Seedling cold and drought hardiness in half-sib families of submaritime Douglas-fir

by

Nicole T. Darychuk
B.Sc., University of Victoria, 2007

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Supervisory Committee

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Abstract

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The submaritime zone of British Columbia experiences summer drought and spring and fall frosts which cause high mortality of seedlings planted for reforestation. Seedlings of half-sib Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) families were studied at 3 test sites in the coast mountains of British Columbia, and in a complementary pot trial on southern Vancouver Island from 2007-2008, with the aim of studying physiological traits related to cold and drought hardiness. These families originated from British Columbia's submaritime zone, and had a range of breeding values. This allowed for comparison of growth and stress resistance traits among progeny with expected differences in performance. In the field trial, spring and fall cold hardiness were assessed using chlorophyll fluorescence after controlled freezing. Growth and mortality data, shoot water potential and carbon isotope discrimination were recorded as measures of drought stress and water use efficiency. In the pot trial, a subset of families was grown under 3 drought levels to study drought hardiness characteristics. Growth, biomass allocation, date of vegetative bud burst, gas exchange, shoot water potential, and chlorophyll fluorescence were measured. Means comparisons between families, treatments and study sites were performed, narrow-sense heritabilities were calculated, and physiological traits were compared using correlation analyses. Family, field site and drought treatment had significant effects on many physiological parameters. Wild stand families tended to have greater field survival and fall cold hardiness than seed orchard families. Field height was negatively correlated with survival and spring cold hardiness. Shoot water potential and water use efficiency showed positive correlations with leader growth in the field. These data have relevance to the selection of families for the BC submaritime Douglas-fir tree breeding program. They can also help further our understanding of how growth and stress resistance traits interact, and provide information on inherent genetic control of these traits.

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Acknowledgments

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Dedication

I dedicate this work to all members of my family – past, present, and future. You all give me so much to be proud of, and inspire me to strive to do the same.

Section 1 – General Introduction

Douglas-fir

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a conifer of great ecological and economic importance, and is planted widely around the world due to its sought-after qualities for timber production. A true landmark of its native range in western North America, this tree flourishes in partial shade, prefers well-drained soil, has some drought resistance, and is found in many habitats across its range (Coward, 1977). There are two regional varieties, a coastal variety, *P. menziesii* var. *menziesii*, and an interior variety, *P. menziesii* var. *glauca*, also known as Rocky Mountain Douglas-fir. The term variety is used to designate different morphological characteristics and ranges within a species (White *et al.*, 2007). The coastal variety occurs from central coastal British Columbia and Vancouver Island, south to mid-California, and inland to the Coast mountains. The crest of the Coast Mountains in Canada and the Cascade Mountains in the United States is the approximate dividing line between the two varieties (Wright, 1976). The interior variety grows from central British Columbia east to the Rocky Mountains, and south along the Rocky Mountain range, with the distribution growing patchier with decreasing latitude. Small patches extend south into the southwest United States and Mexico along the mountain ranges. The elevation of Douglas-fir habitat ranges from sea level on the coast to 3500 m in the southern Rocky Mountains (White *et al.*, 2007).

The coastal variety inhabits more productive forests, alongside western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and grand fir (*Abies grandis*), with salal (*Gaultheria shallon*), huckleberries (*Vaccinium* spp.), Oregon-grape (*Mahonia aquifolium*), sword-fern (*Polystichum munitum*) and other species in the undergrowth. In interior Douglas-fir habitat, mesic sites usually have a dense forest with tree assemblages similar to the coast, while on drier sites, open forest with pinegrass (*Calamagrostis rubescens*) and moss undergrowth is the norm (Canadian Forest Service, 2009). Habitat and food for many organisms is provided by Douglas-fir across western North America. Seeds, cambium, twigs and foliage are an important food source for many insects, birds and mammals (Coward, 1977).

Genecology of Douglas-fir

Douglas-fir has an outcrossing mating system which promotes high genetic diversity (White *et al.*, 2007). Wind pollination enables a high level of gene flow between stands. In a study plot in southern Oregon, as determined by genetic markers, the mean dispersal distance of pollen from trees within the plot that successfully sired offspring was 55 m, and the proportion of offspring sired by pollen from outside the stand was 27% (White *et al.*, 2007). In years of heavy pollen production, appreciable amounts of Douglas-fir pollen have been shown to travel on the wind a distance of several kilometers (Allen and Owens, 1972). Birds and rodents that feed on Douglas-fir seeds likely aid in dispersal of genetic material to new areas (Coward, 1977).

The amount of genetic variability in a species is influenced by several factors. A larger range tends to increase a species' genetic diversity (Wright, 1976). Among the most widely distributed conifers in western North America, Douglas-fir occupies a variety of habitats (Li and Adams, 1989). The amount of environmental diversity in day length, precipitation, temperature, pests and pathogens, and other factors within the range of Douglas-fir increases the genetic variability through differential selection pressures (Wright, 1976; Campbell, 1979; Aitken, 2004). Environmental heterogeneity causes population adaptation via site-specific selection rather than extensive phenotypic plasticity (Aitken, 2004).

There are some generalizations in genetic structure and physiology that differentiate the two varieties of Douglas-fir. Coastal Douglas-fir shows less genetic differentiation between populations than the interior variety (Ponoy *et al.*, 1994). There is much less production of the camphene group of terpenes in the leaf oils of coastal populations than in interior populations (von Rudloff and Rehfeldt, 1980). The coastal variety grows much faster under mild conditions, and is less hardy to extreme winter cold and drought (Wright, 1976; White *et al.,* 2007). Typical height in the wild is 85 m for the coastal variety, and 42 m for the interior variety (Coward, 1977). The interior variety can be split again at about 44° latitude into two races. Provenances of the northern race are characterized by greater cold hardiness, less drought resistance, and slower growth rates than the southern race (White *et al.*, 2007). Using allozymes, Li and Adams (1989) reported genetic diversity within populations of the southern race to be half that of the northern race and coastal variety, while the genetic differentiation between populations of the southern race was two to three times greater than that among northern or coastal populations.

During the Pleistocene epoch (1.6 million – 10,000 years ago), cycles of glacial cover over much of the North American landscape pushed species south and left them in isolated refugia during the ice ages. The three major genetic subdivisions of Douglas-fir likely originated from different refugia populations during the Pleistocene. These refugia probably underwent genetic bottlenecks as they lost variability due to genetic drift (Li and Adams, 1989). The two varieties of Douglas-fir were periodically separated and probably diverged over 0.5 million years ago (White *et al.,* 2007). It is hypothesized that following the Wisconsin glaciation (20,000-15,000 years ago) Douglas-fir migrated north from populations in western Oregon or south-western Washington in the west, and from eastern populations in the great basin and the southern Rocky Mountains (St. Clair *et al.,* 2005).

Much empirical evidence, including analysis of terpene profiles by von Rudloff (1973) and von Rudloff and Rehfeldt (1980), and chloroplast DNA diversity by Ponoy *et al.* (1994), suggests a zone of introgression between the coastal and interior varieties. In the area to the east of the Coast Mountains in southern British Columbia, known as the submaritime or transition zone, the coastal variety is exposed to unusually xeric conditions and some gene flow from the interior variety. Contact between coastal and interior varieties appears to have resumed around 7000 years ago in the submaritime zone in southern British Columbia (White *et al*., 2007). Seven thousand years is relatively little time for enough gene flow to occur for interbreeding of the varieties (White *et al*., 2007). Differences in flowering phenology between the varieties may also limit the amount of intervarietal gene flow (St. Clair *et al*., 2005).

Environmental gradients cause genetic variation through natural selection. In a study in the Oregon Cascades, seedlings of parents from south-facing slopes had earlier bud set, lower growth, and greater root to shoot ratios than seedlings from north-facing slopes (Hermann and Lavender, 1968). This reflects the drier environment found on the southern slopes. St. Clair *et al*. (2005) investigated patterns of variation in coastal Douglas-fir in western Washington and Oregon. They found that provenance variation in shoot emergence, bud set and growth were strongly correlated with geographical trends in elevation and temperature.

Some general geographic trends have been observed in quantitative traits in Douglas-fir (reviewed in Wright, 1976).

1) Cold-warm trend: Occurs from north to south, high-altitude to low-altitude, and continental to coastal clines. Provenances from warmer regions tend to grow faster, and can be less resistant to extreme winter cold.
2) Photoperiod trend: Day length is affected by latitude. In North America, southern provenances usually start growth earlier in the spring, and cease growth later in the fall.
3) Dry-moist trend: Provenances from mesic regions usually grow faster, are less deeply rooted, and have greener foliage (Wright, 1976). Coastal Douglas-fir from dry areas in southern Oregon and northern California grew more slowly than other provenances along the west coast in common garden experiments (Wright, 1976).

There are great climatic differences between elevations (Campbell, 1979; McKenzie *et al.*, 2003). Unlike differences in latitude or continentality, elevational differences cause appreciable climatic differences within small spatial scales, in meters rather than many kilometers. Gene flow is greater between trees of different elevations than of trees of appreciably different latitudes (Wright, 1976). This may prevent nearby populations at different elevations from diverging too greatly. However, gradients in reproductive timing may exist along elevational clines. This would reproductively isolate nearby populations at different elevations, limiting gene flow.

The high amount of environmental heterogeneity over both space and time in a forest ensures a wide range of environments in which seedlings have the opportunity to become established (Campbell, 1979). Differences in soils, frost pockets or shading contribute to microsite differences (Timmis *et al.,* 1994). Phenotypic plasticity doubtless plays a role in seedling success in a variable environment, but in Douglas-fir, this heterogeneity translates into a genetic heterogeneity of trees within a stand through selection, and further fuels the high within-stand genetic diversity of this species.

Tree breeding program

Tree breeding is becoming a more necessary component of forestry practice, due to restriction on harvestable land and increasing global competition (Xie and Yanchuk, 2003). The central goal of the tree breeding program run by the BC Ministry of Forests and Range is to grow trees with higher quality wood at a faster rate (BC Ministry of Forests and Range, 2005). Douglas-fir is one of the ten tree species in BC that are part of an active breeding program. Trees are selected for high growth rate, straight stems, high wood quality, and resistance to insects and pathogens (BC Ministry of Forests and Range, 2005).

The natural variation found in wild stands is selected from to bring out traits of interest to be further developed in the breeding program. Seed or scions are harvested from wild trees that have desirable characteristics. These parents are raised in seed orchards and their progeny are tested for traits of interest in progeny trials. Desired traits are accentuated through selection for breeding over successive generations. However, variation is due only in part to genetic differences. Environment also influences tree phenotype. Of those traits that are genetic in nature, not all have high heritability (Aitken *et al.,* 1996). This makes extensive testing necessary in order to determine good genetic stock (Hadley *et al.,* 2001). Progeny tests may need 5-25 years to assess the genetic quality of the parents (BC Ministry of Forests and Range, 2005). An important characteristic of a parent genotype is a high breeding value (BV). This indicates the value that an individual passes on to its progeny, and thus its value as a breeding parent (Xie and Yanchuk, 2003). Breeding value can be defined as

$$BV = 2h^2_f\,(y-\alpha)$$

where h^2_f is the heritability of family means, y is the progeny mean and α is the population mean of the trait of interest (Xie and Yanchuk, 2003).

After generations, this cycle of selection, testing and breeding leads to genetic gain in traits of interest (Hadley *et al.,* 2001). Genetic gain is defined as the estimated percentage of increase in performance of a specific trait in improved trees over wild-stand material. After selective crosses between trees, the best offspring are put in a seed orchard, which produces seed for reforestation. Each year over 200 million trees are planted in British Columbia for reforestation (BC Ministry of Forests and Range, 2005). Many of these planted seedlings are improved stock from breeding programs (BC Ministry of Forests and Range, 2009).

British Columbia has a varied topography and climate, and thousands of years of natural selection have acted on tree populations. Evidence is compelling that natural selection is responsible for the geographical distribution of cold hardiness traits (Howe *et al.,* 2003). To take advantage of local adaptation, it has proved practical to divide the province into seed planning zones, based on geographic patterns in genetic diversity, determined by provenance tests of trees. The zones are designated based on climate, ecological conditions, and patterns of genetic diversity in the trees. Seed orchards are assigned to seed planning zones to ensure that seedlings are planted in a region that best suits their genetic predisposition (BC Ministry of Forests and Range, 2005). The breeding program for submaritime Douglas-fir must take into account the dry, mountainous conditions in the transition zone and select for resistance to freezing and drought stress as well as height growth.

Drought hardiness

Drought hardiness, or resistance, is defined as the sum of physiological responses that allow a plant to avoid or tolerate the effects of drought (Taiz and Zeiger, 1998). Many factors affect the drought hardiness or resistance of land plants. Plant water status depends less on soil moisture than it does on the balance between water absorbed from the soil and water transpired to the atmosphere. This balance is affected by soil and atmospheric conditions (Vaadia *et al*., 1961). Within the plant, resistance to water stress is made up of mechanisms for drought avoidance and drought tolerance. Drought avoidance strategies increase water uptake or decrease water loss from the plant. These include leaf waxes, small or few leaves, rapid stomatal closure during drought, extensive root growth, and high root / shoot ratios (Kozlowski and Pallardy, 2002). Drought tolerance involves strategies that enhance the ability of tissues to cope with a lack of water. These include a reduction in cell wall elasticity, the adjustment of osmotic potential, and the accumulation of proteins and other molecules that stabilize cellular components (Kozlowski and Pallardy, 2002). Resistance to drought depends both on the plant's genotype and on its acclimation to prior conditions. As expected for such a complex trait, drought resistance is controlled by many different genes (Sung *et al.*, 2003).

Drought stress negatively affects cell elongation and photosynthesis in plants (Vaadia *et al.*, 1961). Not only does drought limit growth for the current growing season, drought-related injury in the form of foliage damage and xylem cavitation can lead to a significant reduction in height growth during the following growing season in Douglas-fir seedlings (Anekonda *et al.*, 2002).

One of the first physiological responses to drought is stomatal closure (Pessarakli, 2005). This response is largely mediated by the plant growth regulator abscisic acid (ABA). ABA is synthesized continuously at a low rate in the mesophyll, and is stored in the chloroplasts (Taiz and Zeiger, 1998). When the mesophyll becomes dehydrated, stored ABA is released into the apoplast, and synthesis also increases (Taiz and Zeiger, 1998). ABA is also synthesized by the roots in response to soil drying, and translocated to the stomata through the transpiration stream (Passarakli, 2005). When ABA reaches the guard cells which line the stomata, it induces an efflux of K^+ and anions, reducing turgor pressure, and closing the stomata (Taiz and Zeiger, 1998). In Douglas-fir seedlings, Johnson and Ferrell (1982) reported an increase in ABA as water stress increased during soil drying. When a shoot water potential of -2.0 MPa was reached, branch conductance decreased significantly, and the ABA content of needles increased (Johnson and Ferrell, 1982). When water potentials reached -5.0 MPa, ABA levels sharply declined. They also found a reciprocal relationship between ABA and a conjugate, and suggested that the conjugate functioned for storage of ABA, and could be converted rapidly to the active form under stress conditions (Johnson and Ferrell, 1982). Both ABA and its primary inactive conjugate, ABA glucose ester, can move rapidly through the plant via the xylem and phloem (Pessarakli, 2005).

