlings on the east and

west facing sides and one row of buffer seedlings on the north and south facing sides.

Experimental seedlings totaled 4,680 at planting (5 blocks x 3 treatments per block x

39 full-sib families x 2 row plots per family x 4 seedlings per family plot). In addition, six auxiliary row-plots of seedlings were randomly located in each raised nursery bed to be used for predawn xylem water potential assessments (see below).

All seedlings were well-watered during the first growing season. Appropriate fertilizer (Peters 20:20:20) and insecticide (Orthene) were applied as needed. The total survival of seedlings at the onset of the second growing season was 88.3% of the original planting stock. Some of the mortality was due to fungal infection in the first growing season. In July 1996, a couple of months after planting, the seedlings showed symptoms of a hypocotyl rot disease (*Fusarium oxysporum*). The disease killed about 10% of the seedlings regardless of family, but upon the advice of Dr. Everett Hanson, Department of Botany and Plant Pathology, Oregon State University, it was brought under control by decreasing the frequency of irrigation, application of fungicide, and by covering the nursery beds with 50% shade cloth for about a month. The fungicides (Captan, Banrot, and Benlate) were applied bi-weekly from May 9 to October 7, 1996, following recommendations given by Dick Miles, USFS Pacific Northwest Research Station, Corvallis, OR.

Drought treatments

Drought treatments were imposed from June through September in the second growing season (1997), by controlled application of irrigation, and by protection from natural rainfall. Timing and frequency of irrigation were based on average predawn xylem water potential readings taken on the auxiliary seedlings. Earlier seedling

studies suggested that significant response of seedlings to drought, including extensive

foliage damage and some mortality, will occur if Ψ_{pd} is kept below -2.0 MPa (White

1987; O'Neill 1999). Therefore, the targeted predawn xylem water potentials were set

to above -0.7 MPa for the control, between -1.0 and -1.5 MPa for the moderate, and

between -2.0 MPa and -5.0 MPa for the severe treatment. These levels of drought

were expected to inflict 40-50% shoot damage and 30% mortality in seedlings in the

severe drought treatment and 20-25% shoot damage with only minor mortality (say

less than 5%) to seedlings in the moderate treatment.

Protection from natural rainfall was largely achieved by placing clear plastic

covers as needed on PVC hoops over the beds. However, severe winds occurring at

nighttime removed plastic covers twice allowing unintentional water into the treatment

beds before this problem was eliminated with more secure plastic holders. Exposure

to the natural rainfall was minimal and no impact was noted in the predawn xylem

water potential readings. The plastic covers were left on for only a minimum duration.

In addition, the hoops kept the covers well above the seedlings (0.5 to 1.5 m) so that

the potential for sun-scald damage, heat-stress, interference with atmospheric gas

exchange, incoming radiation, and injury to seedlings was minimized.

Soil moisture levels were assessed as predawn xylem water potential (Ψ_{pd}) by

using a pressure chamber (Waring and Cleary 1967). Once a week over the course of

the growing season (June through September, 1997), three shoot samples were

collected systematically from three of the six different auxiliary rows in each main plot

(5 blocks x 3 treatments per block x 3 trees = 45 trees per week) and Ψ_{pd} assessed by

pressure bomb (PMS Instruments, Corvallis, OR). On average, the control treatment

was watered once a week until July 27th and then watered twice weekly; the moderate treatment was watered approximately every other week; and the severe treatment only once during the 13 weeks of the drought treatment. If weekly predawn xylem water potentials were at target level in mid-to-late summer, irrigation was kept at every three to four days for the control at approximately 109 L per main plot. However, when water potentials fell below target levels for the control treatment, irrigation was increased by 36 to 72 L/main plot and reduced by the same amount when Ψ_{pd} was above target. Likewise, when the moderate treatment fell below target, main plots were irrigated in a similar fashion.

Measurements

From the total of 4,680 experimental seedlings in the main study, sub-sets of seedlings were used to assess various physiological responses to drought in the second growing season. Each of the traits measured is described below.

Stomatal conductance (g_s)

Stomatal conductance is a measure of the amount of water vapor lost per unit surface area of needles in a specified time period. In this study, diurnal stomatal conductance was measured (i.e., g_s over the course of the day) by a LiCor-1600 (Lincoln, NE) steady-state porometer on a total of 18 trees from the tallest (family 412) and the shortest (family 114) families as grown under the control treatment, in

each of the three drought treatments (3 treatments x 2 families x 3 trees per family =

18 trees). Because measurement of all 18 trees took about 30 to 45 minutes, small

changes in air temperature etc., during the course of each sampling period might have

contributed to minor variation in g_s levels among trees. Porometer readings were first

taken on a small, prepared portion of intact branch needles (say a 3 cm long segment

of branch) located in an upper, well-exposed area on each tree at 8:00, 10:00, 14:00,

and 16:00 hours on September 9, 1997, just prior to cessation of the drought

treatments, when reduction in stomatal conductance was expected to be maximum.

Porometer readings of stomatal conductance were adjusted for the leaf area of the

needles measured inside the porometer cuvette. Leaf area was estimated using a

Decagon Image Analysis System (Pullman, WA). Because the two families did not

differ significantly from each other in g_s values, only pooled values are presented in

the results.

The diurnal increase in negative xylem pressure was assessed on September 9,

1999, concurrently with g_s measurements. However, xylem water potential was not

assessed directly on trees measured for g_s, but rather on immediately adjacent ones.

Xylem water potential readings were taken predawn and at 8:00, 10:00, 14:00, and

16:00 hours.

Cavitation

Staining of functional xylem conduits with safranin dye followed by an

examination of the stem for proportion of area stained is an indirect measure of the

degree of cavitation. In this method, safranin dye was forced through short (3 cm) stem segments using a gravity feed system. A description of this cavitation assessment procedure is presented below:

(1) *Materials and experimental design*

From one randomly selected row-plot of each family within each main plot, the main stem of every-other seedling (2 total) was harvested to assess xylem cavitation. In total, 1044 trees were sampled, or an average of 8.9 seedlings per family per moisture treatment (N ranged from 6 to 10 per family). Each tree was harvested at the ground level, placed into a plastic bag containing moist towels, and immediately taken to an adjacent laboratory for sample preparation. Trees were sampled by block over the period from September 2-12, 1997.

(2) *Safranin dye apparatus*

A low concentration of safranin dye (0.5 g /L) was vacuum filtered through 0.22 μm filter paper so that the stain would pass through the pit membranes of functioning xylem conduits (Ewers 1985; LoGullo and Salleo 1991; Kavanagh and Zaerr 1997). The original protocol from Kavanagh and Zaerr (1997) was modified to increase the uniformity of the results by standardizing the duration of exposure to the dye (2 h), the length of stem segment (3 cm), and the location where the dyed stem cross-section was to be scored for visual cavitation. The standard set-up for dye application consisted of a flask (1-2 L) containing 0.5% safranin dye raised 1 M above the counter, and tubing connecting the dye in the flask to the

stem segment (see below). The end of each tube with the stem segment was placed on an aluminum, mesh-covered drip pan located on the lab countertop. The mesh-covered drip-pans allowed dye that had been transported through the stem to collect away from the stem segment, thus avoiding external exposure to and contamination by the excess dye.

(3) *Sample preparation and scoring*

Following removal from the plastic bags, the stems were placed in water. While in the water, the bark was removed, the distal and proximal ends were trimmed, and a short connecting tube was fit to the proximal end. The first cut under water (i.e., trimming) was 1.5 cm from the basal end to remove any harvest-induced cavitation, while the second cut at the distal end was made to create a 3-cm-long basal stem segment for use in this experiment. The samples were removed from the water just prior to attaching to the stain apparatus, and the water remaining in the connecting tube was replaced with safranin dye with the help of plastic syringes. Each stem segment was attached to a small tube that was attached to the main tubing, which lead to a reservoir containing safranin dye. Suspension of the reservoir, 1 M above the samples, created 0.01 MPa of gravimetric pressure. Each apparatus set-up (flask, main tube, and smaller tubes attached to samples) contained 10-12 stem segments at a time. Three set-ups were run simultaneously.

Dyed stem segments were detached from the tubing and set on blotting paper for a few minutes to drain excess dye. Each stem segment was then

cut cross-wise 0.5 cm away from the proximal end (the end at which dye enters the stem), and the cross-section inspected under a magnifying lens (10x) for staining. Cross-sectional areas of the dyed portion of the stem (i.e., functioning, non-cavitated tracheids) was visually estimated and recorded into 10% classes for each growth ring (first annual growth ring from 1996, closest to the pith, and second annual growth ring from 1997). Because non-stained areas were presumably due to cavitation, 0% to 10% staining (10% class) meant 90-100% cavitation, and vice versa. The pith was excluded from the cavitation assessment as it does not function in water conduction because of natural pith cell degeneration and air filling that occurs developmentally in all seedlings irrespective of drought.

Hydraulic conductivity (K_h)

On September 12, 1997, twenty random trees regardless of family were selected from each drought treatment to measure stem hydraulic conductivity. In addition, the 18 trees measured for g_s on September 9th were also measured for specific hydraulic conductivity (K_s). Results on these two groups of trees will be discussed separately.

Sample preparation for K_h assessment was similar to the procedure presented above for the cavitation method. However, instead of safranin dye, the stem segments were attached by tubing to a reservoir containing a weak solution of oxalic acid (1.26 g/L) to reduce the potential occurrence of bacterial occlusions. Suspension of the

reservoir, 1 M above the samples, created 0.01 MPa of gravimetric pressure (Sperry et al. 1988; LoGullo and Salleo 1991). The solution conducted through the stem was absorbed into lab tissue and weighed after one minute of collection. Three such measurements were taken for each sample and an average flow was calculated. Hydraulic conductivity was then estimated for each seedling using Equation 2.1.

Damage assessment

All seedlings assessed for xylem cavitation in both the moderate and severe drought treatments (5 blocks x 2 treatments x 39 families x 1 family row-plot x 2 trees per row-plot = 780) were scored visually into five 20% damage classes based on intensity of yellowing and browning of shoots in late August 1997 (Table 2.1). Due to some mortality, 84 trees were not available for sampling, thus, only 696 trees were assessed (780-84 = 696 seedlings). Control treatment seedlings were not included in

Table 2.1. Damage score, class and description of shoot damage of Douglas-fir seedlings.

Damage Score	Damage Class	Description
1	0 – 20 %	Dark green
2	21 – 40 %	Yellowish-green
3	41 – 60 %	Yellowish or light-green
4	61 – 80 %	Yellowish to brown
5	81 – 100 %	Brown and brittle

the injury assessment as no damage was observed, therefore, all trees in this treatment were in the first (0-20%) damage class. Each tree was scored separately by two people and the scores were averaged for each tree.

Growth measurements

Seedling height (mm) was measured from ground level to the top of the terminal bud eight times at monthly intervals (May 3 to November 5) during the second growing season and diameter (mm) of the main stem was measured at ground level above the root collar at the end of the season. The last two height measurements and the diameter measurement were made only on one-half of the 4,680 experimental trees because half of the trees (every other tree in a row) were thinned on September 12th following completion of cavitation assessment (1044 of these thinned trees were used for the cavitation assessment). Incidence of second-flushing was not assessed during the second-year drought treatments. However, from field observations, it was apparent that seedlings grown under well-watered conditions had a higher likelihood of second-flushing compared to seedlings grown under severe drought stress, which showed little if any flushes.

STATISTICAL ANALYSIS

The main objective of the research described in this chapter was to investigate average effects of drought treatments on physiological traits; the next chapter

addresses genetic differences in response to drought. Least square treatment means were estimated under an ANOVA model using the GLM procedure (SAS Institute Inc., 1997). While analysis of variance will be discussed in detail in the next chapter, the ANOVA model assumed under the GLM procedure is as follows:

$$Y_{ijklm} = \mu + t_i + b_j + tb_{ij} + f_k + ft_{ik} + fbt_{ijk} + p_{ijkl} + w_{ijklm}$$

where,

Y_{ijklm} is the individual tree value of the mth seedling in the lth sub-plot in the kth family in the jth block in the ith drought treatment,

μ is the overall mean,

t_i is the fixed effect of the ith drought treatment, $E(t_i) = t_i$,

b_j is the random effect of the jth block, $E(b_j) = 0$, $var(b_j) = \sigma^2_b$,

tb_{ij} is the main plot error, i.e., random interaction effect of the ith drought treatment with the jth block, $E(tb_{ij}) = 0$, $var(tb_{ij}) = \sigma^2_{tb}$,

f_k is the random effect of the kth family, $E(f_k) = 0$, $var(f_k) = \sigma^2_f$,

ft_{ik} is the random interaction effect of the kth family with the ith drought treatment, $E(ft_{ik}) = 0$, $var(ft_{ik}) = \sigma^2_{ft}$,

fbt_{ijk} is the random interaction effect of the kth family with the jth block with the ith treatment, $E(fbt_{ijk}) = 0$, $var(fbt_{ijk}) = \sigma^2_{fbt}$,

p_{ijkl} is the random effect of the lth sub-plot in the kth family in the jth block in the ith treatment, $E(p_{ijkl}) = 0$, $var(p_{ijkl}) = \sigma^2_p$,

w_{ijklm} is the random within family plot error of the mth individual tree in the lth sub-plot in the kth family in the jth block in the ith drought treatment, $E(w_{ijklm}) = 0$, $var(w_{ijklm}) = \sigma^2_w$.

The full model presented above is the basic model by which traits listed in Table 2.2 were tested for treatment differences. The full model was applied to height and diameter with no modifications. However, it was necessary to modify the model for the remaining physiological traits. For stomatal conductance, the block component was dropped from the model as it was not included in the sampling design, and

Table 2.2. A summary definition of traits and statistical analyses for assessment of traits.

Trait	Definition	N	Model Design[a]
Stomatal Conductance	Loss of water vapor through stomates	18	Trt + Fam +Trt*Fam
Hydraulic Conductivity	Ability to transport a volume of water through a given length under a given pressure	60	Trt + Blk + Trt*Blk
Cavitation	Area of air-blocked tracheids	1044	Trt + Blk + Trt*Blk + Fam + Trt*Fam + Trt*Fam*Blk
Shoot Damage	Visual stem and foliage damage	696	Trt + Blk + Trt*Blk + Fam + Trt*Fam + Trt*Fam*Blk
Height	Measured from ground to top of terminal bud	2340	Trt + Blk + Trt*Blk + Fam + Trt*Fam + Trt*Fam*Blk
Diameter	Measured at ground level	2340	Trt + Blk + Trt*Blk + Fam + Trt*Fam + Trt*Fam*Blk

[a] Trt = treatments; Blk = blocks; Fam = family.

differences between families (two families) and among treatments were evaluated for each sampling period over the course of the day. The family component was ignored for hydraulic conductivity (both K_h and K_s) as families were not specifically sampled. For cavitation, the model was adjusted to account for a single family plot per block, and for damage, the model contained only two treatments (moderate and severe)(Table 2.2). Fisher's least-significant pairwise t-tests were performed on treatment means with the 'T' option under the MEANS statement of the GLM procedure. All t-tests of significance were carried out at the 0.05 probability level. In addition, the difference between K_h of two damage classes (1 and 2) was tested using Cochran's approximate 't'-statistic for unequal variances (TTEST procedure, SAS Institute Inc., 1990a). However, as distinguishing family variation was not the primary objective of this chapter, only treatment level differences were established with the design presented in Table 2.2.

The second objective of this chapter was to determine the nature of interrelationships among these physiological responses to drought. The degree of relationship between specific hydraulic conductivity and stomatal conductance, between hydraulic conductivity and growth traits, between hydraulic conductivity and damage, hydraulic conductivity and xylem cavitation, and between xylem cavitation and shoot damage of seedlings were evaluated graphically. Phenotypic correlations (at the single-tree level) between K_h and growth traits were also estimated.