Under water stress, photosynthesis decreases (Vaadia *et al.*, 1961). Consequently, light requirements by the photosynthetic apparatus also decrease. To cope with excess light, plants can reduce chlorophyll concentrations, or divert excess light to heat production or increased photorespiration (Pessarakli, 2005). The effect of drought on the reduction of transpiration and photosynthesis in Douglas-fir is well established (Zavitkovski and Ferrell, 1968; Lopushinsky and Klock, 1974; Unterscheutz *et al.*, 1974). Brix (1979) reported that with decreasing water potential, photosynthesis decreased, and stopped completely at water potentials around -5.0 MPa in Douglas-fir seedlings.

Drought acclimation

When stress resistance increases because of previous exposure to a stress, a plant is said to have acclimated, or hardened, to the stress. Mechanisms involved in increasing resistance to drought in response to water stress include osmotic adjustment, molecular level changes, and an increase in root biomass relative to shoot biomass (Kozlowski and Pallardy, 2002). Early root growth has been found to increase drought avoidance ability in Douglas-fir seedlings (Brix, 1979).

In response to drought, the activity of ABA biosynthesis enzymes in both roots and shoots increases (Pessarakli, 2005). Increased presence of ABA in the plant induces numerous biological effects in addition to stomatal closure. Two thirds of genes upregulated by drought have been found to be regulated by ABA or an ABA analogue in *Arabidopsis* (Huang *et al.,* 2008). In response to drought, proteins are expressed that protect cellular components from dehydration damage (Kozlowski and Pallardy, 2002). Water-protein interactions are very important in ensuring proper protein folding and the maintenance of proper conformation and catalytic activity (Kalemba and Pukacka, 2007). Lack of water is therefore a concern for metabolic function, and the synthesis of chaperone proteins that stabilize cellular components is a part of drought response. Among these are the dehydrins, which are thought to interact with and stabilize membranes and proteins to prevent denaturation and aggregation due to dehydration (Kozlowski and Pallardy, 2002; Wang *et al.*, 2002). Late embryogenesis active (LEA) proteins and small heat shock proteins (sHSPs) are induced during drought stress in plants, and stabilize various molecules and structures within the cell (Kalemba and Pukacka, 2007). Ubiquitins are another group of stress-related proteins, which tag for degradation damaged proteins that would otherwise disrupt metabolism (Kozlowski and Pallardy, 2002).

Family variation in drought hardiness

Variation among cultivars in osmoregulation and maintenance of turgor under drought stress has been observed in *Gossypium hirsutum* and *Sorghum* spp. (Morgan, 1984). Anekonda *et al*., (2002) found significant differences between families for growth and drought resistance traits in droughted Douglas-fir seedlings. They reported significant family effects on height, diameter, xylem cavitation, xylem hydraulic conductivity, and foliage damage due to drought. Ferrell and Woodard (1966) found significant differences among provenances in time-to-death under drought treatment in potted Douglas-fir seedlings.

Drought hardiness traits in Douglas-fir have been found to be roughly equivalent in heritability to growth potential traits (Anekonda *et al*., 2002), thus drought hardiness has much potential for improvement through selection and breeding. Significant variation in water use efficiency among families has been reported in conifers, including Douglas-fir (Zhang *et al*., 1994, Aitken *et al*., 1995). This family variation makes selection for water use efficiency using indicators such as C isotope discrimination a possibility in forest tree breeding programs.

Cold tolerance

Different groups of plants respond to sub-zero temperatures in different ways. Tropical taxa accustomed to warm temperatures year-round are especially sensitive to freezing. As temperatures drop a few degrees below zero, ice crystals nucleate in tissues and rapidly rupture plasma membranes, causing cell death (Sakai and Larcher, 1987). Other plants, like many temperate hardwoods, exhibit supercooling, which is the cooling of the solutions of the symplast and apoplast below the freezing temperature point while remaining in liquid phase (Sakai and Larcher, 1987). This mechanism allows plants to tolerate temperatures no lower than -40°C, below which ice forms spontaneously in the absence of ice nucleators (Sakai and Larcher, 1987). Some of the most cold hardy plants are the boreal conifers. In artificial freezing tests, some boreal tree species are able to survive immersion in liquid nitrogen (Howe *et al.,* 2003). These extremely hardy plants exhibit binding of water molecules and cellular dehydration during acclimation, so that freezing occurs extracellularly, thus directing ice formation to where it will do the least harm to cells (Palva and Heino, 1998).

Cold acclimation

Cold acclimation in boreal conifers develops in stages. The first stage is induced by lengthening nights, which are detected via phytochrome signalling (Hopkins and Huner, 2004). First, growth of shoot meristems ceases. Once growth has stopped, tissues begin increasing in frost hardiness (Welling and Palva, 2006). Bud set then occurs, during which the buds are covered by a set of protective bud scales. The buds enter a state of endodormancy, which can only be lifted after a sufficient chilling period (Welling and Palva, 2006). Chilling period is expressed in terms of chilling hours (i.e., number of hours below 5°C) (Grossnickle, 2000). This stage needs to be under way before the first frost, or growing tissues will be vulnerable to severe frost injury, since frost resistance is lowest in actively growing tissue (Sakai and Larcher, 1987). The second stage of cold acclimation is triggered by low temperatures (Sakai and Larcher, 1987). It involves metabolic changes that make cells more resistant to freezing and dehydration damage. After sufficient time at low temperatures, buds are released from endodormancy and enter ecodormancy. During ecodormancy, the buds are dormant and very frost hardy, and they maintain that status until conditions favourable for bud break occur in the spring (Welling and Palva, 2006). A third stage of hardiness in the hardiest conifers is thought to be brought on by freezing temperatures after the first two steps are complete. After this third stage, boreal trees can withstand even the coldest winter temperatures (Sakai and Larcher, 1987).

Due to the completion of the hardening process, there is rarely frost damage in midwinter in boreal trees, as acclimation has occurred and is still in place (Timmis *et al.,* 1994). Instead, most damaging frosts take place either in the spring, after dormancy is released, or in the fall before cold acclimation is complete (Timmis *et al.,* 1994; Jermstad *et al.,* 2001).

The mechanisms of cold acclimation

Conifer cold hardiness is a quantitative trait controlled by a myriad of genes. This trait can be seen as the additive effect of a set of physiological changes at the time of acclimation (Aitken and Hannerz, 2000). Such changes include the conversion of starch to soluble sugars, an increase in organic phosphates, and the accumulation of glycoproteins (Hopkins and Huner, 2004). Various proteins, amino acids, lipids, and antioxidants play roles in cold acclimation (Zwiazek *et al.,* 2001). The plasma membrane composition changes, new proteins are synthesized, sugar content increases, phytohormone levels change and gene expression is altered (Palva and Heino, 1998). The increase in sugars, amino acids and other cellular solutes is necessary to bind water in the cell under low temperatures.

During winter inhibition of photosynthesis, cells are protected from photooxidative damage via long-term xanthophyll mediated nonphotochemical antenna quenching, which involves extensive reorganization of photosystems I and II (Oquist and Huner, 2003). Even with these protective measures, reactive oxygen species (ROS) are still a threat during cold stress. ROS are known to cause extensive damage by reacting with cellular components like lipids, proteins and DNA. In trees that exhibit extracellular freezing, low temperatures, ice crystals, and the accompanying dehydration cause the destabilization of membranes (Palva and Heino, 1998), which leads to greater ROS production (Oquist and Huner, 2003). In pine trees, enzymes that scavenge ROS have been found to accumulate during cold acclimation and have been associated with increased resistance to freezing damage (Suzuki and Mittler, 2006).

The cell wall, as the barrier between the cell and its surroundings, plays important roles in water movement and extracellular freezing. The amount of water in cells decreases during acclimation. This makes freezing less likely to happen inside cells, where it would be lethal (Welling and Palva, 2006). An increase in pectin content reduces the size of cell wall pores, making it more effective at protecting the cell membrane from ice crystals outside the cell. Water in smaller diameter pores is less likely to freeze (Zwiazek *et al.,* 2001). Douglas-fir has also been shown to exhibit a decrease in cell wall elasticity after acclimation (Zwiazek *et al.,* 2001). This may help cells reach osmotic equilibrium with extracellular ice crystals sooner, thereby reducing the large amount of water loss that occurs during prolonged freezing.

In spruce trees, the first phase of hardening has been characterized in part by an increase in the amount of membrane lipids, phospholipids in particular (Sakai and

Larcher, 1987). During the second stage of hardening, there is an increase in the proportion of polyunsaturated fatty acids in membrane lipids (Sakai and Larcher, 1987). Lipids with a greater degree of unsaturation are more fluid under low temperatures, which helps maintain membrane integrity during freezing.

Proteins that become more abundant during acclimation and subsequent cold temperatures include ROS scavenging enzymes, dehydrins, chaperone proteins and pathogenesis-related proteins (Welling and Palva, 2006). A myriad of roles are suggested for dehydrins in overwintering plants, including cryprotective, antifreeze, osmoregulatory, calcium-binding, and radical scavenging functions. Molecular chaperones, such as small heat shock proteins, prevent aggregation and irreversible inactivation of cellular proteins under both low and high temperature extremes (Welling and Palva, 2006). Pathogenesis-related proteins are thought to assist with the tolerance of various stresses in plants, and have been shown to exhibit antifreeze and cryoprotective function as well as antifungal activity (Welling and Palva, 2006).

Tolerance of freezing

The term "freezing tolerance" does not imply the ability to survive freezing throughout plant tissues, as any intracellular freezing is invariably fatal to the cell (Sakai and Larcher, 1987). Instead, the hardiest plants must direct freezing to areas where it will not damage vital structures. During the acclimation process, Douglas-fir and other cold hardy conifers develop mechanisms that limit the formation of ice crystals to outside the cell. Cytosol has a higher solute concentration than does the apoplast. Due to the negative relationship between freezing point and solute concentration, ice first forms in the apoplast (Sakai and Larcher, 1987). Once ice nucleation occurs, the lower vapour pressure of the ice crystal establishes a vapour pressure gradient between the ice in the apoplast and the water in the protoplasts of surrounding cells. This gradient causes cytoplasmic water to migrate out of the cells and attach to the enlarging ice crystal (Hopkins and Huner, 2004). This migration of cellular water continues as the ice crystal grows. As water continues to leave the cell, the solute concentration increases, further depressing the freezing point in the cell. This lowering of cellular osmotic potential in response to cold temperatures has been recorded in Douglas-fir (Zwiazek *et al.,* 2001).

The remaining water is matrically bound to proteins, sugars and other osmotically active molecules in the cell (Sakai and Larcher, 1987). If the amount of water in the cell is sufficiently low, it will remain bound to solutes, and can even form a glass phase at very low temperatures, which will not freeze and damage the cell (Sakai and Larcher, 1987). The extensive migration of water out of cells, while essential to the process of extracellular freezing, does have the physiological consequence of dehydrating the contents of the cytoplasm (Hopkins and Huner, 2004). Freezing stress thus becomes much like drought stress, and this makes dehydration tolerance of protoplasts essential (Zwiazek *et al.,* 2001).

Deacclimation

Acclimation to cold in the fall months involves changes in structure and function to deal with freezing stress, in a very energy-intensive process that takes weeks. Deacclimation, however, occurs very rapidly, in a matter of days, triggered mainly by warm temperatures. Increasing photoperiod may also play a limited role (Kalberer *et al.* 2006). Deacclimation likely has far lower energy requirements than acclimation, involving downregulation instead of upregulation of the biosynthesis of cold hardiness related molecules (Kalberer *et al.* 2006). Warm temperatures and lengthening days in the spring trigger release from dormancy and bud burst (Welling and Palva, 2006). Fluctuating temperatures can result in partial deacclimation, then a degree of reacclimation (Kalberer *et al.,* 2006). Temporary thaws can therefore prematurely decrease bud hardiness via partial deacclimation, which can be detrimental in the event of a spring frost.

Objectives of this study

Douglas-fir is an important tree for forestry both in British Columbia and overseas. This study was undertaken to address the high stress-related mortality of Douglas-fir seedlings planted in the submaritime zone of BC. Understanding when and to what degree stresses like frost damage and drought stress occur and how they limit Douglas-fir seedling success is key to helping plantations flourish. The anticipated impact of climate change on forest habitat is likely to negatively affect the ability of trees to survive and grow in many areas. Forest managers are planning ahead by selecting for appropriate traits in breeding programs to increase seedling success in the field, and help forests regenerate more successfully over the next rotation.

The objectives of this study were to explore family differences among a selection of families of submaritime Douglas-fir in response to cold and drought stress, to identify exceptional families best able to resist or tolerate stress, and to examine relationships between growth and measures of cold and drought stresses to further the understanding of seedling stress physiology in Douglas-fir.

Section 2 – Materials and Methods

Field trial

Field sites

In 2005, 113 submaritime Douglas-fir families were planted at 3 field sites, Blackwater, Birken, and Hurley, in the Coast Mountains near Pemberton, British Columbia. All field sites were former clearcuts, with some slash and brush competition.

Blackwater is in the IDFww biogeoclimatic zone (BGCZ) at lat. 50°32' N, long. 122°30' W, and 390 m elevation. It has a flat slope and a S-SW aspect, and is the driest of the sites with an annual precipitation of 711mm. This site alone was fenced to limit herbivore browsing, and had been burned prior to planting. From visual estimates, there was the least brush competition at this site.

Birken is nearby, also in the IDFww BGCZ, at lat. 50°33' N, long. 122°32' W, and 686 m elevation. Its aspect is SE, with a slope of 40%.

Hurley is in the CWHds ss01 BGCZ, at lat. 50°33' N, long. 123°03' W, and at 923 m, is the highest elevation site. It has a 40% slope and a S-SE aspect, and is the wettest of the 3 sites, with an annual precipitation of 1411mm.

Water use efficiency: Carbon fractionation

To estimate water use efficiency and select families for further study, the CO_2 fractionation of seedlings in the field trial was measured. Current year shoot samples were collected in November 2007 from 2 or 3 trees per family for 98 families at the Blackwater site. Samples were taken from laterals from the top whorl, pointing in roughly the same compass direction. Leaves and bark were removed, and the wood was dried in an oven, ground to a fine powder with a Wig-L-Bug tissue grinder (Crescent, 1212 Abbott Drive, Elgin, IL), packaged into tin capsules, and analysed for $\delta^{13}C$ at the University of California, Davis stable isotope facility. $\delta^{13}C$ was calculated as

$$\delta^{13}C = \frac{\text{plant } ^{13}C/^{12}C}{\text{standard } ^{13}C/^{12}C} - 1$$

where the standard was the Pee Dee Belamnite in South Carolina.

The resulting $\delta^{13}C$ values were used to estimate water use efficiency. Fifty families with a wide range of $\delta^{13}C$ values were selected for further study (Table 1). Of these families, 39 were half-sib, with a poly cross of pollen donors. These 39 families were considered "A" class, and had been selected from wild stand trees as free from obvious growth defects and grown in a seed orchard. Five of the families were full-sib top cross with an estimated 18% gain in height over wild stand trees, and six were open pollinated half-sib wild stand families. In October 2008, shoot samples from these 50 families were taken from 3 trees per family per site (450 trees) and analyzed as before for a more intensive assessment of $\delta^{13}C$ values to compare differences in water use efficiency among families.

Table 1. Seed type and female parent location of 50 submaritime Douglas-fir families planted at 3 field sites. Families are half-sibs except for top crosses. Percent gain is predicted gain in height for the family over wild stand trees.

		Female Parent Location			
Family	Seed Type	Elev. (m)	Latitude (°N)	Longitude (°W)	Geographic Name
511	"A" class	762	49 16	121 14	Tashme
512	"A" class	732	49 20	121 17	Nicolum
577	"A" class	366	49 20	121 25	Silver Lake
659	"A" class	762	50 30	122 45	Birkenhead
707	"A" class	792	49 26	121 23	Ogilvie peak
723	"A" class	945	49 43	121 49	Big Silver
724	"A" class	853	49 43	121 49	Big Silver
726	"A" class	914	49 33	121 36	Cogburn Creek
736	"A" class	914	49 01	121 23	Depot Creek
845	"A" class	400	50 58	125 30	Wahkash Creek
847	"A" class	274	50 59	125 28	Wahkash creek
849	"A" class	550	50 59	125 28	Kurucz Creek
850	"A" class	520	50 59	125 28	Kurucz Creek
896	"A" class	200	49 46	122 17	Skookumchuk
943	"A" class	781	50 33	124 03	Racoon Creek
944	"A" class	877	50 40	123 54	Toba Inlet
946	"A" class	893	50 41	123 57	Daugleigh
949	"A" class	524	50 40	123 56	Toba Inlet
950	"A" class	915	51 35	126 26	Pashleth Creek
1128	"A" class	575	50 03	123 06	Brandywine
1136	"A" class	650	50 07	122 56	Lost Lake
1138	"A" class	750	50 09	123 58	Green Lake
1147	"A" class	457	50 11	123 23	Squamish
1154	"A" class	366	50 32	123 07	Lillooet River
1157	"A" class	293	50 27	122 55	Lillooet River
1159	"A" class	381	50 26	122 57	Ryan River
1167	"A" class	451	50 31	122 28	Darcy
1168	"A" class	396	50 32	122 28	Darcy
1176	"A" class	683	49 54	122 01	Nahatlatch
1177	"A" class	616	49 54	122 01	Nahatlatch
1178	"A" class	671	49 54	122 01	Nahatlatch
1180	"A" class	366	49 56	122 55	Nahatlatch

1194	"A" class	128	49 33	121 26	Yale
1202	"A" class	206	49 08	121 14	Klesilkwa
1204	"A" class	652	49 08	121 14	Klesilkwa
1205	"A" class	213	53 03	127 08	Kimsquit
1207	"A" class	335	53 03	127 08	Kimsquit River
1210	"A" class	262	53 05	127 07	Kimsquit River
1232	"A" class	488	51 20	125 35	Klinaklini
31519	Wild stand	350	50 32	122 30	Devine
31670	Wild stand	700	50 02	121 30	Mowhakam Creek
31902	Wild stand	380	50 20	122 46	Ivey Lake
31957	Wild stand	62	49 00	121 25	Mt. Jarvis Hope
31967	Wild stand	600	50 00	121 25	Four Barrel Creek
45007	Wild stand	350	50 31	122 30	Devine
243x80	Top cross (18% gain)	30	47 00	122 50	Lacey (USA)
38x54	Top cross (18% gain)	212	47 55	121 40	Kellogg Lake (USA)
399x243	Top cross (18% gain)	610	49 13	122 10	Suicide Creek
573x561	Top cross (18% gain)	76	50 12	124 36	Llyod Point
900x101	Top cross (18% gain)	73	50 23	125 37	Knox Bay

Survival and growth

Mortality of trees in the field was recorded yearly each spring from 2005-2008 by BC Ministry of Forests and Range staff. In October 2008, total height, current year growth increment, and root collar diameter were measured at Blackwater, Birken, and Hurley for the selected 50 families, 3 trees per family per site (450 trees).

Cold hardiness

Spring cold hardiness

Cold hardiness sampling dates were determined using climate and seedling performance data at a site near Surrey, British Columbia, Canada (lat. 49°50' N, long. 122°51' W). Pemberton climate data were compared, and the timing of anticipated seedling hardening and dehardening was selected according to climate differences. In the spring, the sampling date was aimed for when tissues would show sensitivity to freezing damage during seedling dehardening before bud burst, in an attempt to see if some seedlings dehardened earlier than others. In May 2008 prior to bud burst, shoot samples were collected from 98 families (93 half-sib "A" class, 5 full-sib top cross), 3 trees per family, at the Blackwater site. Samples were taken from the previous year's growth from lateral branches in the top whorl, from lateral branches pointing in a consistent compass direction. Excised shoots from each tree were transported on ice, cut up and divided into two freezing treatments: -7 °C and -12 °C. Shoot segments were put into 1.5 mL tubes with a few drops of distilled water in the bottom. Before freezing, all samples were dark-acclimated for 20 min and their chlorophyll fluorescence measured using a hand-held chlorophyll fluorometer (Opti-Sciences OS-30p, Hudson, NH) to determine unfrozen fluorescence levels. Samples were then frozen in a programmable freezer from 0°C at a rate of 5°C per hour, held at the minimum temperature for the treatment for one hour, then thawed overnight in a refrigerator. The following day, the samples were acclimated to room temperature in the dark for 20 min, then post-freezing chlorophyll fluorescence was assessed. The chlorophyll fluorometer measured the maximal (F_m) and the minimal (F_o) fluorescence. The photochemical efficiency of photosystem II (F_v/F_m) was derived using the equation:

$$F_v/F_m=(F_m-F_o)/F_m$$

F_v/F_m is an indicator of stress damage to membranes (Maxwell and Johnson, 2000) and correlates well with visual assessment of cold hardiness (Perks *et al.*, 2004). Relative cold hardiness was determined by calculating a ratio of unfrozen to frozen values, using the equation:

$$\text{Cold hardiness}=(F_v/F_m \text{ post-freezing})/(F_v/F_m \text{ pre-freezing})$$

where a larger value means greater cold hardiness.

Fall cold hardiness

In the fall, the sampling date was chosen to be when hardening was well underway, but had not yet reached maximum levels. In October 2008, cold hardiness of the 50 selected families was assessed at Birken and Hurley, with 3 trees per family per site sampled (300 trees). Shoot samples were collected as for spring cold hardiness, and the excised shoot from each individual was divided into two freezing treatments: -17°C and -27°C. Pre-freezing measurement of chlorophyll fluorescence, freezing and subsequent reading of chlorophyll fluorescence proceeded as above for spring cold hardiness assessment.

Shoot water potential

On July 22-24, 2008, predawn shoot water potential was measured at the Blackwater site, between 0500 and 0800 hours, over 3 days. A 3000 series plant water status console (Soilmoisture Equipment Corp., Santa Barbara, CA) was used to sample 3 trees per family for 50 families. Shoot samples were excised from the second whorl, in a consistent compass point orientation, and included current years' growth and at least 5 cm of previous years' growth. About 10mm of rain occurred over the sampling period.

Pot trial

Test material and location

Seedlings from 22 submaritime zone Douglas-fir families were germinated and grown in styrofoam blocks with macro- and micro-nutrients under greenhouse conditions at the Cowichan Lake Research Station, British Columbia in 2007. These 22 families were a subset of the 113 families planted in the field trial. Sixteen of these families were half-sib "A" class families, 3 were full-sib top crosses, and 3 were half-sib open-pollinated wild stand families. Each family had 15 individuals, with a few exceptions that had 13 or 14 individuals. In February 2008, when the seedlings were one year old, they were transferred into two-gallon pots with approximately 1:1:2 peat:perlite:hog fuel and chelated micronutrient mix (Plant Products Co. Ltd., Brampton, Ontario). Seedlings were then transported to the University of Victoria field site (48° 27.6' N, 123° 18' W, 60.1 m) and grown in a randomized complete block design with 3 blocks (Appendix B, Fig. 15). Pots were covered by shade cloth that allowed 50% of photosynthetically active radiation (PAR) to reach pot level. A Li-Cor Li-250A (Lincoln, Nebraska) light meter was used for PAR readings. Temperature was monitored throughout the experiment using thermocouples distributed amongst the seedlings. Temperature readings were stored every 30 min on a datalogger. Each pot was watered manually with 300mL of a solution of 20:7:19 fertilizer delivering 200mg N to each pot, once per month.

Table 2. Seed type and female parent location of 22 submaritime Douglas-fir families grown in a pot trial under 3 drought treatments. Families are half-sibs except for top crosses. Percent gain is predicted gain in height for the family over wild stand trees.

		Female Parent Location			
Family	Seed Type	Elevation (m)	Latitude (°N)	Longitude (°W)	Geographic Name
511	"A" class	762	49 16	121 14	Tashme
512	"A" class	732	49 20	121 17	Nicolum
577	"A" class	366	49 20	121 25	Silver Lake
659	"A" class	762	50 30	122 45	Birkenhead
724	"A" class	853	49 43	121 49	Big Silver
850	"A" class	520	50 59	125 28	Kurucz Creek
943	"A" class	781	50 33	124 03	Racoon Creek
944	"A" class	877	50 40	123 54	Toba Inlet
946	"A" class	893	50 41	123 57	Daugleigh
1138	"A" class	750	50 09	123 58	Green Lake
1147	"A" class	457	50 11	123 23	Squamish
1159	"A" class	381	50 26	122 57	Ryan River
1180	"A" class	366	49 56	122 55	Nahatlatch
1204	"A" class	652	49 08	121 14	Klesilkwa
1205	"A" class	213	53 03	127 08	Kimsquit
1210	"A" class	262	53 05	127 07	Kimsquit River
419	Wild stand	210	51 07	125 35	Knight Inlet
1276	Wild stand	-	-	-	-
7802	Wild stand	-	-	-	-
192x54	Top cross (18% gain)	2000	49 07	121 36	Foley
243x101	Top cross (18% gain)	100	47 00	122 50	Lacey (USA)
299x561	Top cross (18% gain)	900	49 36	123 19	Potlach

Drought treatments

Three watering treatments were administered beginning early June. Treatment 1 seedlings were given 825 mL of water when their pots reached 70% of their weight at field capacity. Treatment 2 seedlings were watered with 425 mL at 50% field capacity, and treatment 3 seedlings were watered with 300 mL at 40% field capacity. These treatments were selected by drying a subset of test seedlings down from field capacity and measuring predawn shoot water potential at different pot weight percentages. Pot weights of 70%, 50%, and 40% of field capacity corresponded to predawn shoot water potentials of -0.3MPa, -1.5 MPa, and -2.5 MPa, respectively, in a subset of seedlings. This method enabled the selection of minimum allowable pot weights that caused a good spread of shoot water potentials between treatments.

Growth and biomass allocation

Seedling height was measured in the spring before bud break, and again in the fall after bud set. Julian date of bud burst was recorded for each seedling on the day that leaves began extending past parted bud scales. Mortality of seedlings was assessed in November 2008. Seedlings were recorded as dead if >50% of foliage was brown. In November, roots were washed to remove all potting medium. Roots and shoots were separated, placed in paper bags and dried in an oven at 60°C. Leaves were removed from stems, so that roots, stems and leaves could be weighed separately.

Gas exchange, photosynthesis, and water relations

A LCA-4 portable, open system infrared gas analyser with a PLC4 leaf chamber (Analytical Development Company Ltd., Hertfordshire, England) was used to measure foliar gas exchange in all seedlings in Treatment 1 and Treatment 3. Measurement of the differences in H_2O and CO_2 concentrations inside and outside the leaf chamber allowed for calculation of net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs). Readings were taken between 1000 and 1400 hours on 3 days, with seedlings in one block measured each day. One seedling/family/treatment/block was measured in early August, and persistent cloudy conditions delayed the measurement of the remaining seedlings until early September. The leaf chamber was clamped over current year's growth on a lateral from the top whorl. Lammas growth was avoided. On each seedling, chamber conditions were allowed to equilibrate on the shoot for 3 minutes, and then 3 readings were taken, 1.5 min apart. These 3 readings were averaged to give final values of parameters.

After gas exchange was measured, the lateral shoot that occupied the leaf chamber was excised, and its midday shoot water potential was measured with a 3000 series plant water status console (Soilmoisture Equipment Corp., Santa Barbara, CA). The leaf area of this shoot portion was measured using a Li-Cor LI-3100 Area Meter (Lincoln, Nebraska). Leaf area was used to calculate A, E and gs.

To assess any membrane damage due to drought conditions, chlorophyll fluorescence of current year needles on all seedlings was measured *in situ* using a hand-held chlorophyll fluorometer (Opti-Sciences OS-30p, Hudson, NH). Foliage to be read was first dark-acclimated for 20 min using leaf clips. Readings were taken between 1000 and 1400 hours in early September. F_v/F_m was calculated as above for cold hardiness.

Statistical analyses

For the field trial and the pot trial, significant effects of family and site or drought treatment for all measured parameters were explored using analysis of variance performed in R (R Development Core Team, 2008). Analyses of variance were performed according to the following models:

Field trial

For cold hardiness:

$$Y = \mu + F_i + S_j + T_k + FS_{ij} + FT_{ik} + ST_{jk} + FST_{ijk} + \varepsilon_{ijkl}$$

For growth traits and carbon fractionation:

$$Y = \mu + F_i + S_j + FS_{ij} + \varepsilon_{ijk}$$

where

μ is the overall mean

F_i is the effect of the *i*th family

S_j is the effect of the *j*th field site

T_k is the effect of the *k*th freezing temperature

ε is the random effort associated with measurements of individual seedlings.

Pot trial

For growth traits, chlorophyll fluorescence, gas exchange parameters and shoot water potential:

$$Y = \mu + F_i + R_j + B_k + FR_{ij} + FB_{ik} + RB_{jk} + FRB_{ijk} + \varepsilon_{ijkl}$$

where

μ is the overall mean

F_i is the effect of the *i*th family

R_j is the effect of the *j*th drought treatment

B_k is the effect of the *k*th block

ε is the random effort associated with measurements of individual seedlings.

Square root and arcsine transformations were performed as necessary to stabilize error variance. For the field trial, variance components were calculated in SAS (SAS Institute Inc., 1996). Narrow-sense heritability estimates (h^2_{ns}) were calculated as in Squillace (1974) using the equation:

$$h^2 = 3*\sigma^2_A/\sigma^2_A+\sigma^2_{A*B}+\sigma^2_E$$

where:

σ^2_A = additive genetic variance component,

σ^2_{A*B} = variance component estimating family by site interaction, and

σ^2_E = environmental variance component.