RESULTS AND DISCUSSION

Drought treatments had an almost immediate impact on soil water availability

as evident from predawn xylem water potential (Ψ_{pd}) readings in the three treatments

(Figure 2.1). Ψ_{pd} in the drought treatments was always lower than in the control,

averaging late in the season (July 21 through September 7, 1997) -1.7 MPa in the

moderate treatment, -2.5 MPa in the severe, and -0.8 MPa in the control.
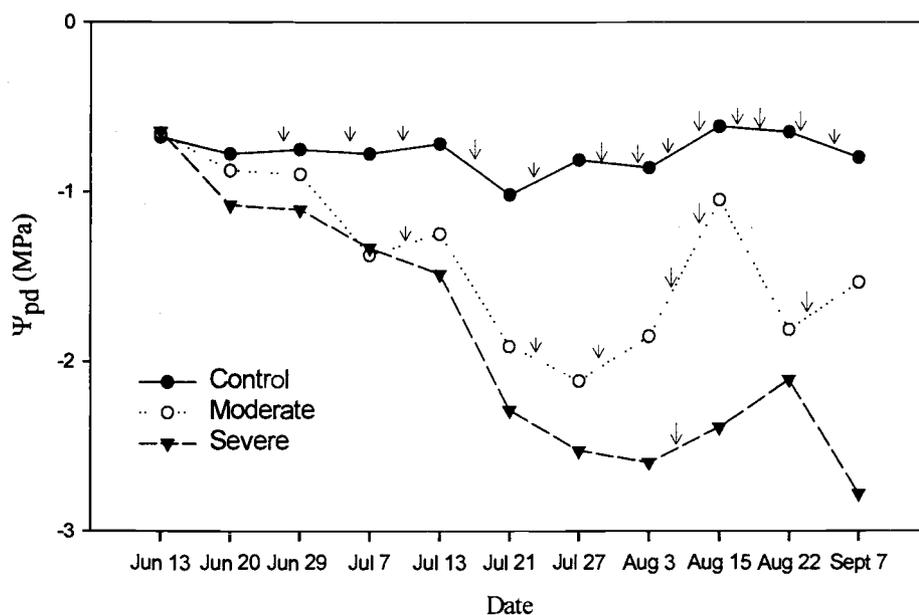
Figure 2.1. Average weekly predawn xylem water potential (Ψ_{pd}) measurements under three drought treatments (control, moderate, and severe) applied in 1997. Watering dates are indicated by down arrows.

Stomatal conductance and xylem water potential

The two families selected for this experiment did not differ significantly from

each other in diurnal g_s values measured at different times of the day; so, only pooled

data will be presented below. On September 9, when the drought treatments were at or near their peak, stomatal conductance of seedlings in the control treatment was much greater throughout the day than seedlings in the drought treatments (Figure 2.2). A high stomatal conductance indicates that the stomata are open and that water is flowing through the stem. Conversely, low g_s indicates that water flow and gas exchange are reduced. The diurnal pattern of stomatal conductance observed in the control seedlings is what is expected under well-watered conditions; where, stomata are closed during the night, begin to open as morning approaches, have peak opening around mid-morning, and then slowly close-down throughout mid-afternoon. This cycle was significantly (p < 0.05) altered under drought, even when the drought was only moderate. Seedlings in the severe treatment, however, barely opened their stomata by 8:00 a.m., and had nearly zero stomatal conductance most of the day.
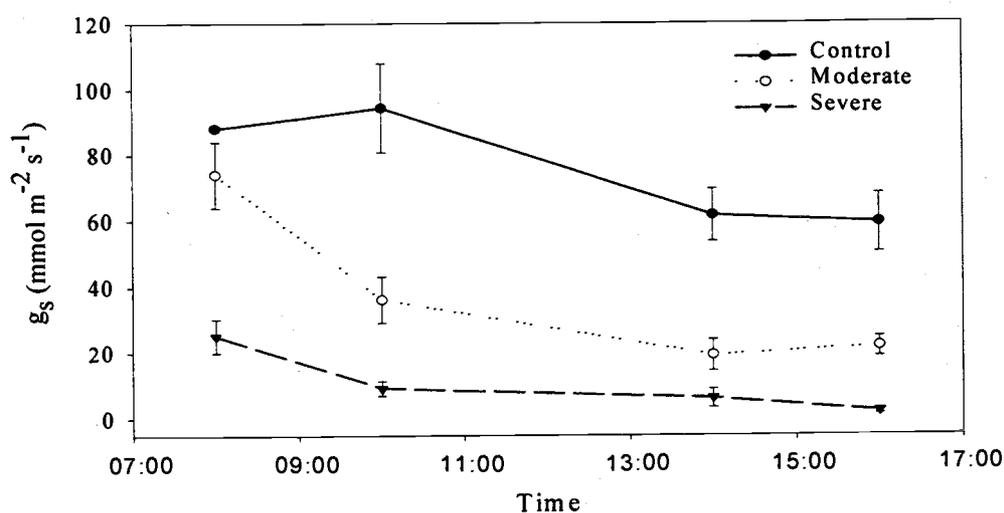
Figure 2.2. Diurnal mean stomatal conductance measurements (mmol of water loss per m^2 of needle surface per second) of seedlings (18 individuals from 2 families) grown in three drought treatments (control, moderate, severe) assessed during the peak drought period. Error bars are standard errors of the means.

A similar reduction in g_s for trees growing on xeric (dry) versus mesic (wet) sites was reported for *Acer grandidentatum* Nutt. over two summers (Alder et al. 1996). In this study, a severe drought during the summer of 1994 caused stems on the xeric site to be significantly ($p < 0.001$) more cavitated than those on a mesic site. They also found that a 5% increase in cavitation from one summer to the next could account for a corresponding decrease in peak g_s and the occurrence of incomplete refilling of stem xylem (i.e., reversed cavitation) due to severe drought. Johnson and Ferrell (1982) found that seedlings can readily recover from short periods of drought. In their study, three-year-old Douglas-fir seedlings were able to recover to control level g_s within 14 days of rewatering following a short period of water stress (9 days at Ψ_x of -5.0 MPa). Therefore, short periods of drought stress may not be severe enough to cause permanent damage to xylem conduits. In addition, Kavanagh et al. (1999) found that three-year-old Douglas-fir seedlings originating from coastal populations in Oregon began cavitation at higher Ψ_x (-3.0 MPa) than a drier source of the coastal variety and two sources of the inland variety (-3.5 MPa; var. *glauca*; inland wet or inland dry) and the loss of conductivity in the coastal wet population was nearly twice that found in inland wet or dry populations at water potentials higher than -4.5 MPa. Several studies indicate stomatal conductance does not recover following prolonged drought. For example, five-year-old *Picea abies* Karst., depending on the extent of xylem cavitation, had reduced g_s with increasingly negative Ψ_x during a series of recurrent drought periods given over the course of a growing season (Borghetti et al. 1989). Compared to their control treatment, they found significantly lower levels of g_s in droughted seedlings despite a 16-day recovery period in which the seedlings were

well-watered. In addition, newly outplanted *Tsuga heterophylla* (Raf.) Sarg., with an

initial maximum g_s of 143.5 mmol m^{-2} s^{-1} (Ψ_x = -1.17 MPa), were unable to re-reach

the initial level of g_s following exposure to Ψ_x great enough to cause cavitation (-1.23

MPa; Kavanagh and Zaerr 1997).

Diurnal patterns of xylem water potential (Ψ_x) in the seedlings closely reflect

expected patterns based on stomatal conductance of the same seedlings (Figure 2.3).

The control treatment showed higher Ψ_x than the drought treatments throughout the

day, and Ψ_x's of seedlings in the moderate drought were intermediate to those in the

control and severe drought treatments. A strong relationship between diurnal xylem

water potential and level of stomatal conductance was predicted in the theoretical

model described by Jones and Sutherland (1991). In addition, a strong relationship
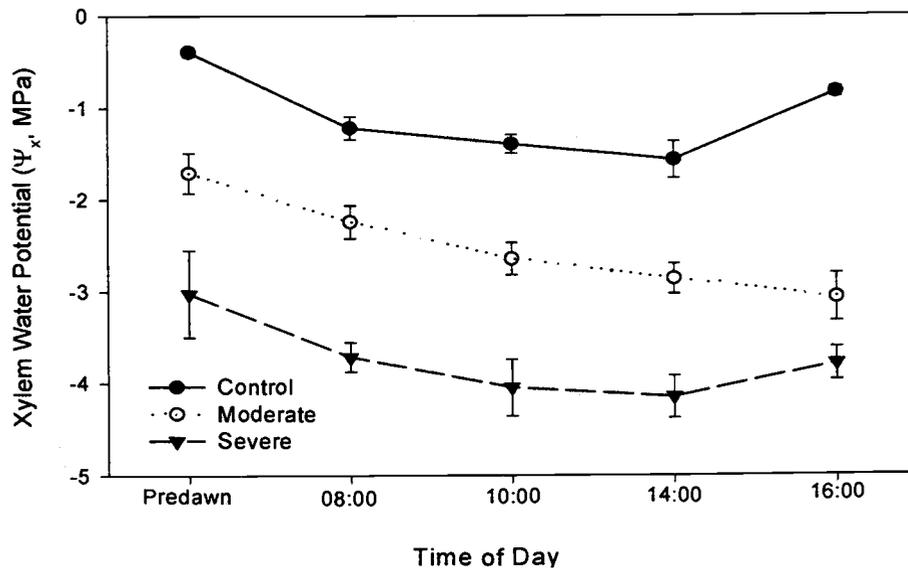
Figure 2.3. Bi-hourly mean xylem water potential measurements (MPa) of seedlings (18 individuals from 2 families) grown under three drought treatments (control, moderate, and severe) on September 9, 1997. Error bars are standard errors of means.

between these two traits has been demonstrated in *Quercus pubescens* Willd.

(Damesin and Rambal 1995), *Betula occidentalis* Hook. (Saliendra, et al. 1995), and

Pinus sylvestris L. (Jackson et al. 1995). All these studies have shown that

increasingly negative xylem water potential induces stomatal closure.

Cavitation

Percent xylem cavitation in the first annual growth ring was greater in the

drought treatments (39% in moderate, and 48% in severe), but differed significantly

from the control (37%) only in seedlings subjected to severe drought (Figure 2.4). The

large amount of cavitation in the first annual growth ring, even in the control

seedlings, was unexpected. Possible explanations for the high amount of cavitation in

this growth ring may be due to the following reasons: a) The pith at the seedling stage

has a spongy texture and is full of large intercellular air spaces (Taiz and Zeiger 1998),

thus, the pith and year-one interface may be largely responsible for the nucleation of

air bubbles into the first annual growth ring; b) 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

by preventing water uptake by the plant regardless of soil moisture (Joseph et al.

1998); c) Overwinter freezing events from the first season to the second also might

have induced cavitation either by freezing xylem sap and forcing air out of solution,

which nucleates cavitation in the subsequent thaw, or by favorable gas exchange

conditions occurring when ground water was frozen (Ewers 1985; Tyree and Sperry
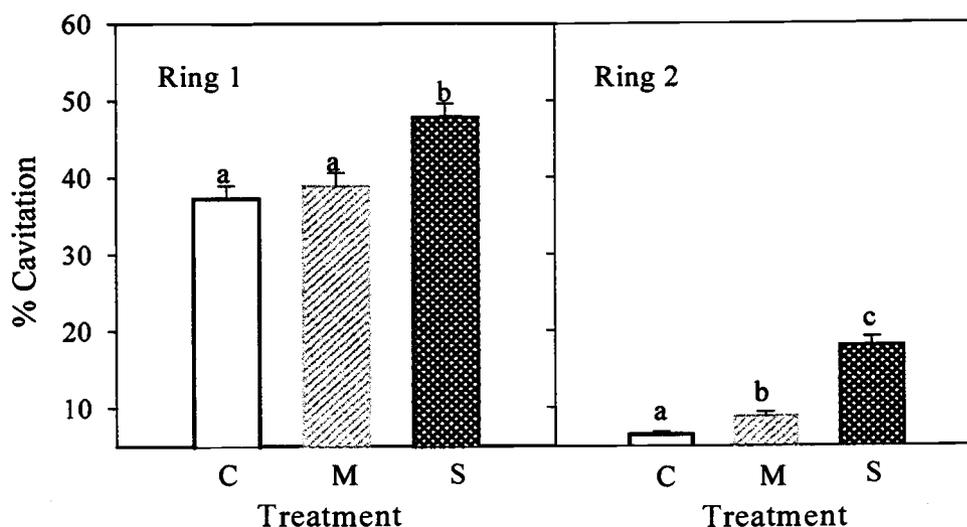
Figure 2.4. Mean percent cavitation of seedlings grown under three drought treatments (control (C), moderate (M), and severe (S)). Error bars are upper standard error of means. When letters over bars differ, mean values for treatments differ significantly ($p < 0.05$).

1990; Sperry et al. 1994). Likewise, subsequent cavitation in the second annual growth ring under strong negative xylem tension could be linked to availability of air emboli within the adjacent xylem tracheids of the first annual growth ring. Exploration of this trend in air seeding from the pith outward across the growth rings may help in understanding the mechanism of cavitation.

Cavitation in the second annual growth ring, differed significantly ($p < 0.05$) among the three drought treatments, with less than 10% cavitation in the control and moderate treatments and about 18% in the severe treatment. These results indicate that water stress was large enough to also cause significant cavitation in this ring.

The frequency distributions of percent cavitation among seedlings for the first annual growth ring were similar across the three treatments (Figure 2.5), confirming

that cavitation in this ring may be largely caused by conditions external to the
imposition of drought in the second growing season. The frequency distributions of
cavitation in the second annual growth ring, on the other hand, are progressively
skewed towards higher levels of cavitation with increasing drought. For example,
nearly 20% of seedlings in the severe drought treatment had more than 35% cavitation,
while less than 3% of seedlings in the control treatment reached this level of
cavitation.
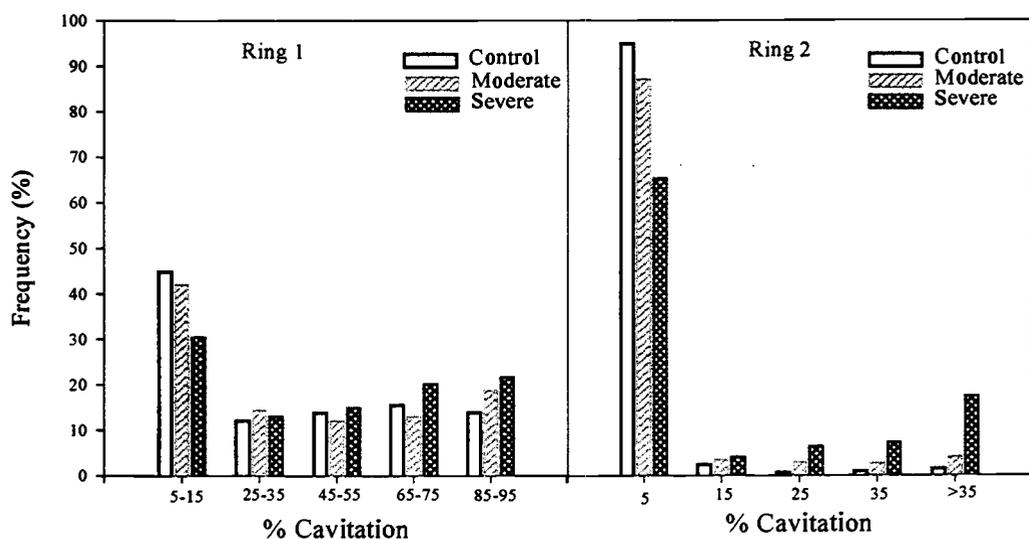
Figure 2.5. Frequency distributions of percent cavitation in the first and second annual
rings of seedlings grown in three drought treatments (control, moderate, and severe) in
the second growing season.

Quantifying the degree of cavitation in Douglas-fir seedlings is important
because of the likelihood that cavitation is irreversible in this species. Previous
research in pines indicated that refilling of cavitated tracheids (i.e., removal of

embolism) may be possible if positive water potentials above the level inducing cavitation occur (Borghetti et al. 1991; Borghetti et al. 1998; Sobrado et al. 1992; Edwards et al. 1994). However, conditions of positive xylem water potential do not typically occur in gymnosperms and there is a possibility that solute encrustation of bordered pit aspirations may disable the pit from opening again. It is presumed unlikely that refilling of Douglas-fir tracheids occurred during the period of this experiment.