For half-sibs, additive genetic variance is generally assumed to be four times the variance due to families. In this case, the pollen donor is a poly mix of males, additive genetic variance is assumed to be three times the family variance. For the cold hardiness h^2 estimates, the family by freezing temperature interaction variance component (σ^2_{A*T}) was added to the denominator:

$$h^2 = 3*\sigma^2_A/\sigma^2_A+\sigma^2_{A*B}+\sigma^2_{A*T}+\sigma^2_E$$

To explain variability in variance components and heritabilities between sites, ANOVAs of individual sites were performed to compare mean squares with variance components at different sites.

To investigate performance tendency of seed types, means and standard errors of traits for "A" class, top cross and wild stand seed types were calculated.

Height and proportion of surviving individuals in the field trial were used to create an index of success, or selection index, of the 50 families at field sites in the submaritime zone. Selection index equals the product of the mean family height and survival proportion after 3 years, both averaged over 3 field sites. A higher survival index indicates superior height growth or survival, or both, at these test sites. The aim of this index was to give an integrated value for family success to aid in selection of families for the submaritime Douglas-fir breeding program.

Linear and polynomial correlation analyses between continuous physiological variables in both field and pot trials were performed in R (R Development Core Team, 2008) and Excel (Microsoft Corporation).

Section 3 – Family Comparisons

Introduction

Climate warming is expected to change forest site characteristics in British Columbia (Nigh, 2006), and longer periods of summer drought are forecast (Mote, 2003). Running north to south along the Coast mountains in British Columbia, the submaritime zone is characterized by cold winters and hot dry summers. Reduced growth and high mortality of seedlings planted for reforestation due to summer drought are currently a concern in this region, and the severity of drought may increase over time as temperatures rise. The effects of increased summer drought will likely be most pronounced on sites with shallow, well drained soils, extreme slopes, and south and west aspects (Case and Peterson, 2005). This dry mountainous region has the potential to become even more inhospitable for seedlings if current trends in climate continue.

A centrally important forest tree in British Columbia, Douglas-fir plays important roles both ecologically and in the forest industry. A breeding program for this species in the submaritime breeding zone has recently been established, to increase growth and survivorship of seedlings planted in reforestation efforts. At low to mid elevations, growth of Douglas-fir is most affected by temperature and precipitation levels during the growing season (Case and Peterson, 2005). As the climate changes, summers in the Pacific Northwest will likely become warmer and drier (Mote, 2003). Two consequences of changing climate are likely to be manifest in plantations of Douglas-fir in the submaritime zone. Firstly, decreasing summer moisture and increasing temperatures will likely lead to increased drought stress, causing reduced growth and increased mortality. Secondly, higher temperatures throughout the year may extend the growing season, making local populations suboptimal in phenology. Consequences of these changes range from suboptimal growth to increased damage from spring and fall frosts.

Tree breeding programs with the aim of improving seedling performance have been implemented in British Columbia for decades. Genetically improved Douglas-fir stock is planted extensively on the coast. Historically, the main objective in Douglas-fir breeding has been to increase gain in height, but there is growing interest in ensuring sufficient stress resistance of deployed seedlings. Tests of seedling performance are necessary to find families to add to the breeding program that have the genetic and physiological characteristics necessary to survive the harsh conditions of the region. There have been many progeny trials in western North America that have explored genetic variation in growth and hardiness in forest trees. Provenance and family differences in height, water use efficiency, and cold hardiness have been revealed in Douglas-fir (Aitken *et al*., 1995; Aitken and Adams, 1996; Zhang *et al*., 1993; Howe *et al*., 2003). A history of genotype tests with potted seedlings also exists in the study of conifer physiology (Timmis and Tanaka, 1976; van den Driessche, 1992). Pot trials are especially attractive for the study of drought hardiness, as they allow fine control of water availability. The relevance of potted experiments to field performance comes into question, however, when large differences between the two environments in nutrition, soil, competition and other container effects are considered.

Measures of drought hardiness

Shoot water potential

The Scholander pressure chamber has been used widely in the measurement of water potential of leaves and shoots (Turner, 1988; Landis, 1989). In this method, the plant tissue is placed in a metal pressure vessel which is pressurized with an inert gas, with the cut base extruding. Pressure is slowly raised in the chamber, until water is seen to be forced from the xylem in the cut end. The pressure at which this occurs is equal but opposite in sign to the water potential of the tissue, often expressed in megaPascals (MPa) (Turner, 1981). The shoot water potential is a good indicator of how much water stress a plant is experiencing at the time of measurement, with more negative values indicating greater stress (Turner, 1988; Landis, 1989). Sampling is only moderately destructive as merely a leaf or small portion of shoot is necessary for measurement, and it is portable and does not require electricity, which makes it attractive for field work.

Water use efficiency: Carbon fractionation

Another approach to studying plant water relations is the estimation of water use efficiency. This can be done by measuring the carbon isotopic fractionation of plant tissues. Fractionation of C isotopes is caused by different diffusivity of $^{13}CO_2$ and $^{12}CO_2$ through the stomatal opening, and more importantly by fractionation by the Calvin cycle enzyme RUBISCO in C3 plants (Farquar *et al.*, 1989). The lighter and more abundant $^{12}CO_2$ diffuses more readily, and is preferentially fixed in photosynthesis (Farquar *et al.*, 1989). When stomata are closed due to water stress, continuing photosynthesis utilizes a greater proportion of $^{13}CO_2$ as the lighter isotope becomes depleted in the substomatal space. This change in proportion of fixed ^{13}C is measured in the wood as $\delta^{13}C$, a dimensionless quantity which refers to the difference in ^{13}C proportion of a sample relative to a standard, which is the Pee Dee limestone formation in South Carolina (Farquar *et al.*, 1989). As $\delta^{13}C$ values are so small, they are presented as "parts per mil" ($= x10^{-3}$ or ‰). These resulting fractionation values can be used to estimate water use efficiency integrated over the growing season.

Leaves are used in some studies to determine $\delta^{13}C$, however, leaf carbon content is metabolically transient and isotope ratios change over the growing season (Chevillat *et al.*, 2005). Leaves of *Solanum tuberosum* have been shown to further fractionate carbon during carbon storage metabolism (Gleixner *et al.*, 1998). Wood deposited over an entire growing season, however, is a good integrative measure of $\delta^{13}C$ for the season (Chevillat *et al.*, 2005). While some studies use cellulose extractions of wood to measure carbon isotope fractionation, untreated wood carbon isotope ratios correlate well with those of extracted cellulose, and have equal merit in determining relative $\delta^{13}C$ among Douglas-fir trees (Aitken *et al.* 1995; Ares *et al.* 2008). Carbon fractionation is a good indicator of water use efficiency in Douglas-fir and correlates well with other methods such as gas exchange and the comparison of water transpired to biomass gained (Ripullone *et al.*, 2004). Ponton *et al.* (2006) found high correspondence between carbon fractionation values and water use efficiency calculated from gas exchange values in a Douglas-fir forest. Carbon fractionation is the best measure to examine water use efficiency over the entire growing season (Ripullone *et al.*, 2004).

Gas exchange analysis

The measurement of the foliar gas exchange of a plant gives valuable information on transpiration and net photosynthetic rates (Pessarakli, 2005). This information can be interpreted in the context of drought response. A portable, open system infrared gas analyser measures the differences in H_2O and CO_2 concentrations inside and outside a cuvette clamped over a photosynthesizing leaf (Long *et al*., 1996). This allows the calculation of the net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) per unit leaf area (Analytical Development Company, 1993). The ratio of transpiration to photosynthetic rate can be used as a measure of water use efficiency, indicating the amount of water transpired per unit carbon fixed (Ponton *et al*., 2006). Closure of stomata and reduction in transpiration and photosynthetic rates can be observed in response to drought (Brix, 1979; Warren *et al*., 2003; Pessarakli, 2005).

Measures of cold hardiness

Whole-plant freeze testing (WPFT)

This technique involves the freezing of all the above-ground tissue of plants in a controlled temperature chamber. Evaluation of damage can be done by visually rating tissue browning, electrolyte leakage, or chlorophyll fluorescence (Burr *et al.,* 2001). A benefit of whole-plant freezing is that the intact plant (or a large portion) is frozen, allowing plant-scale physiological processes like interactions between different tissues and organs to take place during freezing. This helps to show more of the true viability of the whole plant under more natural conditions. However, some constraints of WPFT include a long time period before damage becomes evident (up to 7-10 days), the destruction of a lot of plant tissue, making measurements of the same plant over time difficult, and poor precision due to necessarily small sample sizes (Burr *et al*. 2001). Visual assessments of browning, while fast and simple to perform, are more subjective than other methods of evaluating damage.

Electrolyte leakage (EL)

As freezing damages plasma membranes, their permeability to ions increases (Burr *et al*., 2001). EL analysis measures the resulting amount of ion leakage. Electrolyte leakage is quantified by measuring the conductivity of the solution surrounding stem or foliage tissue with a conductivity meter (Sakai and Larcher, 1987). Membrane permeability to ions is measured post-freezing, and then again after samples are heatkilled to achieve a maximum permeability value (Burr *et al.,* 2001). A comparison of these values allows researchers to determine the amount of damage caused by freezing. Tissue ion concentrations can, however, be affected by differences in nutrient uptake, fertilization, and pollutant exposure (Burr *et al.,* 2001). Previous drought or heat stress can also affect membrane permeability (Burr *et al.,* 2001).

Chlorophyll fluorescence (CF)

The functioning of photosystem II can be measured and used as an estimate of stress-related damage, as photosynthesis is dependent on membrane integrity (Burr *et al*., 2001). Chlorophyll fluorescence is an effective method of measuring vigour of tissues after exposure to any stress, including cold (Rose and Haase, 2002; Perks *et al*., 2004). When light is absorbed by a chlorophyll molecule, it has one of 3 possible fates. It can be utilized to drive photosynthesis, dissipated as heat, or re-emitted as light (chlorophyll fluorescence) (Maxwell and Johnson, 2000). A decrease in the efficiency of photosynthesis affects the amount of fluorescence emitted by photosynthetic tissue (Maxwell and Johnson, 2000), and this can be measured using a simple handheld apparatus. Green photosynthetic tissue is required for analysis. This method requires less time to perform than EL; otherwise, it shares many of the benefits and shortcomings. Researchers should keep in mind all possible membrane or photosystem damage due to stress when using this method.

Heritability and selection

The phenotype of an organism is determined both by its genotype and its environment. One way of quantitatively exploring the genetic influence on phenotype is through the calculation of heritability. Two types of heritability are commonly reported, known as broad sense (H^2) and narrow sense (h^2) heritability. Both measures of heritability are calculated as the genetic component of variance in a trait divided by the phenotypic variance. H^2 comes out of cloning studies, in which genetic variance due to both additive and non-additive genetic effects can be pinpointed. h^2 is calculated using only additive genetic effects in the numerator. As h^2 partitions non-additive genetic effects like dominance and epistasis with environmental variance, it is a more appropriate measure of heritable genetic control through sexual reproduction and breeding (White *et al.*, 2007), and is used in this study.

Traits including bud phenology, emergence rates, cold and drought hardiness, and growth have displayed genetic structure that correlates well with geographical gradients. This correlation likely indicates adaptive significance (St. Clair *et al.*, 2005). There is evidence that natural selection is most intense during the seedling phase of development (St. Clair and Howe, 2005). Campbell (1979) estimated that each mature parent tree in a stand is selected from 2000+ individuals of seedling age or younger. As selection at the seedling stage is so crucial, so is the hardiness of seedlings planted in reforestation programs.

Survival of seedlings in the field over a number of years could be considered a broad surrogate for hardiness. As both growth and survival are of interest to tree breeders, an ideal "selection index" would incorporate both these traits, and would therefore highlight a family's suitability for selection in a tree breeding program.

Results in this section aim to quantify family performance in the classic field progeny test environment, on multiple sites with a high sample size, as well as in a smaller-scale pot trial giving us a better opportunity to investigate physiological processes. Analyses of heritability and other measures of family variation in Douglas-fir add to the knowledge base of genetic control of traits of interest in this species. This was enhanced by the ability to compare detailed physiological measures in the pot trial with field performance. A more applied and practical objective was to assess the growth, hardiness and survival performance of a range of families for the purpose of selection and improvement of stock used in the submaritime zone Douglas-fir breeding program.

Results

Field trial

Means comparisons

Field trial ANOVA results are presented in Table 3. Family (female parent) had no significant effect on growth traits (height, leader growth increment and root collar diameter). Family effects on carbon fractionation ($\delta^{13}C$ (‰)) and predawn shoot water potential were also not significant. Family did, however, have a highly significant effect ($p < 0.001$) on fall cold hardiness. ANOVA results for individual sites are presented in Appendix A. When Hurley data were analysed separately, root collar diameter and carbon fractionation were significantly affected by family (Appendix A, Tables 26 and 27).

Site had a significant effect on every parameter measured at more than one site (Table 3). These included height, leader growth increment, root collar diameter, carbon isotope fractionation, and fall cold hardiness. Average seedling height and root collar diameter were highest at Blackwater, followed by Birken and Hurley (Table 4). Average leader growth increment in 2008, however, was highest at Birken, with Blackwater and Hurley having similar, lower growth (Table 4). Height and leader increment were moderately correlated (R^2=0.6461). Height and root collar diameter were more highly correlated (R^2=0.8243). Family heights are given in Table 5. Mean carbon fractionation ($\delta^{13}C$) was least negative at Blackwater (-23.8 ± 0.069 ‰), indicating greater carbon assimilation per unit water transpired (Fig. 1). Seedlings growing at Birken exhibited more fractionation (-24.5 ± 0.076 ‰), and Hurley had the greatest fractionation (-25.2 ± 0.065 ‰), suggesting less stomatal closure during photosynthesis (Fig. 1). Average fall cold hardiness of families was greater at Birken (0.471 ± 0.021) than at Hurley (0.430 ± 0.021). Families maintained their rankings in growth and carbon fractionation among the 3 sites, as shown by the lack of significant family*site interactions (Table 3). There was a family*site interaction for fall cold hardiness between the sites Birken and Hurley. Families changed ranking in hardiness between the two sites (Fig. 2).

Table 3. ANOVAs of physiological traits of seedlings of 50 half-sib submaritime Douglas-fir families measured in 2008 at 3 field sites (unless otherwise indicated).

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Height (cm)	Family	49	43169	881	1.1664	0.22059	
	Site	2	6940	3470	4.5942	0.01083	*
	Family*Site	97	78990	814	1.0781	0.31356	
	Residuals	300	226598	755			
Leader increment (cm)	Family	49	4566	93.2	1.0907	0.324833	
	Site	2	950	475	5.5598	0.004257	**
	Family*Site	97	8840.3	91.1	1.0668	0.336819	
	Residuals	300	25629.4	85.4			
Root collar diameter (mm)	Family	49	2452.7	50.1	1.228	0.1548	
	Site	2	1882.6	941.3	23.0927	4.70e-10	***
	Family*Site	97	3755.8	38.7	0.9499	0.6107	
	Residuals	300	12228.5	40.8			
$\delta^{13}C$ (‰)	Family	49	46.02	0.939	1.3079	0.09355	.
	Site	2	144.44	72.22	100.569	< 2e-16	***
	Family*Site	97	67.167	0.692	0.9642	0.57577	
	Residuals	295	211.845	0.718			
Predawn shoot water potential at Blackwater (MPa)	Family	49	24.2375	0.4946	0.9024	0.63837	
	Day sampled	2	5.3726	2.6863	4.9008	0.01187	*
	Family*Day sampled	52	27.561	0.53	0.967	0.54897	
	Residuals	45	24.6658	0.5481			
Spring cold hardiness at Blackwater	Family	97	4.278	0.044	1.0467	0.3665	
	Freeze temp	1	108.98	108.98	2586.62	<2e-16	***
	Family* Freeze temp	97	3.919	0.04	0.959	0.5925	
	Residuals	784	33.032	0.042			
Fall cold hardiness at Birken and Hurley	Family	49	7.703	0.157	2.7299	3.76e-08	***
	Freeze temp	1	38.623	38.623	670.667	< 2.2e-16	***
	Site	1	0.262	0.262	4.5425	0.03367	*
	Family* Freeze temp	49	1.836	0.037	0.6507	0.96708	
	Family*Site	49	4.328	0.088	1.5338	0.01521	*
	Freeze temp:Site	1	0.013	0.013	0.2195	0.63966	
	Family* Freeze temp *Site	49	1.802	0.037	0.6385	0.97244	
	Residuals	400	23.036	0.058			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 4. Site differences in growth traits of 450 Douglas-fir seedlings measured in fall 2008 at 3 field sites in the coast mountains of British Columbia.

	Site	Mean	Standard error
Height (cm)	Blackwater	84.08	2.39
	Birken	79.76	2.33
	Hurley	74.44	2.12
Leader increment (cm)	Blackwater	12.43	0.80
	Birken	15.32	0.91
	Hurley	12.08	0.54
Root collar diameter (mm)	Blackwater	22.05	0.53
	Birken	18.29	0.54
	Hurley	17.26	0.51

Family performance

Of the 50 families studied, top cross and "A" class families had the highest mean height (82 ± 2 cm and 80 ± 2 cm, respectively) followed by wild stand families (72 ± 5 cm). Top cross and "A" class families grew taller, on average, than wild stand families. However, for survival, selection index, and fall cold hardiness, this trend was reversed. Wild stand families fared better in these attributes than families that were expected to show height gain. There was no distinction between "A" class and top cross families in survival, selection index and fall cold hardiness. Wild stand survival proportion was highest (0.57 ± 0.02), followed by "A" class (0.48 ± 0.01) and top cross (0.44 ± 0.04). Wild stand families had the highest average selection index of 41.2 ± 2.3 (Table 5). The average "A" class and top cross survival indices were 38.8 ± 1.1 and 35.7 ± 3.1, respectively (Table 5).

Family 944, an "A" class family with a predicted 10% height gain, had exceptionally high height growth and low mortality, giving it the highest selection index at 69.3 (Fig. 9). The female parent tree for this family was from Toba Inlet in the coast mountains of British Columbia (50° 23' 60" N, 123° 32' 24" W, 877m elev.).
Family 399x243, a top cross family with predicted 18% height gain, had good height growth but very poor survival, giving it the lowest selection index at 27.1.

Wild stand families had higher fall cold hardiness, on average. Fall cold hardiness values for wild stand, "A" class and top cross families were 0.60 ± 0.06, 0.44 ± 0.02, and 0.39 ± 0.05, respectively.

In carbon fractionation, "A" class and top cross families had identical average $\delta^{13}C$ values, -24.48 ± 0.05‰ and -24.48 ± 0.15‰, respectively. Wild stand families had more negative average fractionation (-24.86 ± 0.15‰), suggesting a greater tendency for wild stand stomata to remain open during photosynthesis, thus causing a lower water use efficiency. "A" class family 943 had the least negative $\delta^{13}C$ value at -23.94‰, indicating more carbon assimilation during stomatal closure, leading to a higher water use efficiency. The female parent for this family was from Raccoon Creek in the Coast mountains (50° 19' 48" N, 124° 1' 48" W, 781m elev.). Wild stand family 31957 had the most negative $\delta^{13}C$ at -25.22‰.

Table 5. Mean family fall cold hardiness (n=9), height (n=9), survival proportion, and selection indices of 50 submaritime Douglas-fir families of 3 seed types planted at 3 field sites (Blackwater, Birken and Hurley) in 2005 and measured in 2008. Cold hardiness was assessed using freezing trials and chlorophyll fluorescence; values are (post-freezing F_v/F_m / pre-freezing F_v/F_m), with higher values indicating higher cold hardiness. Selection index is calculated as mean family height at 3 field sites * the proportion of individuals in a family that have survived 3 years after outplanting at 3 field sites. Families are half-sibs except for top crosses. Percent gain is predicted gain in height for the family over wild stand trees.

Seed Type	Family	Fall Cold Hardiness	Height (cm)	Survival Proportion	Selection Index
Top cross	243x80	0.381	82	0.53	43.3
(18% gain)	38x54	0.52	92	0.46	42.1
	573x561	0.397	69	0.51	35
	900x101	0.231	73	0.43	31.1
	399x243	0.4	94	0.29	27.1
"A" class	944	0.462	113	0.61	69.3
	1178	0.569	84	0.58	48.8
	849	0.35	90	0.51	45.9
	1168	0.487	90	0.5	45.1
	736	0.304	93	0.47	44.1
	1207	0.425	81	0.53	43.1
	896	0.503	92	0.47	42.8
	1167	0.408	86	0.49	42.2
	847	0.293	77	0.54	42.2
	1147	0.313	80	0.52	41.7
	1205	0.311	78	0.53	41.4
	659	0.598	80	0.51	40.8
	1202	0.35	88	0.46	40.1
	950	0.489	88	0.46	40.1
	512	0.372	85	0.47	39.6
	1159	0.483	76	0.52	39.3
	1128	0.379	96	0.41	39.2
	1157	0.494	76	0.51	39.1
	724	0.377	74	0.52	38.8
	946	0.499	75	0.5	37.6
	1154	0.43	77	0.49	37.5
	577	0.526	74	0.5	37.1
	1180	0.399	74	0.5	36.9
	723	0.434	74	0.49	36.6
	511	0.568	74	0.49	36.4
	850	0.366	90	0.4	36.1
	1232	0.32	70	0.51	35.8
	1177	0.675	70	0.5	35

	1138	0.424	78	0.45	35
	943	0.364	73	0.48	34.9
	949	0.399	79	0.43	34.3
	707	0.416	83	0.41	34.1
	1194	0.447	72	0.46	32.9
	1210	0.41	79	0.41	32.6
	726	0.535	69	0.47	32.3
	1176	0.539	87	0.37	31.8
	1204	0.382	57	0.55	31.3
	845	0.602	81	0.38	30.7
	1136	0.315	62	0.49	30.4
Wild stand	31902	0.418	81	0.58	46.8
	45007	0.419	74	0.61	45.4
	31670	0.668	73	0.59	43.3
	31519	0.633	70	0.61	43
	31957	0.656	68	0.52	35.4
	31967	0.783	66	0.5	33.2

Heritability

Growth, carbon fractionation and cold hardiness data from the field trial were analysed for narrrow-sense heritabilities (h^2_{ns}) (Table 6). Fall cold hardiness was the most heritable trait in the study, with an h^2 of 0.6493 at the Birken site. Heritabilities of traits were highly variable among sites, reflecting large differences in environment between sites (Table 6). Hurley had the highest heritabilities for height growth (h^2=0.3795), root collar diameter (h^2=0.48510), and carbon fractionation (h^2= 0.4855). Birken had the highest heritability for leader growth increment (h^2=0.1465) as well as for fall cold hardiness. Heritabilities calculated from Blackwater data tended to be very low (Table 6).

The differences in h^2 values between sites are corroborated by mean squares in ANOVA results when effects are considered fixed (Appendix A, Tables 14-27). A higher proportion of variance between families compared to within families was observed at different sites for different traits, which led to higher heritabilities at certain sites, and also a more significant family effect in ANOVAs calculated at the same sites.

Table 6. Narrrow-sense heritabilities (h^2_{ns}) and variance components of traits in seedlings of 50 half-sib submaritime Douglas-fir families planted at 3 field sites in 2005 and measured in 2008.

Field site(s)	Random effect	Spring cold hardiness	Fall cold hardiness	Height	Leader increment	Root collar diameter	$\delta^{13}C$
Blackwater	h^2_{ns}	**0.1385**	.	**0.0039**	**0**	**0**	**0**
	Family	0.0020	.	1.103	0	0	0
	Family* Temp.	0.0000	.	.	.	.	.
	Residual	0.0419	.	858	95.38	41.85	0.7192
Birken	h^2_{ns}	.	**0.6493**	**0**	**0.1465**	**0**	**0**
	Family	.	0.0146	0	6.1	0	0
	Family* Temp.	.	0	.	.	.	.
	Residual	.	0.0529	815	117.9	43.22	0.8549
Hurley	h^2_{ns}	.	**0.4047**	**0.3795**	**0.0171**	**0.4851**	**0.4855**
	Family	.	0.0085	85.19	0.247	6.293	0.1033
	Family* Temp.	.	0	.	.	.	.
	Residual	.	0.0543	588.18	43.16	32.62	0.535
Birken and Hurley combined	h^2_{ns}	.	**0.2645**	.	.	.	.
	Family	.	0.0057	.	.	.	.
	Family* Site	.	0.0058	.	.	.	.
	Family* Temp.	.	0	.	.	.	.
	Residual	.	0.0535	.	.	.	.
Blackwater, Birken, and Hurley combined	h^2_{ns}	.	.	**0.0358**	**0.0026**	**0.0777**	**0.1111**
	Family	.	.	9.324	0.0762	1.070	0.0273
	Family* Site	.	.	16.52	1.939	0	0
	Residual	.	.	756	85.54	40.24	0.7094

Note: Cold hardiness= frozen/control F_v/F_m; height, leader increment (cm); root collar diameter (mm); $\delta^{13}C$ (‰).

Pot trial

Means comparisons

Pot trial ANOVA results are presented in Tables 7 and 8. Family had a significant effect ($p < 0.05$) on all growth traits measured on potted seedlings: root biomass, shoot biomass, root / shoot biomass ratio, initial seedling height, and leader growth increment. Family did not have a significant effect on *in situ* F_v/F_m or midday shoot water potential (Table 8).

Drought treatment had a significant effect on root biomass, shoot biomass, root / shoot biomass ratio, *in situ* F_v/F_m, and midday shoot water potential (Tables 7 and 8). Significant effects were unchanged when F_v/F_m was arcsine transformed. Seedlings in Treatment 3, which received the least water, had the lowest biomass, the lowest root / shoot ratio and the most negative shoot water potential, indicating higher water stress (Fig. 3). There was no significant effect of drought treatment on leader growth increment of the seedlings – most leader growth for the season took place in the spring before drought treatments began. Families maintained their rankings in biomass accumulation and allocation between treatments, as evidenced by an absence of significant family*treatment interactions.

Table 7. ANOVA results of physiological traits of potted seedlings of 22 half-sib submaritime Douglas-fir families grown under 3 drought treatments.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Root biomass (g)	Family	21	156.325	7.444	2.8969	0.0001414	***
	Treatment	2	121.238	60.619	23.5903	2.29e-09	***
	Block	2	2.055	1.028	0.3999	0.6712618	
	Family* Treatment	42	89.362	2.128	0.828	0.7547836	
	Family*Block	42	66.619	1.586	0.6173	0.9623659	
	Treatment*Block	4	13.49	3.372	1.3124	0.2692661	
	Family* Treatment*Block	84	217.576	2.59	1.008	0.4795441	
	Residuals	120	308.36	2.57			
Shoot biomass (g)	Family	21	766.06	36.48	10.3201	< 2.2e-16	***
	Treatment	2	49.11	24.56	6.9472	0.001397	**
	Block	2	9.24	4.62	1.3072	0.274414	
	Family* Treatment	42	115.98	2.76	0.7812	0.81858	
	Family*Block	42	152.89	3.64	1.0298	0.437785	
	Treatment*Block	4	14.15	3.54	1.0008	0.410014	
	Family* Treatment*Block	84	216.57	2.58	0.7294	0.937648	
	Residuals	120	424.17	3.53			
Root / shoot ratio	Family	21	2.08306	0.09919	5.6233	3.21e-10	***
	Treatment	2	0.24948	0.12474	7.0717	0.001249	**
	Block	2	0.1495	0.07475	4.2376	0.016662	*
	Family* Treatment	42	0.78991	0.01881	1.0662	0.384381	
	Family*Block	42	0.9334	0.02222	1.2599	0.167259	
	Treatment*Block	4	0.08264	0.02066	1.1712	0.327009	
	Family* Treatment*Block	84	1.99136	0.02371	1.3439	0.068413	.
	Residuals	120	2.11676	0.01764			
Initial height (cm)	Family	21	4553.8	216.8	31.866	< 2.2e-16	***
	Residuals	298	2027.9	6.8			
Leader growth (cm)	Family	21	469.52	22.36	5.4652	6.54e-10	***
	Treatment	2	4.25	2.13	0.5199	0.5959	
	Block	2	17.21	8.6	2.1033	0.1265	
	Family* Treatment	42	128.97	3.07	0.7506	0.8554	
	Family*Block	42	143.31	3.41	0.8341	0.7459	
	Treatment*Block	4	6.02	1.51	0.368	0.831	
	Family* Treatment*Block	83	242.03	2.92	0.7128	0.9495	
	Residuals	120	490.92	4.09			
F_v/F_m	Family	21	0.16633	0.00792	1.5362	0.07784	.
	Treatment	2	0.03197	0.01599	3.1008	0.04864	*
	Block	2	0.01543	0.00771	1.4962	0.22813	

Family* Treatment	42	0.18688	0.00445	0.863	0.70245
Family*Block	42	0.17895	0.00426	0.8263	0.75715
Treatment*Block	4	0.03785	0.00946	1.8355	0.12643
Family* Treatment*Block	84	0.5148	0.00613	1.1886	0.19148
Residuals	120	0.61872	0.00516		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 8. ANOVA results of shoot water potential (MPa) of potted seedlings of 22 half-sib submaritime Douglas-fir families grown under 3 drought treatments, sampled twice in summer 2008.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	21	6.956	0.331	1.0228	0.4726	
Treatment	1	56.743	56.743	175.2144	4.63e-13	***
Block	2	0.416	0.208	0.6424	0.5342	
Month.sampled	1	12.468	12.468	38.4991	1.46e-06	***
Family*Treatment	21	6.076	0.289	0.8935	0.5999	
Family*Block	42	14.9	0.355	1.0954	0.41	
Treatment*Block	2	2.112	1.056	3.2608	0.0545	.
Family*Month	21	5.282	0.252	0.7766	0.7206	
Treatment*Month	1	6.998	6.998	21.6078	8.50e-05	***
Block*Month	2	1.392	0.696	2.1495	0.1368	
Family*Treatment*Block	42	8.723	0.208	0.6413	0.9022	
Family*Treatment*Month	20	5.641	0.282	0.8709	0.62	
Family*Block*Month	5	0.552	0.11	0.3409	0.8834	
Residuals	26	8.42	0.324			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Gas exchange means comparisons

Pot trial gas exchange ANOVA results are presented in Table 9. Family had a significant effect ($p < 0.05$) on stomatal conductance (gs (mol m^{-2} s^{-1})), transpiration rate (E (mmol m^{-2} s^{-1})), and net photosynthetic rate (A (μmol m^{-2} s^{-1})). Family did not have a significant effect on instantaneous water use efficiency (A/E (μmol CO_2 taken up per mmol H_2O released)).