Seedlings grown under the severe treatment during the second growing season of this study were allowed to grow under well-watered conditions the following year to assess recovery from drought (Anekonda, T.S., Oregon State University, unpublished data). Mean percent cavitation in the second annual growth ring of these seedlings increased to 38% after the third growing season (up from 18% at the end of the second growing season). Thus, there is no evidence for occurrence of reversal of drought-induced cavitation in the second ring during the recovery year. Because cavitation increased from the second to the third year despite well-watered conditions, the hypothesis that cavitation occurred due to intrinsic (characteristics of the pith) or environmental (winter freeze, hypocotyl rot, etc.) factors that triggered cavitation is supported, although not explicitly tested in this study.

Hydraulic conductivity

In this study, since the pressure gradient and stem segment length were held constant during K_h assessment, any change in flux is directly related to a change in

hydraulic conductivity. Stem hydraulic conductivity was significantly ($p < 0.05$) lower

for seedlings under the severe drought than for seedlings under the control, while

seedlings in the moderate treatment showed an intermediate level of hydraulic

conductivity (Figure 2.6). Due to the small sample size (20 seedlings per treatment)

and the large amount of variation in hydraulic conductivity of seedlings within

treatments, these results must be considered preliminary.
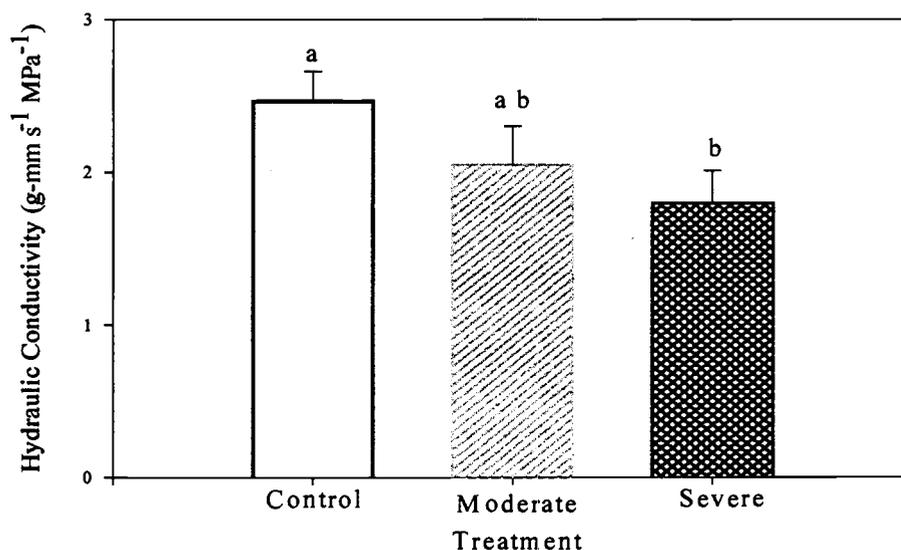
Figure 2.6. Mean hydraulic conductivity (g-mm s^{-1} MPa^{-1}) of seedlings grown under
three drought treatments (control, moderate, and severe). Bars are upper standard error
of means. When letters over bars differ, mean values for treatments differ significantly
($p < 0.05$) based on samples of 20 seedlings from each treatment.

Reduction of hydraulic conductivity in droughted seedlings may be due to four

causes: (i) loss of xylem function due to cavitation, (ii) reduced stem diameter

(Kavanagh et al. 1999), (iii) deposition of solutes, and (iv) a change in the ratio of

percent earlywood to latewood. Under drought conditions, newly formed cells are predominately latewood cells, thus creating a change in the ratio of earlywood to latewood. Any one or all of the above events reduce or change the total area available for water transport, which limits hydraulic conductivity (Sperry et al. 1996).

Hydraulic conductivity results from analyses of the 18 seedlings (9 per family) assessed for stomatal conductance also support the findings of the larger group. Due to the small sample size, difference in hydraulic conductivity between the two families was not statistically significant.

To evaluate if the difference in K_h between treatments was due to loss of functioning cross-sectional area, specific hydraulic conductivity was estimated. Results indicated that K_s was not found to differ significantly between treatments. However, diameter differences for the trees were significant between the control and moderate ($p < 0.05$), and between the control and severe ($p < 0.01$) treatments, but not between the moderate and severe. Despite the significance of diameter differences and, therefore, cross-sectional area, total tree size was still relatively small making it impossible to distinguish differences in K_s with the sample size used in this study. In addition, an exact estimate of functioning cross-sectional area was not available. It is evident that the treatment differences detected in K_h were at least partially due to differences in diameter between the treatments.

Growth traits

Although reduction in second year height (measured on September 30, 1997)
under the drought treatments was small (7%), mean second year height under drought
was significantly different (p < 0.05) from mean height in the control (Figure 2.7).
Mean heights for the moderate and severe treatments, however, were essentially
identical. The small reduction of height under drought and non-significant difference
between moderate and severe treatment can be explained by the timing of initiation of
the drought treatments relative to the seasonal timing of height growth. The drought
treatment was initiated during the first week of June, but the impact of drought on
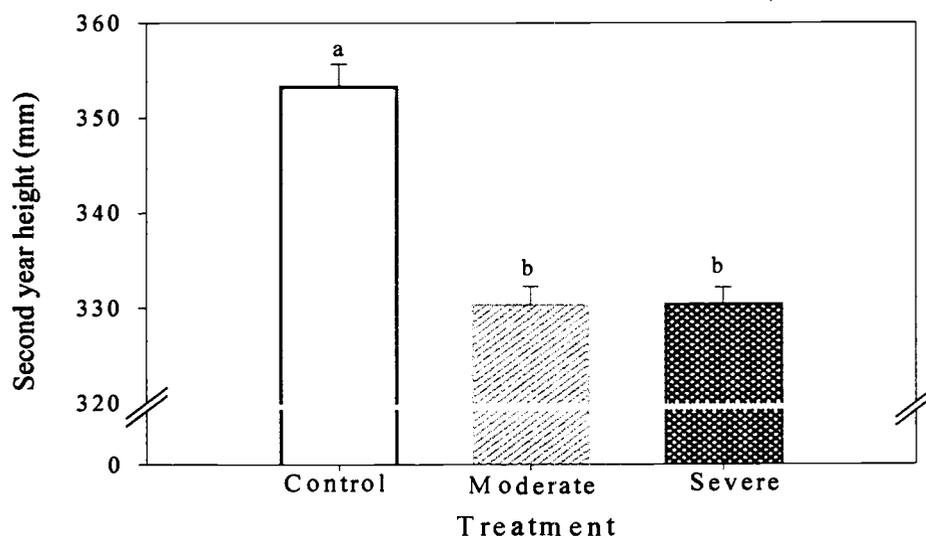
Figure 2.7. Total second-year height of seedlings grown under three drought
treatments (control, moderate, and severe). Bars are upper standard error of means.
When letters over bars differ, mean values differ significantly (p < 0.05).

stem growth was not evident until late July (Figure 2.8) when predawn xylem water

potential levels under drought went below -2.0 MPa. At this point, however, seedling

height growth was nearly complete (i.e., most of the cell, elongation had already

occurred). Only seedlings in the control treatment were able to continue height growth

after this point (perhaps by second flushing), but only for a brief period (until the end

of July), when height growth ceased. The slight decline in mean height of the control

seedlings after September 1, 1997, is due to sampling because only trees left after the

thinning were included in the measurements after this date.
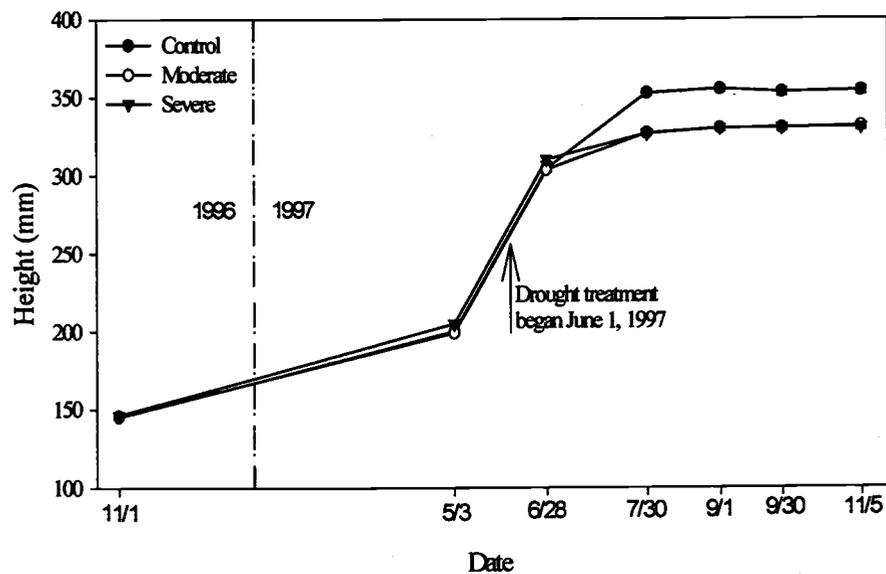
Figure 2.8. Incremental heights in the second growing season (1997) of seedlings grown under three drought treatments (control, moderate, severe). Dates of measurement are given as month/day.

As long as weather conditions are favorable, diameter growth continues well

into late summer and early fall (Vargas-Hernandez 1990). Because seasonal height

growth ended in late July for seedlings grown in the control (Figure 2.8), the duration

of diameter growth is more coincident with the timing of moisture stress than height

growth in this study. Indeed, year-end diameter was significantly reduced in both the

moderate and severe treatments relative to the control, with diameter in the severe

treatment about 11% less than in the control (Figure 2.9). We hypothesize that the

reduction in diameter under drought treatments is due to the production of smaller

latewood tracheids rather than larger earlywood tracheids. In another drought stress

study, when severe water stress was applied to eight-week-old Douglas-fir seedlings, a

21% reduction in diameter occurred in the severe (Ψ_x = -1.12 MPa) treatment relative

to the control (Ψ_x = -0.65 MPa)(Timmis and Tanaka 1976).
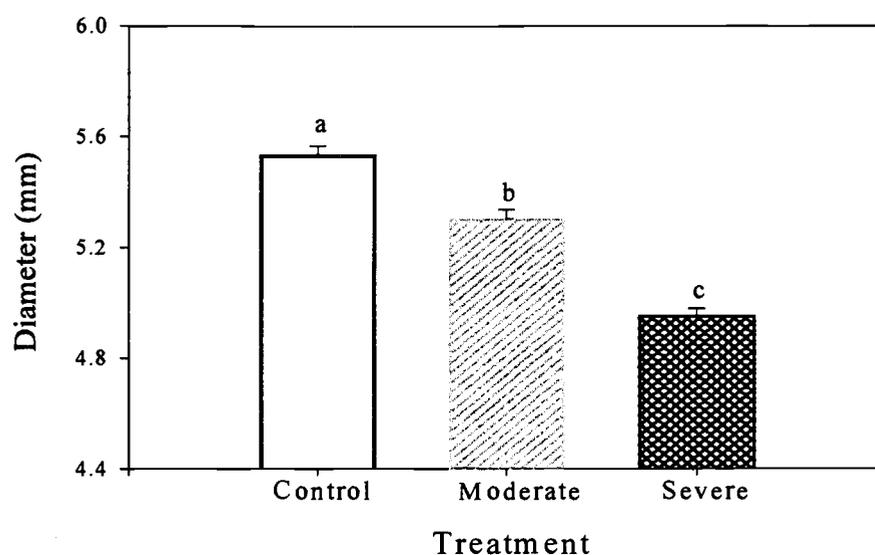
Figure 2.9. Total second-year diameter under three drought treatments (control, moderate, and severe). Bars are upper standard error of means. When letters over bars differ, mean values differ significantly (p < 0.05).

Damage

Average shoot damage score was significantly (p < 0.05) greater for seedlings

in the severe drought treatment than for seedlings in the moderate or control assuming

the control seedlings all received a score of 1 (Figure 2.10). This is estimated to

reflect a 4.5% higher degree of shoot damage under the severe drought treatment.

However, even under severe drought, the damage level was much lower than our

expected damage (i.e., a damage score of 3 or >40% shoot damage) level indicating

that the applied moisture stress, although effective, did not cause dramatic, visible

damage symptoms in the seedlings.
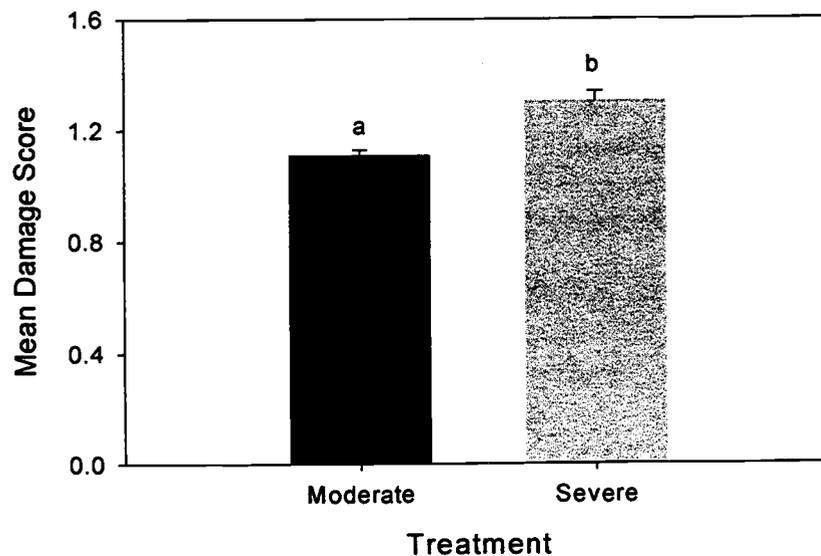
Figure 2.10. Mean shoot damage scores of Douglas-fir seedlings in the second
growing season under moderate and severe drought treatments. Bars are upper
standard error of means. When letters over bars differ, mean values differ significantly
between the treatments (p < 0.05).

Interrelationships among traits

Stomatal conductance measurements of the seedlings in the three drought treatments, made at either 10 a.m. (maximum conductance under well-watered conditions) or 2 p.m. (minimum conductance) on September 9, 1997, were roughly associated with their specific hydraulic conductivity (Figure 2.11). Generally, higher levels of stomatal conductance were related to higher levels of specific hydraulic conductivity, and vice versa. These results suggest that stomatal closure may result at least partially, in response to a drop in specific hydraulic conductivity.
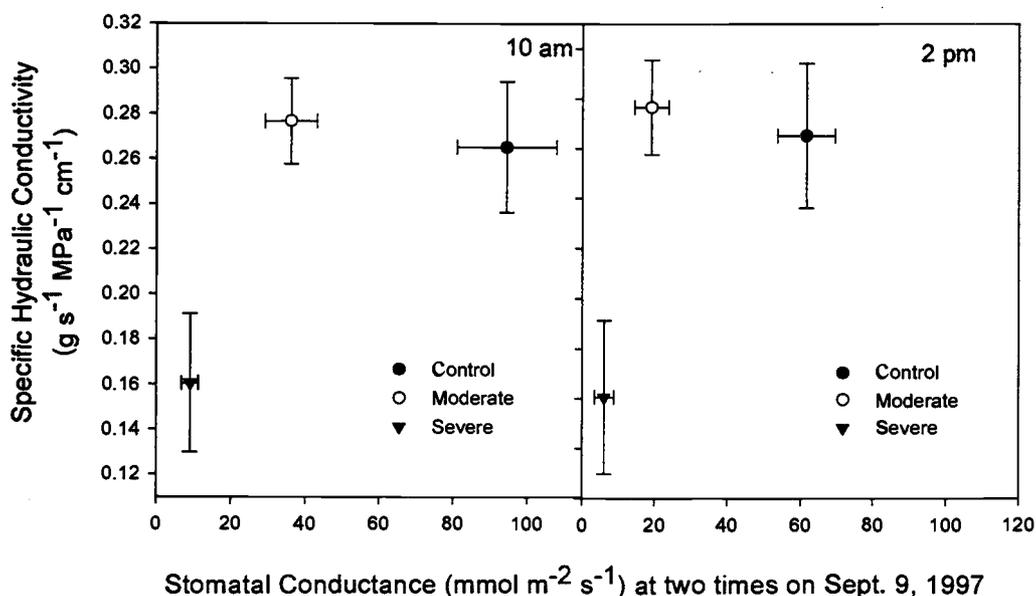
Figure 2.11. Treatment mean specific hydraulic conductivity measurement (g s^{-1} MPa^{-1} cm^{-1}) is plotted by treatment mean stomatal conductance measurements (mmol m^{-2} s^{-1}) made twice (10 a.m. and 2 p.m.) on September 9, 1997, under three drought treatments. Bars are standard error of means.

Hydraulic conductivity was positively correlated with both height (overall r = 0.59), and diameter outside bark (overall r = 0.77) of the seedlings (Figure 2.12). For an approximate 2-fold increase in diameter, conductivity increases 7-to-8-fold. This increase in the conductivity can be attributed to the number of functioning water
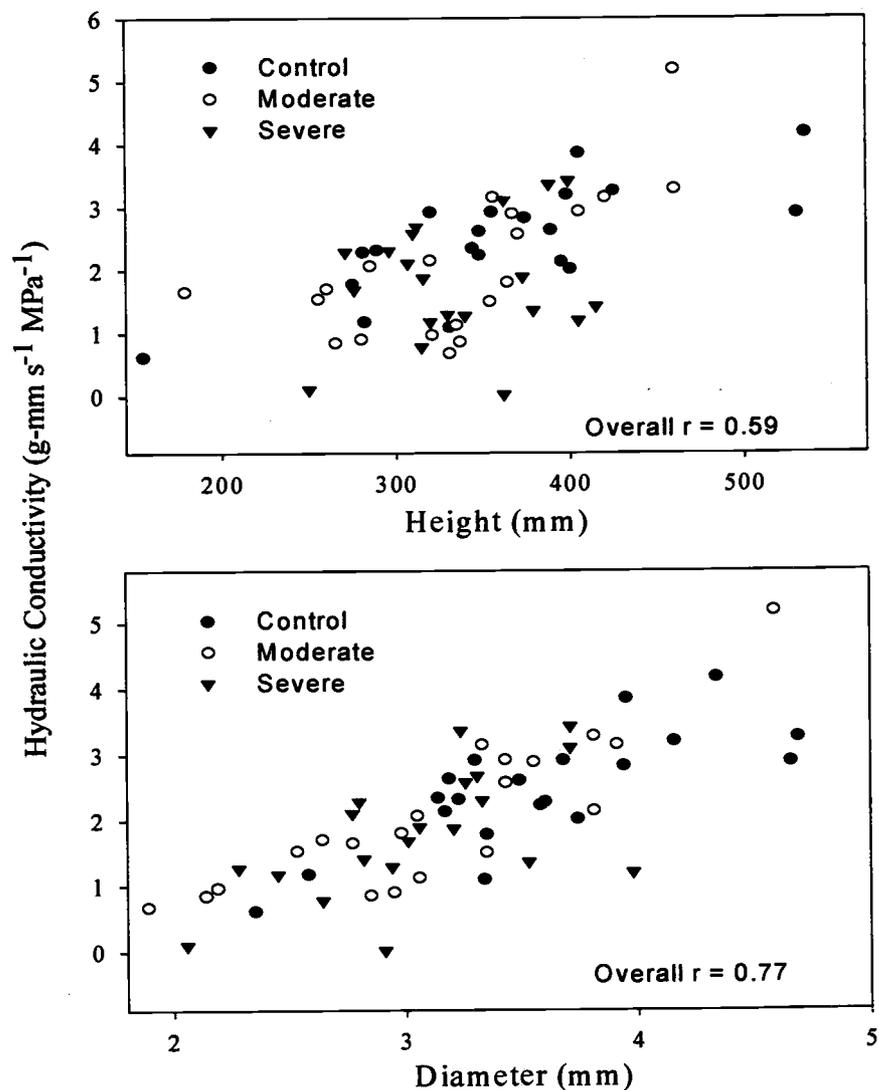
Figure 2.12. Hydraulic conductivity (g-mm s^{-1} MPa^{-1}) plotted over height (mm) and diameter outside bark (mm) of seedlings grown in three drought treatments (control, moderate, and severe).

conducting tracheids in the xylem tissue. Ewers (1985) has suggested that hydraulic

conductivity is proportional to the sum of the tracheid or vessel radii raised to the

fourth power. The size of the radii is related to the type of tracheid and tracheids in

latewood tend to have small radii compared to those in the earlywood (large radii).

Regardless, treatment means of K_h decreased with increasing cavitation from the

control to the severe drought treatment (Figure 2.13).
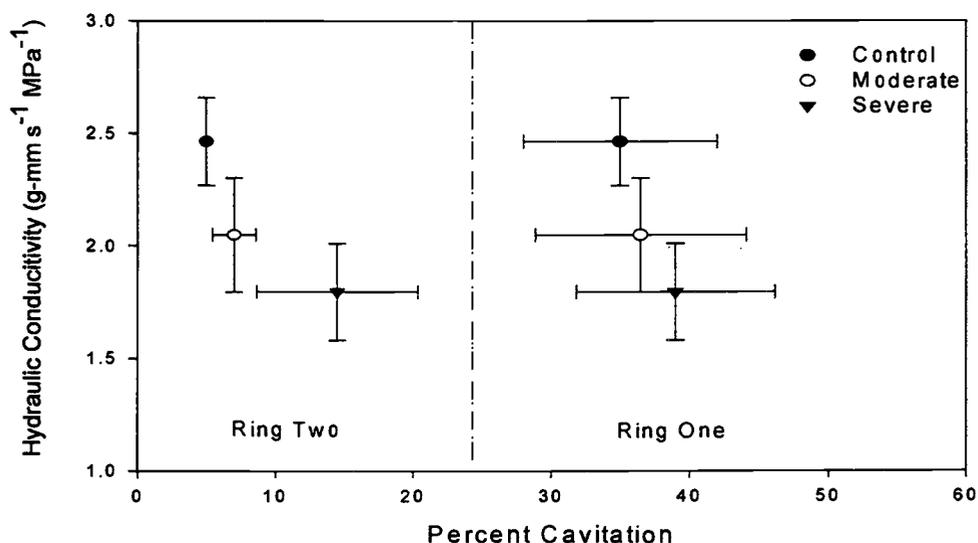
Figure 2.13. Treatment mean hydraulic conductivity (g-mm s^{-1} MPa^{-1}) measurement is
plotted by treatment mean percent cavitation for each annual growth ring (ring one
closest the pith and ring two the current season's growth) under three drought
treatments (control, moderate, and severe). Bars are standard error of means.

Analyses of damage scores on 40 trees assessed for hydraulic conductivity (i.e.,

moderate and severe treatment seedlings only) showed two distinct damage classes (1

and 2), therefore, the average hydraulic conductivity difference between these classes

was estimated. Two data points are missing due to location in the 1.5 data class from

having averaged scores between two people. Hydraulic conductivity of seedlings in

damage class 2 was less than 50%, on average, of seedlings scored in damage class 1

(Figure 2.14). Thus, a 50% decrease in hydraulic conductivity due to drought resulted

in only a modest, absolute increase in visible shoot damage (say from 10% on average

to 30%). Assuming that expression of damage symptoms occurs as a delayed response

to inability of the plants to provide tissues with sufficient water, apparent damage

might be greater with longer exposure to high levels of drought stress (i.e., as a

consequence of long-term disruption in water transport).
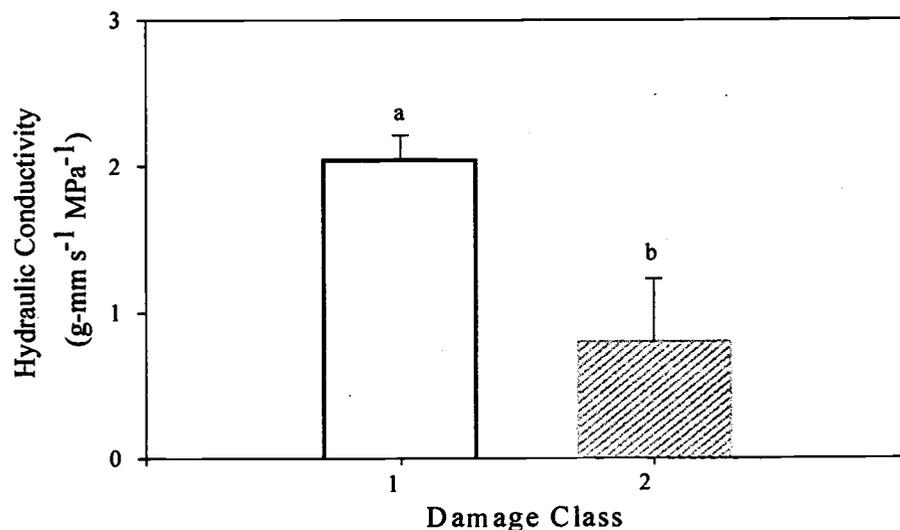
Figure 2.14. Hydraulic conductivity (g-mm s^{-1} MPa^{-1}) for two damage classes (34 seedlings in class 1 and 4 seedlings in class 2) for seedlings grown under moderate and severe drought treatments combined. Bars are upper standard error of means. When letters over bars differ, mean values differ significantly between the classes (p < 0.05).

No relationship was found between xylem cavitation in either annual growth ring and K_s. The lack of relationship may be due to the assessment of cavitation by individual growth ring rather than as an estimate of reduction in the functioning xylem of the entire stem cross-section. A slight positive, but non-significant ($p < 0.05$) relationship, despite a great deal of overlap in the data, was found between xylem cavitation and shoot damage. Thus indicating the possibility that higher levels of xylem cavitation are associated with increased shoot damage. Other potential combinations of interrelationships such as cavitation versus damage, and damage versus height and diameter will be discussed in the next chapter where genetic analyses will be done on these traits.

CONCLUSIONS

Drought had significant impacts on all physiological traits measured (i.e., reduced height and diameter growth, reduced capacity for water transport (as measured by cavitation and hydraulic conductivity), and shoot damage; objective 1), but absolute impacts were relatively smaller than expected given the high level of moisture stress applied. Similarity in diurnal patterns of change in xylem water potential and stomatal conductance measurements made on the same day (September 9, 1997) in different drought treatments suggests that these physiological processes are intimately linked (Waring and Running 1998). Cavitation in the first annual growth ring appeared to be primarily influenced by conditions external to the application of drought treatment in the second growing season such as air-seeding from the pith, root rot, or winter xylem

sap freeze-thaw. Therefore, scoring cavitation in this ring is of limited usefulness for assessing the effects of second season drought on seedlings in this experiment. Cavitation in the second annual growth ring was particularly sensitive to the applied drought suggesting that it is potentially useful for assessing drought injury in Douglas-fir. Hydraulic conductivity of seedlings decreased significantly under severe drought relative to the well-watered control. Even under moderate drought, g_s never attained the levels observed for seedlings grown in the control treatment, and stomates were forced to close earlier in the day to avoid cavitation. The degree of seedling shoot damage under the severe drought was greater than that in the moderate treatment with the control serving as the baseline for comparing moderate and severe. Both height and diameter growth were reduced under drought, but because the drought treatments were initiated midway in the growing season (i.e., assuming the growing season is from budburst to cessation of diameter growth), drought treatments were more coincident with diameter growth, thus drought effects were more apparent on diameter than on height growth.

Predawn xylem water potential, hydraulic conductivity, stomatal conductance, and shoot damage are all interrelated (objective 2). Low xylem water potential (more negative) is an indication of severe moisture-stress, which reduces the hydraulic conductivity of the seedlings. A positive relationship found between specific hydraulic conductivity and stomatal conductance suggests an intimate mechanistic link between these two physiological processes. Severe moisture-stress also damaged xylem tracheids (i.e., increased cavitation) and increased shoot damage.

Large numbers of seedlings were assessed for cavitation, shoot damage, height and diameter, including all 39 families in this study. Therefore, genetic control of these traits and their interrelationships will be discussed in detail in the next chapter.

CHAPTER 3

QUANTITATIVE GENETICS OF SEEDLING DROUGHT HARDINESS, GROWTH POTENTIAL, AND THEIR INTERRELATIONSHIPS IN COASTAL DOUGLAS-FIR

ABSTRACT

Genetic control of drought hardiness (shoot damage and xylem cavitation under drought conditions), growth potential (height and diameter under well-watered conditions), and their interrelationships were investigated in two-year-old, full-sib families (N = 39) of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) seedlings from British Columbia. Seedlings were grown in raised nursery beds and subjected to three soil moisture treatments in their second growing season (June-September 1997): control (well-watered), moderate drought, and severe drought. Xylem cavitation was assessed as the percentage of non-functioning tracheids in each annual growth ring as detected by safranin dye perfusion. The seedlings were also visually scored for shoot injury (needles and stem) in response to drought.

Family variation for percent cavitation in the first annual growth ring was significant ($p < 0.001$) in all treatments, but high family mean correlations between treatments indicated that genetic variation in cavitation of the first annual growth ring is independent of growing season moisture regime. Family variation in cavitation in the second growth ring was significant ($p < 0.0001$) only in the severe drought treatment, suggesting that this trait was particularly sensitive to severe, current-season

drought. Family variation was significant ($p < 0.05$) for both height and diameter under the control treatment.

Despite relatively high ratios of specific to general combining ability variance for most traits, family mean heritabilities for cavitation (second growth ring), shoot injury, and stem height and diameter were moderately high ($h_f^2 = 0.41$ to 0.58). Strong estimated family mean (0.52) and genetic correlations (1.12) between cavitation in the second growth ring and shoot damage, indicates that these traits are largely under the control of the same set of genes. Damage traits are estimated to have a slight, unfavorable genetic correlation with growth potential traits, thus selection for stem growth at the seedling stage is not expected to have a large influence on the drought hardiness of seedlings. Gain in drought hardiness ability (i.e., reduced shoot damage) could be substantial if the selection is based on shoot damage.

INTRODUCTION

Current information on genetic variation of drought hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) originates primarily from geographic variation studies. While specific information regarding the response of Douglas-fir to artificial or seasonal drought is fairly limited, previous research has shown that seedlings from south-facing, and therefore more xeric (dry) sites are less likely to survive summer droughts than those from north-facing, mesic (wet) sites (Ferrell and Woodard 1966). During summer months drought induced moisture-stress not only limits natural regeneration of coastal Douglas-fir seedlings (Hobbs et al.

1980), but also reduces basal area increment in mature trees (Spittlehouse 1985). Successful regeneration on drought-prone sites depends on the inherent ability of genotypes to tolerate moisture-stress. Many traits, morphological to molecular, have been shown to respond to moisture-stress in Douglas-fir (Kozlowski and Pallardy 1997). In general, drought-stress decreases photosynthesis and transpiration (Zavitkovski and Ferrell 1968; Zavitkovski and Ferrell 1970), as well as the percent of live needles (i.e., shoot damage), thus reducing growth potential in coastal Douglas-fir (White 1987). Dark respiration traits investigated in a subsample of the families used in the study described in this chapter, decreased in response to drought, although the response differed among families (Anekonda and Adams, in press). Natural selection on xeric sites has favored drought hardiness ability in Douglas-fir, such that genotypes from dry sites have slower growth rates (White 1987; Joly et al. 1989; Aitken et al. 1995), reduced basal area increment (Spittlehouse 1985), reduced top to root dry mass ratio (Hermann and Lavender 1968; Sorenson 1983), and earlier budset (White 1987) than genotypes from moist habitats.