Drought treatment had a significant effect on all gas exchange parameters: stomatal conductance (gs), transpiration rate (E), and net photosynthetic rate (A); the effect on instantaneous water use efficiency (A/E) was significant when E was square root transformed. Significant effects were unchanged for E and gs after they were square root transformed. A, E, and gs were highest in Treatment 1, which received the most water (Fig. 4).

Significant family*treatment effects for A, E, and gs may indicate different variances between treatments, or rank changes in family performance between drought treatments. Some families that transpired more in Treatment 1 had lower transpiration levels compared to their fellows in Treatment 3, and vice versa (Fig. 5).

Table 9. ANOVA results of gas exchange of 22 half-sib submaritime Douglas-fir families grown under 2 drought treatments, sampled twice in summer 2008.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Stomatal conductance (gs (mol m^{-2} s^{-1}))	Family	21	0.038554	0.001836	3.9221	0.0004566	***
	Treatment	1	0.099175	0.099175	211.8701	1.38e-14	***
	Block	2	0.015532	0.007766	16.591	1.77e-05	***
	Month	1	0.004085	0.004085	8.7269	0.0062903	**
	Family*Treatment	21	0.021311	0.001015	2.168	0.0282579	*
	Family*Block	42	0.056693	0.00135	2.8837	0.0020899	**
	Treatment*Block	2	0.001401	0.000701	1.4968	0.2412173	
	Family*Month	21	0.009508	0.000453	0.9672	0.5244041	
	Treatment*Month	1	0.058457	0.058457	124.8825	7.84e-12	***
	Block*Month	2	0.004607	0.002303	4.9206	0.0147469	*
	Family*Treatment*Block	41	0.031762	0.000775	1.655	0.0820582	.
	Family*Treatment*Month	20	0.019707	0.000985	2.105	0.0344832	*
	Family*Block*Month	8	0.0141	0.001763	3.7653	0.0041482	**
	Treatment*Block*Month	1	0.000001	0.000001	0.0013	0.9715295	
	Residuals	28	0.013107	0.000468			
Transpiration rate (E (mmol m^{-2} s^{-1}))	Family	21	11.0928	0.5282	3.8624	0.0005182	***
	Treatment	1	27.3277	27.3277	199.8174	2.85e-14	***
	Block	2	2.8521	1.426	10.4271	0.0004126	***
	Month	1	7.8637	7.8637	57.4986	2.93e-08	***
	Family*Treatment	21	7.7593	0.3695	2.7017	0.0073958	**
	Family*Block	42	17.3541	0.4132	3.0212	0.0014315	**
	Treatment*Block	2	1.2182	0.6091	4.4538	0.0209209	*
	Family*Month	21	4.7246	0.225	1.645	0.1085039	
	Treatment*Month	1	16.6926	16.6926	122.0547	1.02e-11	***
	Block*Month	2	2.5201	1.2601	9.2135	0.0008422	***
	Family*Treatment*Block	41	10.7848	0.263	1.9234	0.0360276	*
	Family*Treatment*Month	20	5.8843	0.2942	2.1513	0.0306747	*
	Family*Block*Month	8	3.0722	0.384	2.8079	0.0202979	*
	Treatment*Block*Month	1	0.0211	0.0211	0.1543	0.6974508	
	Residuals	28	3.8294	0.1368			
Net photosynthetic	Family	21	69.927	3.33	2.6969	0.007483	**
	Treatment	1	104.354	104.354	84.5162	5.96e-10	***

rate (A (µmol m^{-2} s^{-1}))	Block	2	6.014	3.007	2.4356	0.105878	
	Month	1	49.403	49.403	40.0114	7.66e-07	***
	Family* Treatment	21	53.342	2.54	2.0572	0.037556	*
	Family*Block	42	129.419	3.081	2.4956	0.006328	**
	Treatment*Block	2	10.232	5.116	4.1436	0.026525	*
	Family*Month	21	23.449	1.117	0.9043	0.588453	
	Treatment* Month	1	233.992	233.992	189.5097	5.47e-14	***
	Block*Month	2	1.108	0.554	0.4488	0.642875	
	Family* Treatment*Block	41	65.968	1.609	1.3031	0.233035	
	Family* Treatment* Month	20	57.726	2.886	2.3376	0.019205	*
	Family* Block*Month	8	35.705	4.463	3.6147	0.005283	**
	Treatment* Block*Month	1	0.042	0.042	0.0343	0.85448	
	Residuals	28	34.572	1.235			
Instantaneous water use efficiency (A/E (µmol CO_2 taken up per mmol H_2O released))	Family	21	67.796	3.228	0.9564	0.5352472	
	Treatment	1	3.499	3.499	1.0365	0.3173625	
	Block	2	5.662	2.831	0.8386	0.4428829	
	Month	1	62.853	62.853	18.6194	0.0001796	***
	Family* Treatment	21	66.186	3.152	0.9337	0.5582699	
	Family*Block	42	98.416	2.343	0.6942	0.8607026	
	Treatment*Block	2	3.562	1.781	0.5277	0.5957302	
	Family*Month	21	41.33	1.968	0.583	0.8970155	
	Treatment* Month	1	151.18	151.18	44.7851	2.90e-07	***
	Block*Month	2	13.297	6.649	1.9696	0.1583768	
	Family* Treatment*Block	41	52.068	1.27	0.3762	0.9978301	
	Family* Treatment* Month	20	52.272	2.614	0.7742	0.72015	
	Family* Block*Month	8	61.673	7.709	2.2837	0.0506686	.
	Treatment* Block*Month	1	0.044	0.044	0.013	0.9098951	
	Residuals	28	94.519	3.376			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Family performance

"A" class families 944 and 943 were the top performers in growth and survival, and water use efficiency, respectively, in the field trial. These two families also displayed exceptional resistance to water stress in the pot trial. They exhibited the two least negative average midday shoot water potentials in the pot trial, -1.54 and -1.65 MPa. The family with the most negative mean shoot water potential was wild stand family 419, at -2.28 MPa. This family also had the second lowest root / shoot ratio in the study (0.78).

"A" class family 1147 had the highest net photosynthetic rate ($A = 4.13$ µmol m^{-2} s^{-1}). The family with the highest instantaneous water use efficiency was "A" class family 1180, with an A/E of 3.19 µmol CO_2 taken up per mmol H_2O released. The lowest net photosynthetic rate and water use efficiency were displayed by wild stand family 419 ($A = 1.96$ µmol m^{-2} s^{-1}, $A/E = 0.75$ µmol CO_2 taken up per mmol H_2O released). "A" class family 1210 and top cross 299x561 had the highest and lowest transpiration rates, $E = 1.213$ and 0.773 mmol m^{-2} s^{-1}, respectively.

Discussion

Site effects on seedling performance

High water use efficiency implies greater assimilation of carbon per unit water lost (Aitken *et al*., 1995). In this study, seedlings were most water use efficient at the driest site. As water availability was likely higher at sites with greater rainfall, seedlings' stomata likely remained open longer, allowing greater carbon fractionation. This suggests that decreased water availability at drier sites induced prolonged stomatal closure, increasing seedling water use efficiency. Korol *et al*. (1999) measured carbon fractionation in *Pinus radiata* and also found that trees had higher water use efficiency on sites that received less rainfall.

Blackwater had the greatest height and root collar diameter of the three sites, suggesting higher long-term vigour of seedlings. This site received the least rainfall, but a prior burn on the site, lack of brush competition, a deer fence, low elevation and a flat aspect likely contributed to greater seedling growth. Birken and Hurley were sloped and had more competing vegetation, which negatively affects crop seedling growth and survival in forest plantations (Rose *et al*., 2006). Additionally, the higher elevation at

Birken and Hurley likely meant cooler temperatures during the growing season. Birken seedlings had the greatest leader growth, and may have capitalized most on what was a wetter than normal summer.

Seedlings growing at Birken had greater fall cold hardiness than those growing at Hurley, which is surprising because Birken is at a lower elevation. A possible explanation is that because Birken was a drier site, trees growing there experienced a harsher summer drought and the acclimation to drought also enhanced cold hardiness. Water use efficiency was higher at Birken than Hurley, implying greater drought (Fig. 1). Several studies suggest that summer drought has the potential to influence fall cold hardiness. Although bud set phenology was not measured, the possibility exists that greater drought at Birken induced earlier bud set as seen in Khan *et al.* (1996). However, at Birken, fall cold hardiness was positively correlated with leader growth (see Section 4), thus hardier families may not necessarily set bud earlier. An alternate link between drought stress and fall cold hardiness is osmotic adjustment, which gives tissues a stronger affinity for water. In redbud (*Cercis canadensis*), Griffin *et al.* (2004) found greater abundance of soluble carbohydrates in water-stressed plants. Appreciable osmotic adjustment has been observed in drought-stressed Douglas-fir (Kozlowski and Pallardy, 2002), and may be a mechanism for increased survival in a xeric environment (van den Driessche, 1992). Any osmotic adjustment that occurred during drought acclimation in the summer could have preconditioned seedlings for increased fall cold hardiness later in the year. Many genes are known to be induced by both drought and cold stress (Shinozaki *et al.*, 2003). Candidate genes that are induced by water deficit in conifers have been found to map near cold hardiness quantitative trait loci in Douglas-fir (Howe *et al.*, 2003). Cross-talk between signalling for drought stress and cold stress could allow one stress to induce acclimation for the other. Several studies support the hypothesis that cellular changes induced by summer drought at Birken increased their fall cold hardiness. In a study by Timmis and Tanaka (1976), Douglas-fir seedlings that were grown under greater drought had enhanced fall cold hardiness. O'Neill *et al.* (2000) found that Douglas-fir seedlings grown under summer drought incurred significantly less cold injury in the fall than well-watered seedlings.

There was a family*site interaction for fall cold hardiness at Birken and Hurley. Aitken *et al*. (1996) found family*site interactions for fall cold hardiness in Douglas-fir saplings, and attributed them to differences in timing of cold acclimation between the two sites. While the first stage of cold acclimation in the fall is due to photoperiod, the second stage is in response to temperature (Weiser, 1970; Smallwood and Bowles, 2002). Local differences in temperature could have caused different rates of acclimation. As Birken is the lower elevation site, it seems unlikely that colder fall temperatures accelerated cold acclimation relative to Hurley. Another explanation is that drier conditions at Birken caused increased cold hardiness in a different subset of families than photoperiod and temperature-induced cold acclimation alone. The families that underwent more osmotic adjustment to tolerate drought could have had greater hardiness to cold (Timmis and Tanaka, 1976).

Genetic effects on seedling performance

Significant variation in growth and hardiness traits within and among families is well documented in Douglas-fir. In this study, when all sites were included in analyses, family had a significant effect only on fall cold hardiness. Differences in soils, competition and shading contribute to microsite differences (Timmis *et al.,* 1994), which may have caused more phenotypic variation in growth traits, masking family variation in most traits. Significant family variation in fall cold hardiness was found in Douglas-fir by Aitken *et al*. (1996).

Wild stand families tended to have higher fall cold hardiness than families predicted to have height gain. This could be because families that grew taller ceased growth later in the season, giving them a longer growing period, but leaving them more vulnerable to freezing damage in the fall. Stevenson *et al*. (1999) found that Douglas-fir trees which had been selected for faster growth continued growing later into the fall and acclimated to cold later in the fall than wild stand trees. In this study, wild stand families also tended to have a higher proportion of individuals survive in the field than did top cross families. This suggests a trade-off between height and survival (see Section 4), which has been observed in other studies on conifers (reviewed by Howe *et al*., 2003). In wild stand trees, natural selection may have emphasized cold hardiness, while during selection by humans, the balance may have been tipped more toward increased height gain at the expense of traits like cold hardiness.

In the field trial, wild stand families had less discrimination against $\delta^{13}C$ than breeding program families. Less discrimination against ^{13}C could be due to stomata being closed for a greater proportion of time or to higher photosynthesis. Leader growth and $\delta^{13}C$ were positively correlated at Hurley (see Section 4). In *Pinus contorta* saplings, stem volume positively correlated with $\delta^{13}C$ (Guy and Holowachuk, 2001). Perhaps greater height gain in the submaritime zone breeding program families will also increase water use efficiency. In *Larix occidentalis*, Zhang *et al.* (1994) observed high variability in water use efficiency among families and correlations with height and diameter which suggest that selection for water use efficiency would benefit height gain. Aitken *et al.* (1995) found that the water use efficiency of Douglas-fir populations grown in a common garden was very closely related to mean annual precipitation at the seed source. Genotypes from wetter environments had less ^{13}C discrimination (i.e., higher water use efficiency) than genotypes from drier environments. Guy and Holowachuk (2001) reported similar findings in *Pinus contorta* saplings. Genotypes from wetter environments are genetically predisposed to greater height growth, likely in response to greater competition (Wright, 1976). In areas where drought or cold are severely limiting, a tree will have a greater chance of survival if it shortens its growing season, possibly limiting height growth, but avoiding otherwise lethal stresses (St. Clair *et al.,* 2005). This has been observed in very dry, high elevation or continental locations in North America that have a high occurrence of such stresses. Populations from dry regions have been shown to have significantly less volume production than provenances from wetter locations (White and Ching, 1985). In wetter, milder regions, trees invest more in height growth to aid in light competition with neighbouring trees (St. Clair *et al*., 2005).

Certain families consistently performed well in traits of interest for field success in both field and pot trials. Families 943 and 944 performed well in growth, survival, water use efficiency and resistance to water stress. Consistent high ranking in both field and pot trials suggests a strong genetic basis for performance in these families.

Growth and water stress in potted seedlings

Leader growth increment was not linked to drought treatment or measures of water stress in the pot trial. In drought trials, van den Driessche (1992) and Anekonda *et al.* (2002) also found that leader growth increment correlated poorly with drought, because most of the height growth occurred before the period of intense drought stress in the summer. In our study, there was a link between drought treatment and biomass production. The seedlings in the harsher drought treatments had lower root and shoot biomass than seedlings that received more water. These findings imply that leaders extended in the spring, before drought treatments were implemented, whereas biomass accumulation continued through the summer and was negatively affected by drought conditions. Khan *et al.* (1996) also found that biomass was significantly lower in water-stressed seedlings, but also saw a drought treatment effect on seedling height. Bower *et al.* (2005) found that annual ring width in young Douglas-fir plantations was lower in response to drought, with much higher sensitivity at drier sites.