The ability to withstand drought is linked to the internal dynamics of xylem tissue under water-stress conditions. For example, moving across an environmental gradient from the Coast Range to the Cascade Mountains, a trend of decreasing stomatal conductance, specific conductivity, and leaf specific conductivity was found among coastal Douglas-fir populations (Panek 1996). Coupled with this, Panek (1996) noted that, moving in the same direction across the gradient, the proportion of xylem tracheids that are closed due to air-blockage caused by drought-induced tension on the water column (cavitation) steadily increased. Since the proportion of xylem

cavitation is related to a variety of physiological mechanisms, which in turn affect drought survival ability, it is helpful to understand the effect of drought-stress on cavitation induction.

One way of expressing the propensity for cavitation due to drought is through vulnerability curves, whereby the percent loss of maximum water flow in stems (hydraulic conductivity) is plotted against increasing water tension (i.e., xylem water potential, Ψ_x). Kavanagh et al. (1999) estimated vulnerability curves for coastal and inland populations of Douglas-fir and found that seedlings from coastal populations, on average, began to cavitate (lose hydraulic conductivity) at a lower water tension (Ψ_x = -3.0 MPa) than inland populations (Ψ_x = -3.5 MPa). This difference in cavitation initiation between sources from moist and dry habitats implies that populations naturally adapted to drier sites are less vulnerable to cavitation than populations adapted to wet sites.

Because Douglas-fir breeding programs in the Pacific Northwest are planning to expand the existing small and numerous breeding zones into fewer, larger breeding zones, broadly adapted Douglas-fir genotypes will be needed. Developing larger breeding zones essentially means moving genotypes further away from their source locations, including transfers to more stressful conditions (e.g., droughty sites). The efficient development of drought-hardy genotypes requires accurate information on heritability of drought hardiness traits and their interrelationships with other traits of interest, particularly stem growth.

Currently, no information on the heritability of drought hardiness traits is available for coastal Douglas-fir. To address this shortcoming, the Pacific Northwest

Tree Improvement Research Cooperative (PNWTIRC) recently subjected seedling families of coastal Douglas-fir to three moisture stress regimes (Seedling Drought Physiology Study). The goals of this research were to identify key physiological mechanisms associated with short-term moisture stress responses in seedlings, to evaluate the genetics of drought hardiness traits, and to develop criteria for screening improved families for drought hardiness in breeding programs.

Chapter 2 of this thesis reported the response of seedlings to drought applied in the second growing season. The results suggested that xylem cavitation in the second annual growth ring and shoot damage are both promising measures of sensitivity to drought (i.e., drought hardiness), while seedling height and diameter growth may be little influenced by moderate or even severe drought applied in late spring or early summer. Wide variation was observed among individuals for cavitation in the first annual growth ring, but this variation seemed to be only marginally related to drought, as relative family performance for this trait did not change across treatments. Therefore, in this chapter, the genetics of two key traits signaling susceptibility to summer drought were investigated: xylem cavitation in the second annual growth ring and visible shoot damage. In addition, because breeding programs emphasize improved growth rate, growth potential and interrelationships of growth potential with drought hardiness traits were also investigated. Specifically, the research objectives addressed are:

1. Determine the nature and extent of genetic variation in drought hardiness (i.e., resistance to xylem cavitation and shoot damage) and growth potential (height and diameter) traits in seedlings of coastal Douglas-fir,

2. Determine the degree of genetic association between drought hardiness traits and their relationship with growth potential, and

3. Evaluate the potential for genetic improvement of seedling drought hardiness in Douglas-fir breeding programs and the expected correlated response in drought hardiness if selection is for growth potential and vice versa.

MATERIALS AND METHODS

Detailed descriptions of the treatments, methods and measurements of physiological traits are given in chapter 2. Presented below is a brief summary.

Plant materials and experimental design

Seeds of the 39 full-sib families used in this study were obtained from the British Columbia Ministry of Forestry. Parent trees used in the crossing program (see mating design below) came from southwestern British Columbia (Vancouver Island and the coastal mainland) sampling areas of high, medium, and low summer moisture availability. Therefore, the seedling progenies are expected to show wide variation in their response to drought-stress conditions.

Seeds from the families were sown into raised nursery beds at Oregon State University in Corvallis, Oregon, grown for the first year under well-watered conditions, and exposed to three levels of moisture during their second growing

season. The experimental design was a split-plot replicated in five blocks. Main plots consisted of the three watering regimes (treatments), that were applied June-September, 1997. The nursery beds were covered temporarily during rainy periods and watering regulated on the basis of predawn xylem water potential readings (Ψ_{pd}) taken weekly on auxiliary seedlings. Average Ψ_{pd}, when moisture-stress reached the targeted level until the end of the drought treatments, was -0.8 MPa in the control (watered once or twice per week), -1.7 MPa in the moderate drought (watered every other week), and -2.5 MPa in the severe drought treatment (watered once during the treatment period). Within the main plots, each of the 39 families was represented by 8 trees in two randomly-located, 4-tree family row-subplots. Experimental seedlings totaled 4,680 at planting (5 blocks x 3 treatments per block x 39 full-sib families x 2 row plots per family x 4 seedlings per family subplot). The total survival of seedlings at the onset of the second growing season was 88.3%.

Cavitation assessment

In early September, the main stem of two seedlings in one subplot of each family was harvested from all main plots for xylem cavitation measurement. On average, each family was represented by 8.9 (range 6-10) individuals per treatment. The proportion of xylem that was cavitated in each stem segment was assessed indirectly. Using a gravitational feed system, safranin dye was passed through a 3 cm length of stem. Two hours after staining, dyed stem segments were detached from the system, cut 0.5 cm away from the proximal end and the cross-section inspected under

a magnifying lens (10x). The proportion of dyed area, representing functional, non-cavitated tracheids, was visually estimated for the first and second annual rings separately into 10% classes. Non-stained cross-sectional area was presumed to be due to cavitation, therefore, 0-10% staining meant 90-100% cavitation and vice versa. Because pith cells do not function in water transport, the pith was excluded from cavitation assessment.

Growth and damage assessments

Height growth was completed by the first of August in the second growing season (chapter 2), and height and diameter of all seedlings were measured on September 30, and November 5, 1997, respectively. Because shoot damage has been shown to be a good indicator of assessing injury due to drought (White 1987), seedlings assessed for cavitation were also scored for percentage of shoot injury (i.e., shoot damage). Shoot damage appeared to be nearly absent in the control seedlings, and was, therefore, not measured in this treatment. Shoot damage was scored visually (into 20% classes) in late August on all seedlings grown under the moderate and severe drought treatments. Because xylem cavitation in these treatments was assessed on two seedlings from one subplot of each family, shoot damage of only these seedlings will be analyzed in this chapter. The darker green the foliage, the healthier the seedling, and the lower the damage score. Seedlings with brown or yellow foliage were scored into higher classes of damage.

Description of traits

In total, genetic variation in ten traits is examined in this chapter (Table 3.1).

Results from chapter 2 suggested that cavitation in the first annual growth ring was

primarily due to causes other than imposition of drought in the second growing season.

That is, the cavitation found in the first annual growth ring may be due to such causes

as air seeding from the pith, the occurrence of freeze-thaw induced air bubble

formation, conditions permitting gas exchange (i.e., a pull on the water column

inducing tension) while ground water is frozen, or in response to a root disease

(*Fusarium*, controlled later in the first season) that infected the seedlings in the early

Table 3.1. Description of xylem cavitation, shoot damage and growth traits assessed under three drought treatments during the second growing season.

Trait	Drought Treatment	Symbol	Trait Description	Unit
Xylem Cavitation	Severe	R1_S	First annual growth ring	%
		R2_S	Second annual growth ring	%
	Moderate	R1_M	First annual growth ring	%
		R2_M	Second annual growth ring	%
	Control	R1_C	First annual growth ring	%
		R2_C	Second annual growth ring	%
Shoot Damage	Severe	Dam_S	Visual shoot damage	%
	Moderate	Dam_M	Visual shoot damage	%
Growth Potential	Control	Ht_C	Height measured from ground level to tip of the terminal bud	mm
		Dia_C	Diameter of the main stem measured above the root collar at ground level	mm

months after germination, creating drought conditions internal to the seedling by limiting water uptake ability. The first annual growth ring is included in this chapter in order to evaluate whether there is genetic variation in the expression of this trait and to further explore the degree to which the trait is influenced by the drought treatments. Xylem cavitation in the second annual growth ring was identified in chapter 2 as a good candidate for assessing drought hardiness, because the drought treatments appeared to have a large effect on the amount of cavitation observed in this ring (e.g., only 4% mean cavitation in the control versus 18% in the severe drought treatment). Cavitation in the control treatment (both in the first and second growth rings) was included in the analyses in order to examine genetic variation in the propensity for cavitation when there is no drought stress. Although mean percent shoot damage was low in both drought treatments (12.5% in the moderate and 16.8% in the severe), it increased significantly with increasing moisture stress. Genetic variation in growth potential was examined by analyzing height and diameter in the absence of drought stress.

Mating design

The British Columbia Ministry of Forestry originally constructed four disconnected, six-parent half-diallel mating sets ((6x5)/2 = 15 full-sib families per diallel) in series #6 of their crossing program in 1978. From these crosses, seed was available in storage to make up four smaller (five-parent) half diallels, consisting, with

one exception, of 10 crosses each (Table 3.2). Seed was unavailable from one cross in one of the half-diallels resulting in the total of 39 ((4 x 10) - 1) full-sib families.

Table 3.2. The mating design for one half-diallel derived from crossing five parents. The resulting 10 full-sib families are shown as $F_{\#\#}$.

Female Parents	Male Parents				
	1	2	3	4	5
1		F_{12}	F_{13}	F_{14}	F_{15}
2			F_{23}	F_{24}	F_{25}
3				F_{34}	F_{35}
4					F_{45}
5					

STATISTICAL ANALYSIS

Analysis of Variance

To evaluate the nature and extent of genetic variation in the measured traits (Table 3.1; objective 1), all traits were subjected individually to analysis of variance and variance components and genetic parameters estimated. Analyses of variance were conducted assuming the following random effects model:

$$Y_{ijklm} = \mu + b_i + d_j + f_{jk} + (bd)_{ij} + (bf)_{ijk} + (pf)_{ijkl} + e_{ijklm} \qquad \text{Equation 3.1}$$

where,

Y_{ijklm} is the value of each trait for the *m*th tree in the *l*th sub-plot of the *k*th family within the *j*th diallel in the *i*th block,

μ is the overall mean,

b_i is the random effect of the ith block, $E(b_i) = 0$, $var(b_i) = \sigma^2_b$,

d_j is the random effect of the jth diallel, $E(d_j) = 0$, $var(d_j) = \sigma^2_d$,

f_{jk} is the random effect of the kth family within the jth diallel, $E(f_{jk}) = 0$, $var(f_{jk}) = \sigma^2_{f(d)}$,

$(bd)_{ij}$ is the random interaction effect of the jth diallel with the ith block, $E(bd_{ij}) = 0$, $var(bd_{ij}) = \sigma^2_{bd}$,

$(bf)_{ijk}$ is the random interaction effect of the kth family within the jth diallel with the ith block, $E(bf_{ijk}) = 0$, $var(bf_{ijk}) = \sigma^2_{bf(d)}$,

$(pf)_{ijkl}$ is the random effect of the lth sub-plot within the kth family within the jth diallel in the ith block, $E(pf_{ijkl}) = 0$, $var(pf_{ijkl}) = \sigma^2_{pf}$,

e_{ijklm} is the random error of the mth tree in the $ijkl$th sub-plot, $E(e_{ijklm}) = 0$, $var(e_{ijklm}) = \sigma^2_e$.

The interaction of block with diallel ($(bd)_{ij}$) was excluded in the final analyses as it was non-significant in all traits except Dam_M, so the sums of squares associated with this component were pooled with the $(bf)_{ijk}$ component. Shoot damage and xylem cavitation traits were also analyzed according to Equation 3.1, but since these traits were sampled from only one sub-plot, $(pf)_{ijkl}$ was excluded from the analysis. Analyses first employed the GLM procedure of the SAS statistical software package (SAS Institute Inc. 1997) to test the significance of family differences using $(bf)_{ijk}$ as the error term (denominator) in F-tests. Type I and Type III sums of squares were

nearly identical. Therefore, all tests in this study are based on Type I sums of squares.

Variance components of the linear model were estimated using the restricted

maximum likelihood (REML) method of the SAS VARCOMP procedure (SAS

Institute Inc. 1990a).

Initial analysis indicated that the frequency distributions of residuals for both

shoot damage and cavitation in the second annual growth ring were significantly

skewed from normality. No transformation sufficiently corrected the non-normality,

therefore, the analysis was performed on non-transformed 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 to be significant at the 5 percent level for these traits, may actually be two to

three percent less-significant (i.e., 7 to 8 percent level; Steel et al. 1997). All traits

were standardized prior to estimation of variance component analyses, so that their

mean was equal to zero and standard deviations equal to 10 (PROC STANDARD

procedure; SAS, Institute Inc. 1990b). Standardization minimized scale effects of the

traits when they needed to be combined (see below). Because standardization is not a

type of transformation, it does not affect the actual values of the data in relation to the

variance associated with each model component. So, analyses of variance were

performed on the standardized data and no back-transformation was necessary.

Both GCA and SCA effects contribute to family variation within diallels (f_{jk})

(Griffing 1956). The GCA effect distinguishes between the average effect of parents

involved in a cross, while the SCA effect quantifies the deviation of the mean of a

specific cross from the average general combining ability of the two parents.

Expressed mathematically, f_{jk} (from Equation 3.1 above) is a combination of g_j, g_k, and s_{jk}; where, g_j and g_k are general combining ability effects of the jth and kth parents, respectively, and s_{jk} is the specific combining ability effect of the full-sib family (or cross) resulting from the jth and kth parents.

The main difficulty with diallel mating designs is apportioning the family within diallel variance ($\sigma^2_{f(d)}$) into GCA and SCA variance components. In the past, estimation of GCA and SCA variances has been facilitated by special computer programs such as DIALL, which lack the ability to handle large data sets (e.g., high numbers of replications or sites; Schaffer and Usanis 1969). Recently, Johnson and King (1998) proposed a relatively simple method using two VARCOMP analyses in SAS to effectively partition full-sib family variances into GCA and SCA variances.

In the first VARCOMP analysis (VARCOMP 1) of each trait in this study, the data were analyzed according to Equation 3.1 and a REML estimate of the family within diallel variance ($\sigma^2_{f(d)}$) obtained. The expectation of $\sigma^2_{f(d)}$ is the covariance of individuals within full-sib families minus the covariance of individuals from different full-sib families in the diallel (Johnson and King 1998). The covariance of individuals within a full-sib family is equal to one half of the additive variance plus a quarter of the dominance variance ($1/2\ \sigma^2_A + 1/4\ \sigma^2_D$). The covariance of individuals from different full-sib families is equal to zero if all full-sibs are unrelated. However, within the five parent half-diallels in this study, the 10 full-sib families within a diallel are interrelated in such a way that individuals within any given full-sib family have a half-sib relationship with individuals in six out of the nine ($6/9 = 2/3$) remaining families (Table 3.2). Half-sibs have a covariance equal to 1/4 of the additive variance

(1/4 σ^2_A). Therefore, the value of covariance for individuals from different full-sib

families for a five-parent, half-diallel is equal to 1/6 σ^2_A (or, 2/3 x (1/4 σ^2_A)). Thus,

$$\text{VARCOMP 1 } \sigma^2_{f(d)} = (1/2\ \sigma^2_A + 1/4\ \sigma^2_D) - (1/6\ \sigma^2_A) \qquad \text{Equation 3.2}$$

$$= (1/3\ \sigma^2_A + 1/4\ \sigma^2_D)$$

To separately estimate σ^2_A and σ^2_D, a second VARCOMP (VARCOMP 2) is

required. In addition to the terms in Equation 3.1, the linear model for the

VARCOMP 2 analysis included four dummy variables (P1 – P4) created to account

for the GCA effects of the parents in the diallels (Johnson and King 1998). Only four

dummy variables are needed in a five-parent diallel mating design because 4 variables

are sufficient to account for the GCA differences among the five-parents. Because the

parental dummy variables partition out GCA effects from $\sigma^2_{f(d)}$ effects in VARCOMP

2, the family within diallel variance ($\sigma^2_{f(d)}$) in this analysis equals σ^2_{SCA} or one quarter

of the dominance variance (1/4 σ^2_D). Thus,

$$\sigma^2_{f(d)} \text{ from VARCOMP 2} = 1/4\sigma^2_D = \sigma^2_{SCA} \qquad \text{Equation 3.3}$$

From Equations 3.2 and 3.3, σ^2_A can be estimated by,

$$\sigma^2_A = 3 * (\sigma^2_{f(d)} \text{ in VARCOMP 1} - \sigma^2_{f(d)} \text{ in VARCOMP 2}) \qquad \text{Equation 3.4}$$

Also note that, from Johnson and King (1998),

$$\sigma^2_A = 4 * \sigma^2_{GCA} \qquad \text{Equation 3.5}$$

and,

$$\sigma^2_{GCA} = 1/4 \ \sigma^2_A \qquad\qquad \text{Equation 3.6}$$

Johnson and King (1998) pointed out that σ^2_{GCA} cannot simply be estimated from

VARCOMP 2 by summing the variance attributed to each dummy variable.