Root growth and root / shoot ratio were lower under more severe drought treatments in this study. A lower root / shoot ratio under drought conditions is unusual in Douglas-fir (Kozlowski and Pallardy, 2002). However, Tinus (1996) found root growth potential to be lower in water stressed seedlings than in unstressed seedlings, and zero in severely water stressed seedlings. van den Driessche (1992) found no correlation between root growth capacity and survival in Douglas-fir seedlings grown under xeric conditions. Treatment 3 may have had the lowest root / shoot ratio because shoots completed most of their growth in the spring when water availability was higher. The roots may have suffered more drought treatment effects because they grew in the summer. Lack of turgor pressure due to drought decreases elongation in plant tissues (Vaadia *et al.*, 1961). The hard, dry potting medium in severely droughted pots may have compounded the difficulties for root elongation.

As expected, midday shoot water potentials were lowest in Treatment 3. Both pre-dawn and midday shoot water potentials of Douglas-fir seedlings were significantly affected by drought treatments in a study by Khan *et al.* (1996), in which seedlings that received less water had more negative water potentials.

Gas exchange

Reductions in stomatal conductance, transpiration rate, and photosynthetic rate under water stress have been well characterized in Douglas-fir and other conifers (Brix, 1979). In this study, all three parameters were lower under drought conditions. Transpiration rate closely corresponded with stomatal conductance, as the rate of diffusion of water through stomata relies greatly on the aperature size. Net photosynthetic rate and stomatal conductance had a lower correlation, indicating that rates of CO_2 assimilation and respiration varied somewhat independently of stomatal aperature. Instantaneous water use efficiency – the ratio of net photosynthesis to transpiration – was lower in drought stressed seedlings. This suggests a greater uncoupling of photosynthesis from stomatal conductance under water limited conditions. Reduction of photosynthesis due to changes in mesophyll metabolism occurs in response to drought (Flexas and Medrano, 2002). Such non-stomatal limitation of photosynthesis may have occurred in Treatment 3, reducing carbon fixation proportionately more than transpiration. Impaired ATP synthesis and subsequent impairment of ATP-dependent regeneration of ribulose bisphosphate in the Calvin cycle has been implicated in limiting photosynthesis under moderate drought (Flexas and Medrano, 2002). Photoinhibition also occurs in high light under extreme drought stress, often accompanying complete stomatal closure (Flexas and Medrano, 2002).

Pot trial and field trial

Although the families in the pot trial were a subset of the families used in the field trial, there emerged no strong links in family growth or hardiness characteristics between the two experiments. Different and much more variable conditions in the field, seedling age differences between the trials and low family sample size in the pot trial likely exacerbated differences between the trials. However, some wild stand versus selected family trends did match in both trials. Some families that performed well in the field trial also performed well in the pot trial. In the field trial, family 943 had the highest water use efficiency, and family 944 had the highest height and survival. In the pot trial, these families had the least negative water potentials during imposed drought, indicating superior avoidance of water stress. In these cases, the pot trial showed merit in selecting families that also did very well under field conditions.

Heritability and selection

Sloped sites with more brush competition had higher heritabilities (h^2) for growth and water use efficiency. Heritabilities for height, root collar diameter, and water use efficiency were highest at Hurley. Fall cold hardiness and leader growth increment heritabilities were highest at Birken. At Blackwater, heritabilities were very low with the exception of spring cold hardiness. Why were heritabilities so variable between sites? More phenotypic variability in a study means a lower calculated heritability (McKeand *et al.*, 2008). When ANOVAs are calculated individually by site (Appendix A, Tables 14-27), within-family phenotypic variation is revealed to be very different between sites. Significant effects of family on root collar diameter and carbon fractionation when Hurley data are analysed separately indicate that variability in these traits due to environmental effects was lower at this site. The correlation between water use efficiency and leader growth was greatest at Hurley as well (see Section 4). As the highest elevation site with the most precipitation, the least growth, and the lowest water use efficiency, Hurley had the best combination of characteristics to minimize individual variation in these traits. Mari *et al.* (2003) found that increased access to nutrients raised heritabilities for growth traits in *Picea abies* seedlings. Soil data were not collected in the current study, but it is possible that differences in water and nutrient availability between sites influenced heritabilities. In *Picea mariana*, Flanagan and Johnsen (1995) found a strong negative correlation between height and water use efficiency at a dry, low productivity site, but found no correlation between the two on more productive sites. Interestingly, the traits that had the highest mean values among sites at Birken, fall cold hardiness and leader growth, had the lowest within-family variation and the highest heritabilities at Birken. Seedlings at Blackwater had the greatest height, root collar diameter, and water use efficiency, but heritabilities for these traits were negligible at this site. Therefore, trait magnitude seems unrelated to trait variability between sites in this study.

Heritability calculations were done assuming similar parentage structure for all families, but a small subset of families (top crosses) were full sibs instead of half sibs.

This may have caused slightly higher heritabilities than warranted. High within-site environmental heterogeneity at our field sites likely kept h^2 values low compared to more controlled studies. Anekonda *et al.* (2002) found heritabilities of height and diameter of 2 year old Douglas-fir seedlings grown under 3 drought treatments in nursery beds to be 0.49 and 0.46, respectively. In the following year, the heritabilities of height, diameter and growth increment of the 3 year old seedlings were 0.61, 0.42, and 0.25. In a study on 26 year old Douglas-fir at 4 progeny test sites by Ukrainetz *et al.* (2008), heritability for height was 0.23.

Strong genetic clines in cold hardiness of coastal Douglas-fir associated with climate have been found previously, suggesting high rates of natural selection for this trait (Howe *et al.*, 2003). The high heritability for fall cold hardiness in my study reflects this strong selection pressure. In my study, the heritability of fall cold hardiness was higher than that of spring cold hardiness. The two parameters were, however, measured at two different field sites - the difference in environmental variance between the two sites likely affected heritabilities. Anekonda *et al.* (2000) found the opposite, that the heritability of cold hardiness was stronger in the spring than in the fall. Aitken and Adams (1996) found heritability of fall cold hardiness of needles to be 0.36 in coastal Douglas-fir. This value is comparable to the heritabilities calculated at both sites and at Hurley. The heritability calculated at Birken alone was higher.

In a meta-analysis of narrow-sense heritabilities in forest trees compiled from 67 published studies, Cornelius (1994) found that heritabilities of height and diameter were generally below 0.4 and most frequently in the range of 0.1-0.3. This corresponds with the growth trait heritabilities found in my study. In all growth and form traits assessed, Cornelius (1994) concluded that there was enough additive genetic variation for selection to facilitate considerable genetic gains, even when screening is within a relatively small population.

There was a significant family effect on carbon fractionation when measured at Hurley. Provenance differences in carbon fractionation have been observed elsewhere in Douglas-fir (Zhang *et al.*, 1993; Aitken *et al.*, 1995). Anekonda *et al.* (2002) found strong genetic correlations between different drought hardiness traits (cavitation, foliage damage, xylem hydraulic conductivity, growth increment), and hypothesized that these traits are under a degree of shared genetic control. Heritability for water use efficiency at Hurley was high. Potential therefore exists for breeding for drought hardiness traits in the Douglas-fir breeding program for deployment to the dry submaritime zone.

Climate change and managed forests

Periods of drought are expected to worsen in British Columbia as climate changes (Mote, 2003; Nigh, 2006). A suggested management strategy for this region is the planting of Douglas-fir genotypes that are tolerant of a warmer and drier climate (Case and Peterson, 2005). It is prudent to plan ahead for future climate scenarios by selecting for appropriate traits now in breeding programs. This study has found some families that had consistently good performance in growth, hardiness and survival, and also some very poor ones that should be avoided. Anekonda *et al.* (2002) found that growth potential when water availability was high was nearly uncorrelated with drought hardiness. This suggests that selection for drought hardiness may have no negative effect on height gain under non-drought conditions. In breeding programs, we should select families that have higher water use efficiency when soil water is limited, but still grow well under low stress conditions. Differences between the three sites in this study offers a good range of environments, which encourages the application of these findings to a breeding program for the entire submaritime zone.

Section 4 – Physiological Comparisons

Introduction

Cold and drought hardiness

Different abiotic stresses often elicit similar effects on plants at the cellular level. Both drought and freezing stress cause desiccation of plant cells, destabilization of membranes and proteins, and oxidative damage (Thomashow, 2001; Welling and Palva, 2006). It is therefore expected that there are similarities in the regulation, gene expression and biochemistry of drought and cold response (Shinozaki *et al*., 2003).

Plant growth regulators

Abscisic acid (ABA) induces stomatal closure in response to drought stress (Taiz and Zeiger, 1998). Leaf ABA concentrations have been found to change in response to drought stress in Douglas-fir. In potted Douglas-fir seedlings, ABA levels increased with decreasing water potential, until water potential reached -5.0 MPa, when ABA levels dropped (Johnson and Ferrell, 1982). During cold acclimation, ABA levels increase in both herbaceous and woody plants (Welling and Palva, 2006). An increase in sensitivity to ABA, however, may play a greater role than absolute ABA concentrations in growth cessation and dormancy in trees (Welling and Palva, 2006). Exogenous application of ABA has been shown to induce cold hardiness in trees in the absence of cold temperatures (Welling and Palva, 2006). Of the genes known to be induced under both cold and drought stress, some respond to ABA concentrations, while others are ABA-independent (Shinozaki *et al*. 2003). In a microarray study in *Arabidopsis*, Huang *et al.* (2008) found that two thirds of the genes upregulated by drought were regulated by ABA or an analogue. Additional microarray and *in silico* analysis by these authors suggests that after ABA, jasmonic acid most significantly affects drought-related gene expression, followed by auxin, cytokinin, ethylene, brassinosteroids, and gibberellins which have smaller but biologically significant effects. The levels of gibberellin, a plant growth regulator involved in shoot elongation, have been found to decrease under short days, likely due to reduced biosynthesis (Welling and Palva, 2006). This coincides with the timing of growth cessation in preparation for dormancy in woody plants.

Gene expression

Considerable crosstalk has been found among signal transduction pathways in response to cold, drought, salinity and ABA treatments in plants (Shinozaki *et al*. 2003). Genetic and physiological overlaps in response to drought and cold stress could allow one stress to induce acclimation for the other.

The CBF/DREB1 cold response pathway (or CBF regulon) is a regulatory pathway that has been characterized in *Arabidopsis*, and appears to be conserved among plant species (Welling and Palva, 2006). It regulates the expression of genes that lead to increased tolerance of freezing, drought, and salt stress in plants (Thomashow, 1999; Welling and Palva, 2006). It appears to be a master switch for a cascade that induces a multitude of biochemical effects involved in stress tolerance, including the transcription of membrane stabilization proteins and increased proline and soluble sugar levels (Thomashow, 2001). The CBF/DREB1 genes appear to be induced by cold temperatures. Transcripts have been shown to accumulate minutes after exposure to cold in *Arabidopsis* (Thomashow, 2001). *Arabidopsis* plants overexpressing the CBF regulon are also more tolerant to freezing, drought and salinity stress (Liu *et al*., 1998). Only a handful of genes controlled by the CBF/DREB1 pathway have been identified. Future work holds much promise for greater understanding of how this pathway works in *Arabidopsis*, as well as its implications for genetic engineering of stress resistance in crop plants (Thomashow, 2001). In Douglas-fir, genes that are induced by water deficit have been found to map near cold hardiness quantitative trait loci (Howe *et al*., 2003), which may suggest similar joint regulation of cold and drought response genes in conifers.

Biochemistry

As gene expression often overlaps between stresses, so too does metabolism in response to stress. Antioxidant metabolism in response to oxidative stress is similar between drought and cold stress responses (Sung *et al*., 2003). Compatible solute production also occurs in response to both drought and cold stress (Sung *et al*., 2003). Compatible solutes are small osmotically active compounds like sugars and amino acids that act during desiccation stress to stabilize protein and membrane bilayer structure. They are also non-toxic osmolytes that lower the osmotic potential of the cytosol, holding on to water and helping to maintain turgor (Morgan, 1984; Thomashow, 1999; Sung *et al.*, 2003). Water stressed redbud (*Cercis canadensis*) has been shown to accumulate a greater abundance of soluble carbohydrates than well watered plants (Griffin *et al.*, 2004). Genotypes with the ability to photosynthesize more under water stress conditions could potentially accumulate more carbon stores from which to synthesize such osmotically active compounds.

Late embryogenesis abundant (LEA) proteins and small heat shock proteins (sHSPs) are produced in seeds and elsewhere in response to various stresses (Kalemba and Pukacka, 2007). LEA proteins have been found to protect proteins from aggregating under water stress and hot and cold temperature stresses in *C. elegans* (Goyal *et al.*, 2005). Accumulation of LEA proteins and sHSPs is induced by cold, drought, salinity and oxidative stress in plants (Kalemba and Pukacka, 2007). Homologs of LEA proteins have been found to be expressed in response to cold temperatures in plants (Thomashow, 1999).

Dehydrins are proteins that accumulate in response to drought, salinity and freezing stress, all of which dehydrate plant cells (Welling and Palva, 2006). Some dehydrins have been found to be induced by ABA in orchid (*Spathoglottis plicata*) (Wang *et al.*, 2002).

Maintenance of membrane fluidity is important in response to both high and low temperature stress. The addition of more saturated fatty acids to thylakoid membranes has been implicated in increased heat tolerance in *Arabidopsis* (Hugly *et al.*, 1999) while during cold stress, addition of unsaturated fatty acids to the thylakoid membranes and other cell membranes is crucial for the maintenance of membrane fluidity (Wu *et al.*, 1997; Welling and Palva, 2006).

Exposure to one stress affecting response to another

Exposure to one stress can affect plant response to other stresses. Summer drought has been observed to influence fall cold hardiness. In a study by Timmis and Tanaka (1976), summer drought increased the cold-hardening capacity of Douglas-fir seedlings. Stresses may also induce changes in phenology of growth cessation and release from dormancy, which in turn influence plant hardiness to stress, as actively growing tissues are the most vulnerable to stress. Drought conditions have been found to induce earlier bud set in Douglas-fir (Khan *et al.* 1996). Selection for earlier bud-burst may be related to drought in the late summer: trees may initiate growth before water becomes limiting (St. Clair *et al.* 2005). A trade-off exists, however, early bud burst in trees may increase vulnerability to damage from spring frost (Campbell and Sugano 1979, O'Neill *et al.* 2001).

Strongly clinal genetic structure has been observed in Douglas-fir wild stands (White *et al.*, 2007). Campbell and Sorensen (1978) found that seedling height and length of the growing period decreased with increasing elevation and distance from the ocean in 2 year old coastal Douglas-fir from Washington and Oregon. There are steep genetic clines in cold hardiness along temperature gradients (Howe *et al.*, 2003) and drought hardiness along moisture gradients (St. Clair and Howe 2007). Silen and Mandel (1983) observed clinal genetic control of growth within two breeding zones in Oregon, Vernonia and Molalla. They found that for every 1,000 ft in elevation gain in parental location of origin, progeny were 5.9 (Vernonia) and 6.3 (Molalla) inches shorter. For every mile parents were located northwards, progeny were 0.45 and 0.29 inches shorter. As frost risk is strongly associated with elevation (Timmis *et al.*, 1994), this suggests that natural selection for cold hardiness has created a trade-off between growth and hardiness.