Presumably this is because partitioning σ^2_{GCA} into four components (i.e., by using 4

dummy variables) results in inaccurate estimates of each of the individual components

and their sum.

An F-statistic to test the significance of GCA variance was calculated by

running a second GLM analysis in SAS with the same model used under VARCOMP

2. Because the GCA effect has been separated from the SCA effect by the inclusion of

the P1-P4 dummy variables, the average of their mean squares reflects the mean

squares for the GCA effect. The appropriate F-statistic can then be calculated as

follows (R. Johnson, USFS, PNW Res. Sta., Corvallis, OR, personal communication):

$$\text{F-statistic for GCA effects} = \frac{\left(\begin{array}{c}\text{Average mean squares for}\\ \text{dummy variables (P1 - P4)}\end{array}\right)}{(\text{Mean squares for f(d)})} \qquad \text{Equation 3.7}$$

$$= \ MS_{GCA} \ / \ MS_{SCA}$$

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]) with a missing family in one

of the diallel sets, where d represents the number of diallels and n is the number of

parents per diallel set. The variance of SCA was tested by dividing the mean squares

for f_{jk} in VARCOMP 2 by the mean square for block x family interaction.

Heritability

The individual tree, narrow sense heritability of each trait was estimated

following King et al. (1998):

$$h_i^2 = \sigma_A^2 / (\sigma_d^2 + (2*\sigma_{GCA}^2) + \sigma_{SCA}^2 + \sigma_{bf(d)}^2 + \sigma_{pf}^2 + \sigma_e^2) \quad \text{Equation 3.8}$$

This estimate of heritability should be appropriate to making selections of individuals

irregardless of the diallel of origin because the variance associated with diallel set is

included in the denominator (phenotypic variance) of the above equation.

The family heritability (h_f^2) of each trait was estimated as:

$$h_f^2 = \sigma_f^2 / [\sigma_d^2 + \sigma_f^2 + (\sigma_{bf(d)}^2/b) + (\sigma_{pf}^2/bp) + (\sigma_e^2/bpt)] \quad \text{Equation 3.9}$$

where b is the number of blocks (5), p is the number of sub-plots per full-sib family in

a block (2), and t is the harmonic mean number of trees (average of 1.89 across traits;

range 1.68 to 2.00) per sub-plot. Note, because both cavitation and shoot damage

traits were sampled from one sub-plot, σ_{pf}^2 is equal to zero in Equations 3.8 and 3.9.

Genetic correlation

The extent to which two traits are under the same additive gene control is

estimated by genetic correlations (objective 2). Genetic correlations (r_A) between

height and diameter and between shoot damage and cavitation in the second annual

growth ring were estimated following Burdon (1977):

$$r_A = \frac{Cov_{A(1,2)}}{\sqrt{\sigma^2_{A(1)} * \sigma^2_{A(2)}}}$$

Equation 3.10

where $Cov_{A(1,2)}$ is the covariance between the additive effects of traits 1 and 2, and, $\sigma^2_{A(1)}$ and $\sigma^2_{A(2)}$ are the estimated additive variances of the respective traits. When both traits were measured on the same individuals, σ^2_A was estimated for the sum of the two traits ($\sigma^2_{A(1+2)}$) following the procedure of Johnson and King (1998) outlined above. $Cov_{A(1,2)}$ was then estimated using the following equation:

$$Cov_{A(1,2)} = (\sigma^2_{A(1+2)} - \sigma^2_{A(1)} - \sigma^2_{A(2)}) / 2$$

Equation 3.11

To evaluate the genetic association between growth potential and drought hardiness traits, genetic correlations were estimated between stem growth (height and diameter) in the control treatment and cavitation and shoot damage under the severe treatment. Analogous to methods described by Burdon and Apiolaza (1998), the additive genetic covariance ($Cov_{A(1,2)}$) between growth potential (1) and drought hardiness (2) traits was calculated by first summing the plot means of the corresponding traits in the control and severe treatments of each main plot. The treatment sums were then analyzed using the VARCOMP 1 and VARCOMP 2 procedures and σ^2_A for sums of the two traits ($\sigma^2_{A(1+2)}$) was estimated. The $Cov_{A(1,2)}$ was then derived by subtraction as in Equation 3.11, where $\sigma^2_{A(1)}$ and $\sigma^2_{A(2)}$ are the estimated additive variances for each trait in the respective treatments. Estimated genetic correlations were then calculated using Equation 3.10.

Direct and correlated responses

The ability to genetically improve seedling drought hardiness by selection was evaluated by calculating expected direct responses (i.e., reduction) in stem damage and cavitation when either trait is selected against. Expected correlated response in drought hardiness traits was also calculated when improved growth potential (i.e., height and diameter) is the target of selection. Both direct and correlated responses were estimated assuming that the best 20.5% (8 of the 39 families) of the parents were selected based on their GCA. The expected response to direct selection (R_y) may be calculated as (Shelbourne 1969):

$$R_y = 2i(h^2_{f(y)})\sigma_{p(y)} \qquad \text{Equation 3.12}$$

where i is the selection intensity expressed in standard deviations (i = 1.4); $h^2_{f(y)}$ is the family heritability of the response trait (i.e., shoot damage, y); and, $\sigma_{p(y)}$ is the estimated phenotypic standard deviation of family means for the response trait. However, since the distribution of both cavitation and shoot damage data exhibited non-normality, inclusion of the selection intensity variable (i) was found to upwardly bias the results, therefore, a more conservative calculation for estimating gain from selection was used (Falconer and Mackay 1996):

$$R_y = 2(h^2_{f(y)})SD_{(y)} \qquad \text{Equation 3.13}$$

where $SD_{(y)}$ is the selection differential of the response trait (shoot damage, y). $SD_{(y)}$ was estimated as the mean of the selected families (N = 8) minus the mean of all 39 families (N = 39).

Expected correlated response (CR_x) to selection was also calculated using the selection differential rather than the selection intensity approach as (Falconer and Mackay 1996):

$$CR_x = b_{(A)yx}R_y \qquad \text{Equation 3.14}$$

and,

$$b_{(A)yx} = r_{A(x,y)}\left[\frac{\sigma_{A(x)}}{\sigma_{A(y)}}\right] \qquad \text{Equation 3.15}$$

were $\sigma_{A(x)}$ and $\sigma_{A(y)}$ are the square roots of the estimated additive variance for the selected (y) and response (x) traits, and $r_{A(x,y)}$ is the estimated genetic correlation between the selected and response traits.

RESULTS AND DISCUSSION

Variation and frequency distribution of family means

Ranges among family means were large for cavitation, shoot damage and growth potential traits and family variances were significant (p < 0.05) for all but cavitation in the second annual growth ring in the moderate and control treatments, and for shoot damage in the moderate treatment (Table 3.3). Significant family variation for cavitation in the first annual growth ring in all treatments including the

control, suggests that cavitation in this ring has a high degree of genetic determination

in all treatments. However, family variation for cavitation in the second annual

growth ring was significant (p < 0.0001) only in the severe treatment. Ranges among

family means for both second annual ring cavitation and shoot damage were greater in

the severe drought than in the moderate drought treatment. Ranges in family means

for first annual ring cavitation, on the other hand, were uniformly large in all

treatments.

Table 3.3. Means and ranges of growth and damage traits assessed under severe, moderate, and control drought treatments.

Treatment	Trait[a]	Family mean	Range	F-value (p-value)
Severe	R1 S	48.3	6.0-77.0	2.84 (0.0001)
	R2 S	17.9	5.0-60.6	2.82 (0.0001)
	Dam S	16.9	10.0-36.7	1.82 (0.0372)
Moderate	R1 M	38.9	9.0-83.6	2.34 (0.0005)
	R2 M	8.7	5.0-19.3	1.00 (0.4442)
	Dam M	12.5	10.0-20.0	1.37 (0.4599)
Control	R1 C	36.7	10.6-75.8	2.53 (0.0004)
	R2 C	6.4	5.0-12.5	0.91 (0.6972)
	Ht C	353.3	299.8-440.5	5.62 (0.0001)
	Dia C	5.5	4.6-6.7	3.11 (0.0001)

[a] A description of these traits can be found in Table 3.1.

The frequency distributions of family means for cavitation in the first annual

growth ring were similar in all three treatments (Figure 3.1). As with variation among

individual trees (chapter 2), the frequency distribution of cavitation in the first annual

growth ring among family means was not influenced by drought. On the contrary,

cavitation in the second growth ring showed differential, treatment-specific frequency

distributions (Figure 3.1). In addition, while no families had mean cavitation greater

than 20% in the control and moderate treatments, mean cavitation exceeded 20% in

44% of the families in the severe drought treatment.
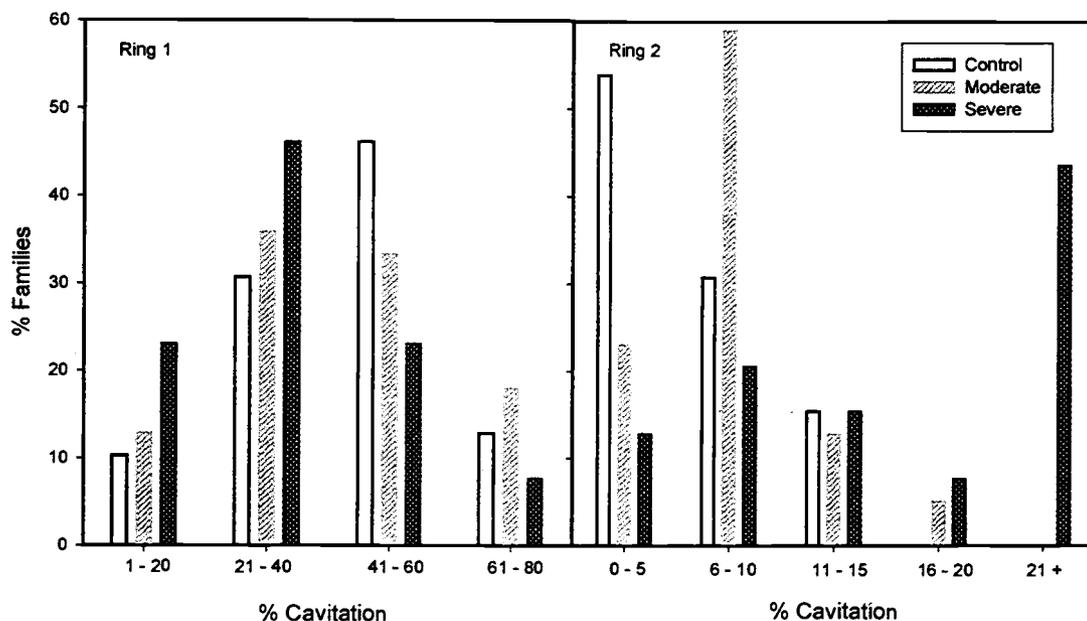
Figure 3.1. Frequency distribution of family means for percent xylem cavitation in the
first and second annual growth rings of seedlings subjected to three levels of drought
(control, moderate, and severe).

The low, non-significant family mean correlations between cavitation in the

second growth ring in the control and the drought treatments indicate that family

differences in ring two cavitation differed depending on whether there was drought or

not (Table 3.4). Estimated correlations of family means between treatments for

cavitation in the first growth ring, however, were moderately strong (mean = 0.61)

indicating that relative differences among families in cavitation were similar in the different treatments (Table 3.4). There was little, if any, relationship between annual growth rings within or between treatments.

Table 3.4. Estimated correlations between family means for percent cavitation in different growth rings (R1= ring 1, R2 = ring 2) of seedlings subjected to three moisture regimes (C = Control (well-watered), M = moderate drought, S = severe drought) in the second growing season.

Trait	R1_C[a]	R1_M	R1_S	R2_C	R2_M
R1_M	0.66****				
R1_S	0.54***	0.64****			
R2_C	0.02 ns	-0.01 ns	-0.14 ns		
R2_M	0.05 ns	0.10 ns	-0.07 ns	0.06 ns	
R2_S	-0.11 ns	-0.14 ns	-0.11 ns	-0.19 ns	0.27 ns

[a] Significance is denoted as: *** = p < 0.001; **** = p < 0.0001; ns = non-significant.

The similarity of frequency distributions of family mean cavitation in the first annual growth ring and the moderately strong correlations of these means between treatments, indicates family differences in cavitation of the first annual ring were not influenced by the drought treatments. Significant family differences in cavitation in the second annual ring, however, were only observed in the severe drought, indicating that differential drought hardiness was the cause of the differences. The very different frequency distribution of family means in the severe drought and the low estimated genetic correlations between cavitation in the second annual ring in the severe drought

and other treatments supports this hypothesis. Because only cavitation in the second annual growth ring, and shoot damage when seedlings were subjected to severe drought, appear to reflect genetic variation in drought hardiness, only these traits will be compared as measures of drought hardiness.

Family means for height and diameter were also moderately to strongly correlated between treatments (mean = 0.83 (range 0.81 to 0.84) and 0.66 (range 0.62 to 0.69) for height and diameter, respectively) suggesting that drought treatments had little effect on total seedling growth (chapter 2). Since the growth traits assessed were greatest in the control, it is assumed that the potential for growth is best expressed under well-watered conditions. Therefore, only two drought hardiness traits (R2_S, Dam_S) and two growth potential traits (Ht_C and Dia_C) will be considered in the remainder of this chapter.

Combining ability and heritability estimates

Estimates of general and specific combining ability variances and narrow-sense and family heritabilities for the two drought hardiness and two growth potential traits are presented in Table 3.5. Variance component estimates derived from VARCOMP 1 and VARCOMP 2 analyses of the data are given in Tables C.1 and C.2, respectively of Appendix C, and the calculations for determining the significance of GCA variance are presented in Table C.3 of this appendix. σ^2_{SCA} was significant for R2_S, Ht_C, and Dia_C indicating that dominance effects are important for these traits. The

Table 3.5. Estimates of general combining ability (σ^2_{GCA}) and specific combining ability (σ^2_{SCA}) variances, the ratio of σ^2_{SCA} to σ^2_{GCA} (x 100; %SCA), and individual (h^2_i) and family (h^2_f) heritabilities for cavitation in the second ring (R2_S) and shoot damage (Dam_S) in the drought treatment, and seedling height (Ht_C) and diameter (Dia_C) in the control.

Trait	σ^2_{SCA}[a]	σ^2_{GCA}[b]	SCA %	h^2_i	h^2_f
R2_S	0.869**	12.515*	6.944	0.46	0.57
Dam_S	4.056 ns	3.073 ns	131.988	0.12	0.41
Ht_C	8.367***	5.762**	145.210	0.28	0.47
Dia_C	5.119**	1.623 ns	315.404	0.07	0.58

[a] Significance of variance components: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = non-significant; see also Table C.2 in Appendix C.
[b] See Table C.3 in Appendix C for estimation of F-statistic for testing σ^2_{GCA}.

dominance effect includes non-additive gene action as well as non-genetic and maternal effects (Falconer and Mackay 1996). Because of the limited number of families tested, genetic parameters are estimated with considerable error in this study. Nevertheless, the high amount of σ^2_{SCA} observed in three of the four traits suggests that much of the genetic variation in these traits will remain unexploited in breeding programs that rely entirely on additive variance.

While the results shown here suggest that σ^2_{SCA} is stronger than σ^2_{GCA} for growth potential traits at age two, Yeh and Heaman (1987) found that the GCA variance in seven-year-old Douglas-fir trees was six times larger than SCA variance based on field progeny tests in British Columbia. Similarly, Yanchuk (1996) found that growth trait σ^2_{GCA} was three times greater than σ^2_{SCA} based on both seven- and

twelve-year-old Douglas-fir trees. A possible explanation for this is that %SCA declines with age as observed in *Pinus radiata* D. Don for both height and diameter from age two to seven (King et al. 1998). In support of the results presented in the current investigation, Yanchuk's study also showed a higher SCA ratio for diameter than for height, suggesting a greater association of dominance effects with diameter than with height.

Individual tree, narrow-sense heritability estimates ranged from 0.07 for Dia_C to 0.46 for R2_S (Table 3.5). Estimates for Ht_C and Dia_C are within the range usually observed for growth traits in coastal Douglas-fir (Yea and Heaman 1987; Stonecypher et al. 1996; Yanchuk 1996). The estimated h^2_i for R2_S is relatively high (0.46), but may be upwardly biased because of the non-normality of this trait. Family heritabilities ranged from 0.41 for Dam_S to 0.58 for Dia_C. Extensive family variation and moderate family heritabilities indicate that substantial genetic response could be expected in all these traits from family or parent-tree selection, even if only general combining ability is utilized (e.g., as in a wind pollinated seed orchard).

Genetic and family mean correlations

Estimated genetic ($r_A = 0.74$) and family mean ($r_f = 0.75$) correlations between height and diameter were strong and positive (Table 3.6). The agreement between the size and sign of the genetic and family mean correlations suggests that both genetic and environmental factors that affect family means for height and diameter may be expressed through similar physiological mechanisms (Falconer and Mackay1996).

Estimated correlations between height and diameter shown here are in agreement with several other studies in pines and other conifers (Cornelius 1994) and more specifically in Douglas-fir (Vargas-Hernandez 1990).

Table 3.6. Genetic (below diagonal) and family mean (above diagonal) correlations among and between growth and damage traits.

Trait	Ht_C	Dia_C [a]	R2_S [b]	Dam_S
Ht_C	--	0.75****	0.01 ns	-0.07 ns
Dia_C	0.74	--	0.01 ns	0.00 ns
R2_S	0.02	0.18	--	0.52****
Dam_S	0.52	0.18	1.12	--

[a, b] Significance of family mean correlation: **** = $p < 0.0001$; ns = non-significant;

The two drought hardiness traits also appear to be strongly and positively intercorrelated ($r_f = 0.52$, $r_A = 1.12$). The strong, positive genetic correlation indicates that these two traits are largely under control of the same set of genes (objective 2). That is, susceptibility to damage of shoot under severe drought is probably largely a function of reduced water conducting ability as a result of cavitation. If both traits are largely reflecting the expression of the same drought hardiness genes, the choice of which trait to assess in screening for seedling drought hardiness is a trade off between selection accuracy and cost of assessment.

There is little evidence of correlation between growth potential and drought hardiness traits. At the family mean level, estimated correlations between growth

potential and drought hardiness were near zero. Estimated additive genetic correlations between these two sets of traits appear to be weakly positive, at most. The $r = 0.52$ correlation may be somewhat anomalous, as family mean correlation between height and damage was near zero, and all other additive genetic correlations between growth potential and damage were very small. Thus, the evidence indicates that there may be a slight unfavorable genetic correlation between growth in favorable conditions and drought hardiness.

Direct and correlated responses for drought hardiness

Because shoot damage and cavitation are essentially reflecting the same drought hardiness genes, and have similar family heritabilities, shoot damage should be the trait of choice when selecting for drought hardiness ability considering it is a much easier trait to measure. Despite low σ^2_{GCA}, substantial reduction in shoot damage is still expected if selection is for the least damaged families. In addition, because of the high genetic correlation between cavitation and shoot damage, cavitation is also expected to be substantially reduced. Although these estimates must be viewed with caution because of the non-normality of the drought hardiness traits, selection of the top 20.5% of the parents with the least shoot damage in two-year-old seedlings is expected to result in a 24.6% decrease in mean shoot damage at age two in the following generation, and a 55.7% reduction in mean cavitation, 3.8% reduction in mean height, and a 1.2% reduction in mean diameter. If selection for increased drought hardiness ability was done using cavitation as the selected trait, a 78.2%

decrease in cavitation, 34.5% decrease in shoot damage, 0.2% decrease in height, and a 1.7% decrease in diameter are expected.

Because most breeding programs focus on improved growth potential, the impact of height and diameter selection on drought hardiness should also be considered. Selection for increased height at age 2 is expected to result in a 1.9% increase in mean cavitation and a 21.6% increase in mean shoot damage. Similarly, selecting for increased diameter, is expected to increase cavitation and shoot damage by 19.4% and 8.2%, respectively. At face value, the ~20% increase in damage traits due to selection for growth potential is not trivial. However, since the family mean of these two traits is roughly 18%, an absolute increase of < 4.0% to either shoot damage or cavitation is estimated when selection is based solely on growth potential traits. Because the genetic correlations between these traits is small enough, there should be little difficulty in finding genotypes that are both drought hardy and have good growth potential, as long as both traits are measured.

CONCLUSIONS

Drought hardiness at the seedling stage, as measured by xylem cavitation or shoot damage under severe drought applied during the growing season, has a considerable amount of genetic variation as shown by the large range in family means. In fact, the drought hardiness traits possessed as much or more genetic variation on a relative scale than growth potential traits (second year height and diameter under well-watered conditions). Although estimates of narrow-sense heritabilities were probably

biased (upwardly) by non-normal distributions of both drought hardiness traits, the estimated individual tree heritabilities were twice the magnitude for cavitation than for stem damage, indicating that cavitation is under stronger genetic control. However, at the family level, heritabilities were similar in magnitude between these two traits.

Evidence suggests that the genetic association between growth potential traits and drought hardiness traits is weak, at most, in the seedling stage. Still, results indicated a slightly unfavorable relationship between these traits. This would likely create less drought hardy trees with increased height or diameter if selection is based solely on growth potential.

The gain in drought hardiness could be substantial if either shoot damage or xylem cavitation is selected against. Selection against shoot damage is less costly and its estimated family heritability was nearly equivalent to that found for xylem cavitation. Therefore, for selection purposes, shoot damage is the recommended drought hardiness trait of choice between the two hardiness traits investigated here. Given the strong genetic correlation between these two hardiness traits, selection based on one trait will improve the other trait as well. If selection is solely for growth potential, a small decrease in hardiness is expected. So, in areas where summer drought is likely to be a problem, both traits should be selected for simultaneously (e.g., by using selection index).

CHAPTER 4

GENERAL CONCLUSIONS

THESIS CONCLUSIONS

Seedling physiology of coastal Douglas-fir was strongly influenced by drought treatments applied in this study. All physiological mechanisms assessed here did not respond to drought in the same fashion, nor did they appear equally useful for screening Douglas-fir for drought hardiness. The main conclusions of this thesis are as follows:

1) In response to severe summer drought, predawn xylem water potential, hydraulic conductivity, and stomatal conductance decreased, while cavitation and shoot damage increased.

2) There is a nearly identical mean difference between cavitation in the control and cavitation in the severe treatments for both annual growth rings. So cavitation in both rings is responding to drought, although the frequency distribution among trees suggests that drought has more influence on the second annual growth ring than the first as evidenced by the differential effect among trees in the second ring.

3) The relative performance of families for cavitation in the first annual growth ring did not change between drought treatments indicating that factors other than the imposed drought may have influenced the family variation in cavitation in this ring.

4) Drought treatments had relatively limited impact on stem growth in the second growing season, but more so on stem diameter than height. Diameter growth was more impacted presumably because it occurred primarily during and beyond the onset of severe drought stress.

5) Families differed greatly and significantly for both cavitation in the second annual growth ring and shoot damage when grown under the severe drought treatment.

6) Despite high proportions of SCA variance for most traits, moderately strong family heritabilities and family variation suggests that both seedling drought hardiness and growth potential traits can be readily improved in this species using breeding methods that rely on GCA.

7) Cavitation in the second annual growth ring and shoot damage appear to be largely under control of the same set of genes ($r_A = 1.12$). The choice of which trait to utilize in selection for seedling drought hardiness is a trade-off in selection accuracy versus cost. Direct selection for reduced shoot damage is expected to result in a 24.6% reduction in mean family shoot damage in the next generation, with a modest unfavorable influence on height (3.8% decrease) and diameter (1.2% decrease) growth.

8) A minimal, but positive, unfavorable association was found between damage due to drought and growth potential in the absence of drought. Correlated response due to selection for stem growth also indicates a slight unfavorable reduction in drought hardiness ability.

FUTURE RESEARCH

Cavitation assessments made in this study were based on subjective scoring, therefore, the scoring is less accurate than quantitative measurements. Quantifying cavitation would require the use of image analysis, which can be very time consuming and costly. Alternatively, specific hydraulic conductivity that also quantifies xylem function can be used. In this study, specific hydraulic conductivity was assessed only on a limited number of seedlings and was adjusted by the entire cross-sectional area rather than the area still functioning in water transport, therefore, it was not possible to determine the usefulness of this trait in screening for drought hardiness. Future studies should examine and compare specific hydraulic conductivity and cavitation as measures of drought hardiness.

Assessment of cavitation in the annual ring of the current year's growth (the second annual growth ring in this study) when seedlings are subjected to drought appears to be useful for screening Douglas-fir for drought hardiness. However, such an assessment is destructive. It would be useful in future research to compare branch and stem cavitation in order to establish hardiness relationships between these organs. A strong correlation in cavitation between branches and stems would mean that branches could be utilized for cavitation measurements, thus avoiding destructive sampling of seedlings.

Future studies should also focus on establishing drought hardiness relationships between juvenile seedlings and mature trees. A "Sapling Drought Hardiness Study" conducted by the PNWTIRC is currently attempting to provide some

insight on the response of annual growth ring traits (ring density and ring mass) to naturally occurring moisture stress. Because the same 39 families used in this study were also investigated in the sapling drought hardiness study, juvenile-mature correlations between drought hardiness traits can be estimated. Therefore, there is a need to determine how well hardiness screening in nursery beds predicts survival in droughty field sites as well as determining the relationship of seedling drought hardiness to hardiness over the life-span of the tree.

Genetic correlation between traits in Douglas-fir have been shown in several previous studies to vary from one population to another (Kleinschmit and Bastien 1992), therefore, it is useful to determine whether correlations between drought hardiness and growth potential traits differ in different populations. Traits that are indicative of drought hardiness ability across a range of populations may then be employed in breeding programs. In addition, by establishing juvenile-mature drought hardiness trait correlations, it would be possible to make selections at the seedling stage given a favorable correlation, thus saving both planting space and years of expense.

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APPENDIX A: VULNERABILITY CURVE EXPERIMENT ON THREE-YEAR OLD DOUGLAS-FIR SEEDLINGS

Vulnerability curves are a way of expressing the loss in hydraulic conductivity as a function of decreasing xylem water potential (Ψ_x); the function is characteristically curvilinear. Previous studies have found that variation in the curvilinear response exists both between species (Sperry and Tyree 1990; Cochard 1992) and within species (Sperry and Saliendra 1994; Alder et al. 1996). In this study, two of the thirty-nine full-sib families from the PNWTIRC's "Seedling Drought Physiology Study" were assessed with an objective of determining between family variation in vulnerability to cavitation following Sperry et al. (1988). The best (412) and the worst (112) performing families were selected based on second and third year shoot damage scores and third-year height. If variation existed between families, then it would be possible to explain a family's disposition to shoot damage in relation to cavitation and initiation point. Six trees from each family were sampled from the well-watered (control) beds to assess vulnerability to cavitation. The selection of trees within family was not strictly random, because trees selected had to have at least a 13 cm-long, second-year shoot increment in order to fit inside the cylindrical pressure chamber (PMS Instruments, Corvallis, OR). The length requirement may have created some bias. Flushed stems were not included because the junction of second flush and the primary shoot created large areas of air entry under pressurization (see below), thus inflating the likelihood of cavitation. After assessing initial conductivity following Kavanagh et al. (1997), the stem segments were pressurized at eight progressively increasing pressure levels (3.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 8.5 MPa), each for 30

seconds. After 30 seconds of pressure, the hydraulic conductivity (K_h) at each

pressure level was calculated by measuring the amount of water that passed through a

known length of stem from a constant pressure reservoir of weak oxalic acid. The

duration of time after each pressurization event and measurement of K_h varied (5 to 20

minutes) due to significant pith bubbling (i.e., de-pressurization of cells). As

Pammenter and Willigen (1998) have suggested, an exponential sigmoidal equation

was used to fit the vulnerability curves for each tree. The percent loss of conductivity

was calculated using the following equation:

$$\% \text{ loss in conductivity} = 100 \ (K_{max}-K_h)/K_{max} \qquad \text{Equation A.1}$$

where, K_{max} is the maximum hydraulic conductivity of the segment typically equal to

the initial measurement, but may sometimes be reached after pressurization due to

reversal of cavitation. K_h is the hydraulic conductivity at the specific xylem

pressurization. The following equation was used to fit the relationships between

%loss of conductivity and Ψ_x:

$$\% \text{ loss in conductivity} = 100/(1 + \exp(a(\Psi_x - b))) \qquad \text{Equation A.2}$$

Taking the natural log and rearranging gives:

$$\ln \ (100/\% \text{ loss in conductivity} -1) = a\Psi_x - ab \qquad \text{Equation A.3}$$

where, Ψ_x is one of the eight pre-determined xylem pressurization levels; a is the slope

of the curve and it is constrained to a 100% maximum of percent loss in hydraulic

conductivity; and, ab is the ordinate intercept. This equation allows for derivation of

biological significance by relating the effects of the coefficients to characteristics

related to vulnerability loss. Pammenter and Willigen (1998) related coefficient a to

the range of water potentials over which conductivity is lost. For example, the lower

the coefficient, the larger the range of xylem water potential in which cavitation

occurs. In addition, coefficient b determines the water potential corresponding to a

50% loss in conductivity.

Vulnerability curves were estimated for each tree using Equation A.3 (Figure

A.1). Two samples from family 112 where dropped from analysis due to ramicorn

branches, high pre-existing cavitation, and unavailability of a suitable alternate

sample. Family 412, which is the most drought hardy family, appears to have more

variation among individuals in the shape of the vulnerability curve. Despite this

variation, all individual-tree curves had a strong fit with an R^2 ranging from 0.941 to

0.997 (Table A.1). Paired t-tests revealed no significant family differences at any

given pressurization point. So, a combined vulnerability curve including

measurements from all 10 seedlings is shown in Figure A.2. The b values ranged from

-5.706 to -6.553 MPa among individual trees over both families (Table A.1). For the

pooled data, this value was -6.185. The value of Ψ_x corresponding to this pooled

value of b is 1.2 MPa larger than that found by Kavanagh et al. (1999) in shoots of

three-year-old Douglas-fir seedlings. The discrepancy in the value of the 50%

cavitation point may be due to the following reasons: (1) different equations used to

analyze the data; (2) the maximum hydraulic conductivity point for this study was

reached after exposure to higher xylem pressure levels than those used in the other

study; (3) it is likely that prior cavitation either caused by winter soil of sap freezing
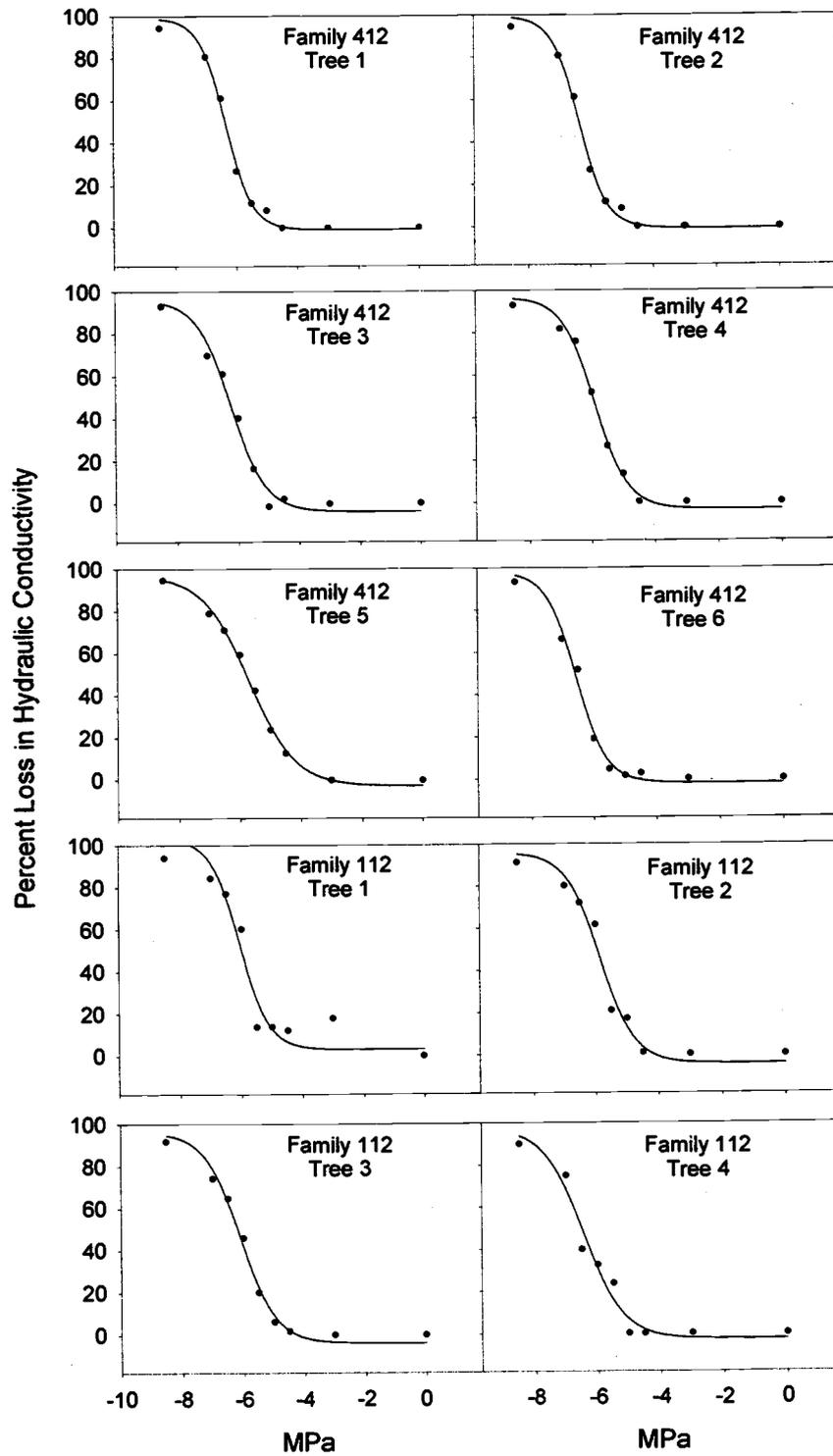
Figure A.1. Vulnerability curves expressed as the percent loss in hydraulic conductivity per drop in xylem water potential for two full-sib families of coastal Douglas-fir.
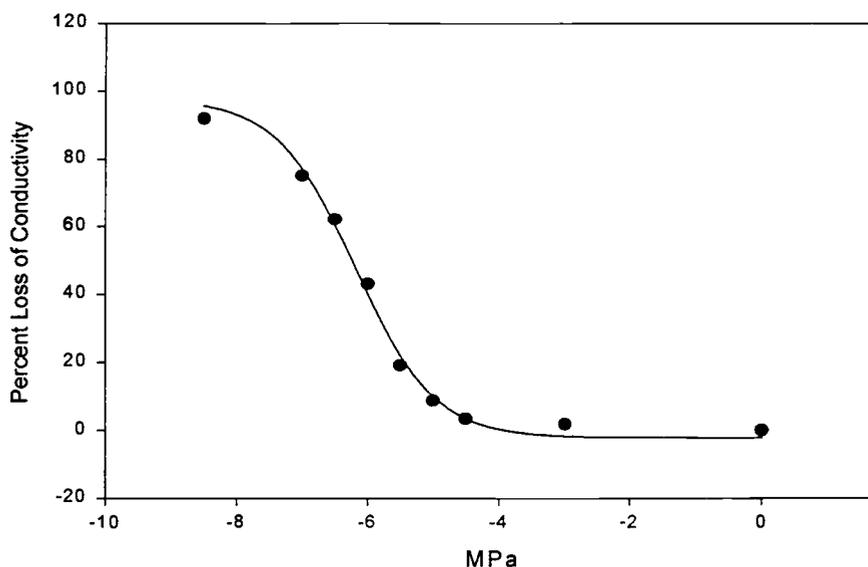
Figure A.2. Mean vulnerability curve for three-year old coastal Douglas-fir seedlings.

Table A.1. Values of coefficients *a* and *b* (at which 50% reduction in conductivity occurred) derived from Equation A describing the vulnerability curves for three-year-old Douglas-fir seedlings during late-summer for one drought hardy (412) and one drought susceptible (112) family, and pooled family data values.

Family	Tag	*a*	*b*	R^2
412	425	2.315	-6.346	0.994
	203	2.315	-6.346	0.994
	715	1.774	-6.237	0.985
	763	1.931	-5.907	0.994
	764	1.324	-5.706	0.997
	697	2.105	-6.553	0.990
112	438	2.131	-6.058	0.941
	439	1.848	-5.857	0.977
	754	1.724	-6.105	0.992
	305	1.579	-6.454	0.970
Pooled Data		1.661	-6.185	0.994

(Ewers 1985; Sperry et al. 1994) or by root rot during the early part of the first growing season (Joseph et al. 1998) may have increased susceptibility to cavitation in the second-year assessments of this study; or, (4) a real difference between the populations used in these two studies. In this study, maximum hydraulic conductivity was selected within the 0 to -4.5 MPa range depending on the degree of increase from initial conductivity measurement due to pressurization. Pressurization can sometimes reverse low-tension embolisms (Borghetti et al. 1991; Sobrado et al. 1992), therefore, causing a rise in hydraulic conductivity from that noted at the initial measurement point. The results from these vulnerability curve measurements indicate that Douglas-fir has a fairly gentle vulnerability curve suggesting that cavitation occurs over a broad range of xylem pressure and that 50% of the stem is cavitated at -6.2 MPa. Because this low level of xylem water potential rarely occurs in the field, Douglas-fir is able to occupy xeric sites and is adapted to withstand summer drought.

APPENDIX B: SAS CODE

Shown below are examples of the SAS codes used in the GLM and the variance component procedures for all traits assessed in chapter three. Test command lines indicate the hypothesis (H) and the error term (E) used to test the significance of variance in each corresponding factor. The P1 through P4 variables included in model 2 represent the parental components within each diallel.

SAS code for GLM procedure used for Model 1

```
proc glm data=control;
 where treatment='Control';
 class block family diallel plot;
 model Height=block diallel family(diallel)
      block*family(diallel) plot*family(block diallel);
 random block diallel family(diallel) block*family(diallel)
      plot*family(block diallel);
 test H=block                        E=block*family(diallel);
 test H=diallel                      E=family(diallel);
 test H=family(diallel)              E=block*family(diallel);
 test H=block*family(diallel)        E=family*plot(block diallel);
run;
```

SAS code for GLM procedure used for Model 2

```
proc glm data=severe;
 where treatment ='Severe';
 class block family diallel P1 P2 P3 P4;
 model Damage = block diallel P1(diallel) P2(diallel) P3(diallel)
      P4(diallel) family(diallel) block*family(diallel);
 random block diallel P1(d) P2(d) P3(d) P4(d) family(diallel)
      block*family(diallel);
 test H=block                        E=block*family(diallel);
 test H=diallel                      E=family(diallel);
 test H=family(diallel)              E=block*family(diallel);
run;
```

SAS code for VARCOMP (1) procedure used for Model 1

```
proc varcomp data=severe method=REML;
 where treatment ='Severe';
 class block family diallel;
 model Damage = block diallel family(diallel)block*family(diallel);
run;
```

SAS code for VARCOMP (2) procedure used for Model 2

```
proc varcomp data=severe method=REML;
 where treatment ='Severe';
 class block family diallel P1 P2 P3 P4;
 model Damage = block diallel P1(diallel) P2(diallel) P3(diallel)
      P4(diallel) family(diallel) block*family(diallel);
run;
```

APPENDIX C: MODELS USED IN VARCOMP 1 AND VARCOMP 2 ANALYSES

The following tables list the variance components derived from the two VARCOMP runs (VARCOMP 1 shown in Table C.1 and VARCOMP 2 shown in Table C.2) used in the analysis of genetic parameters for drought hardiness and growth potential traits. The VARCOMP 1 model was run without partitioning the family within diallel component into general combining ability (GCA) and specific combining ability (SCA). The VARCOMP 2 model, however, partitioned the family within diallel component into these two variances by including four dummy variables to represent the parental effects. Plot mean values for the two damage and two growth potential traits are given in each table. Variances for these traits at the plot mean level is used to estimate genetic correlations between these traits as well as family heritability.

Also, because it was not possible to use the statistical software to test the significance of the GCA variance, an effort was made to calculate the significance manually from information provided from the analysis of variance for the second run. Results from the manual calculations are presented in Table C.3.

Table C.1. Analysis of variance from VARCOMP 1 for growth and damage traits.

Data Level	Trait[a]	σ^2_b[b]	P-value	σ^2_d[c]	P-value	$\sigma^2_{f(d)}$[d]	P-value	$\sigma^2_{bf(d)}$[e]	P-value	σ^2_{pf}[f]	P-value	σ^2_e[g]
Individual	R1_S	0.746	0.2984	4.896	0.0429	16.749	0.0001	0.000	0.5619			78.963
tree	R2_S	0.000	0.7672	3.205	0.1287	17.555	0.0001	15.938	0.0032			64.380
	Dam_S	3.187	0.0372	0.360	0.3651	8.154	0.0372	21.657	0.0024			67.368
	R1_M	6.831	0.0001	10.259	0.0021	12.851	0.0005	13.962	0.0258			60.727
	R2_M	4.651	0.0020	0.000	0.8996	0.000	0.4442	0.000	0.4410			96.335
	Dam_M	10.208	0.0001	0.109	0.3706	0.940	0.4599	22.237	0.0133			68.085
	R1_C	0.787	0.2191	9.283	0.0050	11.693	0.0004	0.000	0.5961			80.446
	R2_C	1.131	0.0956	0.000	0.5963	0.000	0.6972	0.000	0.8240			99.092
	Ht_C	29.585	0.0001	11.579	0.0004	12.801	0.0001	0.029	0.5102	1.531	0.2745	53.668
	Dia_C	14.600	0.0001	2.770	0.0646	10.531	0.0001	4.956	0.0773	7.626	0.0355	63.306
Plot Mean	R2_S	0.000	0.7968	4.135	0.1146	20.361	0.0003					77.000
	Dam_S	3.618	0.0377	0.305	0.3611	9.444	0.0401					87.611
	Ht_C	49.732	0.0001	19.617	0.0003	21.244	0.0001					24.641
	Dia_C	29.598	0.0001	5.990	0.0511	21.196	0.0001					50.947

[a] Under the severe drought treatment, R1_S is cavitation in the first annual growth ring, R2_S is cavitation in the second annual growth ring, and Dam_S is shoot damage; under the moderate drought treatment, R1_M is cavitation in the first annual growth ring, R2_M is cavitation in the second annual growth ring, and Dam_M is shoot damage; under the control treatment, R1_C is cavitation in the first annual growth ring, R2_C is cavitation in the second annual growth ring, Ht_C is height, and Dia_C is diameter.

[b] variance due to random effect of blocks.

[c] variance due to random effect of diallel.

[d] variance due to random effect of family within diallel.

[e] variance due to random interaction effect of the family within diallel with the block.

[f] variance due to random effect of the family within diallel with the plot.

[g] random error of plot-plot variance within families within blocks.

Table C.2. Analysis of variance from VARCOMP 2 for growth and damage traits with the family within diallel component partitioned by parental variance.

Data Level	Trait[a]	σ^2_b[b]	σ^2_d[c]	$\sigma^2_{P1(d)}$[d]	$\sigma^2_{P2(d)}$[e]	$\sigma^2_{P3(d)}$[f]	$\sigma^2_{P4(d)}$[g]	$\sigma^2_{f(d)}$[h]	P-value[i]	$\sigma^2_{bf(d)}$[j]	σ^2_{pf}[k]	σ^2_e[l]
Individual tree	R2 S	0.000	0.000	0.000	13.206	3.747	5.390	0.869	0.1548	17.386		63.451
	Dam S	3.209	0.000	0.000	0.213	0.423	4.144	4.056	0.0645	21.759		67.292
	Dia C	14.598	1.842	3.745	0.000	0.000	0.000	8.367	0.0001	4.968	7.598	63.321
	Ht C	29.636	5.957	4.106	2.721	0.302	6.898	5.119	0.0001	0.027	1.530	53.672
Plot Mean	R2 S	0.000	0.000	0.000	16.176	4.564	8.711	0.000	0.4747			75.738
	Dam S	3.618	0.000	0.000	0.721	1.542	5.450	2.939	0.2230			87.611
	Ht C	49.732	10.270	7.074	5.061	0.655	10.959	8.243	0.0009			24.641
	Dia C	29.598	4.082	7.699	0.000	0.000	0.000	16.752	0.0081			50.947

[a] See Table C.1 for description of traits.

[b,c,j,k,l] See Table C.1 for description of these variance components.

[d,e,f,g] GCA variance component for parental dummy variables 1 to 4 (P1 to P4) within diallel.

[h] Variance due to family within diallel or variance due to SCA.

[i] Test of significance of SCA.

Table C.3. Calculation of significance of GCA variances for growth and damage traits.

Parents	Mean Squares of Traits[a]							
	Individual-tree basis				Block mean basis			
	R2_S	Dam_S	Ht_C	Dia_C	R2_S	Dam_S	Ht_C	Dia_C
P1	26.6	48.9	581.4	664.8	13.1	56.2	246.6	341.4
P2	1003.2	230.7	606.7	233.7	706.9	181.2	269.4	125.0
P3	399.2	182.4	329.6	432.4	319.3	166.8	136.2	213.4
P4	162.2	312.4	487.6	384.2	155.0	154.8	195.6	191.2
GCA average[b]	397.8	193.6	501.3	428.8	298.6	139.7	211.9	217.8
SCA[c]	131.2	168.6	148.5	204.0	77.5	109.8	62.5	105.6
$F = MS_{GCA}/MS_{SCA}$[d]	3.0	1.1	3.4	2.1	3.9	1.3	3.4	2.1
Significance[e]	*	ns	**	ns	**	ns	**	ns

[a] See Table C.1 for description of traits. Mean square values were estimated using the second General Linear Model (GLM 2) of the variance components presented in Table C.2.

[b] Sum of mean squares for P1 to P4 divided by 4.

[c] Mean squares of SCA is equal to mean squares for family within diallel component in GLM 2.

[d] The ratio of the mean squares of GCA and the mean squares of SCA.

[e] Significance is denoted as: * = $p < 0.05$; ** = $p < 0.01$; ns = non-significant.