The objective of this portion of my study was to explore relationships among measured indicators of growth, drought resistance and cold hardiness and improve understanding of the relatedness of these traits. Understanding the relationships between growth and hardiness to stress, and the interactions among different stresses, is important for forest tree breeding programs. In British Columbia, many harsh environments exist where spring and fall frosts and summer drought cause high mortality of seedlings planted for reforestation. Ensuring sufficient hardiness and survival of seedlings is an important goal for healthy forests now and into the future.

Results

Correlation analysis

Field trial

Strong correlations were found among growth and drought and cold hardiness traits, measured in 2008 in 50 families growing in the field trial. Of particular interest were physiological variables which correlated with growth and survival of seedlings in the field. Two of the most important traits for seedlings used in reforestation, field survival and height, exhibited a negative correlation at all 3 field sites (R^2=0.1174) (Fig. 9), thus taller families had poorer survival. Most families followed this trend; however, the negative correlation was much stronger when the outlier, Family 944, which had exceptionally high survival and height, was excluded from the analysis (R^2=0.2506) (see Section 3 results).

Correlations between traits often varied widely between sites. At Blackwater, the driest site, less negative predawn shoot water potential correlated with greater leader growth (R^2=0.3283) (Fig. 6). A less negative shoot water potential indicates less water stress in a seedling. Shoot water potential was only measured at Blackwater.

Hurley, the highest elevation site, had the highest correlation between water use efficiency and leader growth. Less negative $\delta^{13}C$ (‰) correlated with greater leader growth at this site (R^2=0.1388) (Fig. 7). Blackwater had a similar correlation (R^2=0.1247), while Birken had a much lower correlation (R^2=0.0519). Less negative $\delta^{13}C$ indicates greater water use efficiency over the entire growing season.

Fall cold hardiness was positively correlated with leader growth at Birken (R^2=0.1330) (Fig. 8), thus faster growing families were more cold hardy in October. Correlation between these traits was highest at Birken, which had the highest mean leader growth of the 3 sites in 2008 (Table 4).

There was no correlation (R^2=0.0005) between family spring cold hardiness measured at Blackwater and fall cold hardiness measured at Birken and Hurley. Spring cold hardiness was negatively correlated with height measured at Blackwater (R^2=0.1641) (Fig. 10). Fall cold hardiness was negatively correlated with water use efficiency over the growing season at Birken and Hurley (R^2=0.2436) (Fig. 11), thus families which had greater water use efficiency over the growing season tended to be less cold hardy.

Pot trial

In potted seedlings from 22 families (Table 2) grown under 3 drought treatments, seedling height was highly correlated with shoot biomass (R^2=0.7376). Shoot biomass showed a lower but substantial correlation with root biomass (R^2=0.5819). Seedling height had a moderate correlation with root biomass (R^2=0.2618).

Rates of gas exchange – measured as stomatal conductance (gs (mol m^{-2} s^{-1})), transpiration rate (E (mmol H_2O m^{-2} s^{-1})), and net photosynthetic rate (A (µmol CO_2 m^{-2} s^{-1}) – were found to correlate with patterns of accumulation and allocation of biomass within seedlings. Families with greater leaf biomass tended to have a lower transpiration rate per unit leaf area. Leaf biomass was negatively correlated with transpiration rate (R^2=0.5459) (Fig. 12). Families with a higher root / shoot biomass ratio tended to have a higher transpiration rate per unit leaf area. Root / shoot biomass ratio was positively correlated with transpiration rate in droughted seedlings from Treatment 3 (R^2=0.5717) (Fig. 13).

While the three measures of gas exchange used were highly positively correlated, transpiration rate had a tighter relationship with biomass and stomatal conductance than did net photosynthetic rate. Stomatal conductance and transpiration had a very strong positive correlation (R^2=0.9267). Stomatal conductance and net photosynthetic rate had a lower but still substantial correlation (R^2=0.6664). Leaf biomass had a tighter correlation with E than with A (R^2=0.2531). The same was true for root / shoot biomass ratio; correlation with A was lower at (R^2=0.2467). As E exhibited the closest relationships with biomass allocation traits, these correlations are displayed in Figures 12 and 13.

Mean family chlorophyll fluorescence in the pot trial, measured as F_v/F_m, was negatively correlated with family survival proportion (R^2=0.4187). Lower F_v/F_m indicates reduced quantum yield of PSII due to stress-induced damage to photosynthetic membranes. Families that had individuals die had lower mean chlorophyll fluorescence than families that had no mortality (Fig. 14), thus families that exhibited more photosynthetic membrane damage had higher mortality in the pot trial.

Discussion

Relationships between growth, hardiness, and survival in the field

Both growth rate and stress resistance are important for survival, but these two traits have different relative importance depending on environmental conditions. Correlations from the field trial suggest physiological and functional relationships among traits of interest, including height, survival, growth of the central leader, shoot water potential, water use efficiency and cold hardiness.

My findings suggest a trade-off between growth and stress resistance in Douglas-fir seedlings. Height was negatively correlated with survival at all three sites. Within this trend, however, there was substantial variation, and one exceptional outlier, Family 944, had by far the best growth and survival in the study. There was also a trade-off between height and cold hardiness. Seedlings that were more cold hardy in the spring were shorter. This may have been because hardier seedlings burst bud later in the spring, reducing the risk of frost injury, but possibly reducing the length of the growing season. Aitken and Adams (1997) found date of bud burst and spring cold hardiness in Douglas-fir seedlings to be highly correlated.

The trade-off between growth and stress resistance has evolutionary underpinnings. Moist mild coastal environments may favour height growth for selective advantage, while harsher interior environments (summer drought, colder spring and fall) may favour more stress resistance at the expense of height growth. In areas with severe drought or cold conditions, success depends more on survival of stress than on competitive height growth (St Clair *et al.,* 2005). This is observed in very dry, high elevation or continental locations in North America that have a high occurrence of such stresses. Plants with a shorter growing season can avoid harmful spring and fall frosts at the expense of height growth. Trees in drought-prone areas can close their stomata to use less water, consequently limiting photosynthesis. Increased allocation of biomass to roots in response to drought could also influence shoot biomass. Populations from a dry region in southern Oregon have been shown to have 30% less volume production than other Pacific Northwest provenances (White and Ching, 1985). Conversely, in milder regions like mesic coastal environments, a better competitive strategy is to invest in height growth so as not to be overtopped by neighbouring trees (St. Clair *et al*., 2005).

Evaluation of data for some stress responses, however, showed positive correlations between height and the ability to avoid or tolerate stress at different times of the year. At Birken, families that grew more in 2008 were more cold hardy in the fall. Aitken *et al*., (1996) found the opposite, that taller 9 year old Douglas-fir had higher fall cold injury. Their study, like ours, experienced highly variable correlations between traits depending on the site tested. Some sites showed no relationship between height and fall cold hardiness (Aitken *et al*., 1996).

The ability of seedlings to tolerate water limitation may confer greater growth potential. In this study, growth correlated positively with two measures indicating avoidance of drought stress. Less negative water potential, and higher water use efficiency both correlated with increased growth in the field trial. Hurley, the highest elevation site, and Blackwater, the driest site, had the highest correlations between leader growth and water use efficiency. Taller seedlings, on average, appeared to fix more carbon per unit water transpired. Zhang *et al*. (1993) observed a high correlation between δ^{13}C and height in 15 year old Douglas-fir. They found no correlation between height and photosynthetic rate, suggesting that water use efficiency, not higher photosynthetic capacity, was benefiting growth. Anekonda *et al*. (2002) found that growth potential when water availability was high was nearly uncorrelated with drought hardiness. The water potential measurements showed that the less water stressed seedlings tended to grow more. In *Larix occidentalis*, Zhang *et al*. (1994) reported that water use efficiency as estimated by carbon fractionation had a strong positive correlation with height and diameter in 12 year old trees. Significant family variation in water use efficiency has been reported in conifers, including Douglas-fir (Zhang *et al*., 1994, Aitken *et al*., 1995). Genetic variation in δ^{13}C coupled with a positive correlation with growth makes selection for δ^{13}C a possibility for increasing growth in forest tree breeding programs.

What conferred this lower water stress to faster growing families? Family had a significant effect on root biomass and root / shoot biomass ratio in the pot trial (see Section 3). While no positive correlations were observed between root biomass or root / shoot ratio and leader growth in the pot trial, it is possible that in older seedlings in a field environment, root characteristics may have helped some families access more water than others. In *Picea sitchensis* seedlings, Mari *et al*. (2002) found that variation among families in height and root dry weights was greater under restricted access to nutrients than under free access to nutrients. As the field sites were likely more nutrient poor than the fertilized pot trial, family variance in nutrient use efficiency may have compounded differences in growth in the field trial. Seedlings with better developed root systems would have had better access to water and nutrients, allowing for increased growth.

There was no correlation between spring and fall cold hardiness in this study. Spring and fall cold hardiness in Douglas-fir were found to be independent in a study by Anekonda *et al.* (2000), who hypothesized differing genetic control of hardiness in spring and fall. Studies of quantitative trait loci suggest they are controlled by different suites of genes (Jermstad *et al.*, 2001).

A negative correlation between cold hardiness and water use efficiency emerged from this study. Families that had higher water use efficiency during the growing season tended to have lower fall cold hardiness. This correlation could be due to covariance with other traits that affect both $\delta^{13}C$ and cold hardiness, as no direct link is immediately obvious.

Drought physiology of potted seedlings

Leaf area is a principal determinant of water use (Simpson, 2000). In the pot trial, families with higher leaf biomass, and presumably leaf area had lower stomatal conductance, transpiration and net photosynthesis per unit leaf area. As seedlings with more leaf area likely have more stomata overall than seedlings with fewer leaves, more water loss from transpiration would occur, and stomata would close in response to water stress earlier.

Root / shoot ratio was also positively related to stomatal conductance and gas exchange in potted seedlings. Increased root biomass could allow more water uptake, while decreased shoot biomass would reduce transpirational water loss, resulting in more water available for the plant. Families with a higher root / shoot ratio exhibited drought induced stomatal closure later than families that had fewer roots in relation to shoots. This indicates a successful avoidance of drought through allocation of relatively more biomass underground. A lower leaf area and higher root / shoot ratio therefore has the potential to decrease water use in seedlings. This could increase survival of seedlings under extreme drought. No relationship between biomass allocation and survival was observed in the pot trial, but the possibility exists that biomass allocation in response to drought played a role in growth and survival in the field.

Both biomass and stomatal conductance had a tighter correlation with transpiration than with net photosynthesis. While water loss due to transpiration depended greatly on stomatal opening, rates of CO_2 assimilation and respiration varied independently of stomatal aperture. Reduction of photosynthesis due to changes in mesophyll metabolism occurs in response to drought (Flexas and Medrano, 2002). Such non-stomatal limitation of photosynthesis likely reduced carbon fixation proportionately more than transpiration. Impaired ATP synthesis and subsequent impairment of ATP-dependent regeneration of RuBP has been implicated in limiting photosynthesis under moderate drought (Flexas and Medrano, 2002). Under extreme drought stress, photoinhibition occurs, often accompanying complete stomatal closure (Flexas and Medrano, 2002).

There was a strong relationship between damage to photosynthetic membranes and survival of potted seedlings. A higher F_v/F_m indicated higher quantum yield of PSII, and less stress-induced damage to photosynthetic membranes. Families with higher F_v/F_m had better survival. This suggests that measurement of chlorophyll fluorescence of leaves during the growing season could be a predictor of seedling survival under drought conditions.

There were no strong correlations between corresponding traits in the field and pot trials in this study. Large differences in trait correlations existed even between field sites. The very different site conditions caused great differences in performance. Different ages of trees between the two experiments and a low sample size in the pot trial added to differences between the trials. Water potentials in the pot trial were measured at midday, not predawn as in the field trial. The transient nature of measured water potentials and gas exchange should be taken into account. They reflect the current conditions and change from day to day – day sampled was the only significant effect for water potential measured at Blackwater. Conditions during water potential sampling at Blackwater were likely not dry enough to tease out many differences, as there had been a recent rain.

Seedling performance in pot studies often does not correlate well with field performance (Mari *et al.*, 2002). Field environments are inherently more variable, and the different ages of trees studied is a confounding factor. The potted seedling trial offered a controlled environment to explore family differences in seedling physiology under convenient and uniform conditions. Such studies are useful for fine scale physiological work controlling drought and nutrient treatments. Biomass allocation and gas exchange provide means to study the principles of conifer physiology in depth, if one is willing to place less emphasis on field performance. However, there are opportunities to make a pot trial possibly more relevant to the field. Larger sample size, more severe drought that started earlier, and reduced fertilization of potted seedlings may have improved correlations between the pot and field studies. In addition, testing cold hardiness and $\delta^{13}C$ of potted seedlings could provide stronger links to field results.

When selecting for traits of interest in tree breeding programs, information regarding the interactions between different stresses and between stress and growth is important to ensure planted seedlings have both the hardiness to survive, and the vitality to grow quickly. This study found both positive and negative relationships between growth and resistance to stress. Future study of the genetic and biochemical basis for such physiological relationships is crucial and holds much promise for the greater understanding of stress physiology, as well as the improvement of tree and agricultural crops.

Section 5 – Conclusions

There is evidence that natural selection is most intense during the seedling phase of development (St. Clair and Howe, 2005). The understanding of physiological processes at this stage is therefore crucial, not just for understanding how selection and genetic differentiation operate in the wild, but for improving success of trees planted by humans. This study helped to elucidate some of the complex interactions between different traits of interest in Douglas-fir seedlings. This information can be used to direct further research in stress physiology, as well as inform tree breeders of trends in growth and hardiness that are meaningful for reforestation. The negative correlation between height and survival of seedlings in the field is especially pertinent.

This work and other recent studies are challenging ideas of trade-off between growth and stress resistance. Especially compelling are trends that indicate growth improves with stress resistance, for example positive correlations of height with water use efficiency and cold hardiness. Coupled with genetic variation in traits, this means that through selection of elite genotypes, breeding stock with both good height growth and good survival can be developed for deployment to harsh regions.

In this era of warming climate, it is important to respond to current environmental conditions as well as preparing for the drier summers and increasing drought forecast by climate models. The development of seedlings that can perform well in their environment is an important component of ensuring a healthy productive forest, to the benefit of the environment and the forest industry. Family performance data can also be applied directly to the submaritime Douglas-fir breeding program. “A” class families 943 and 944 had very good height growth and drought hardiness in this study. Top cross family 399x243, while having good height growth, had very poor survival. This information can help guide selection and ultimately improve the survival and performance of seedlings in BC’s submaritime zone.

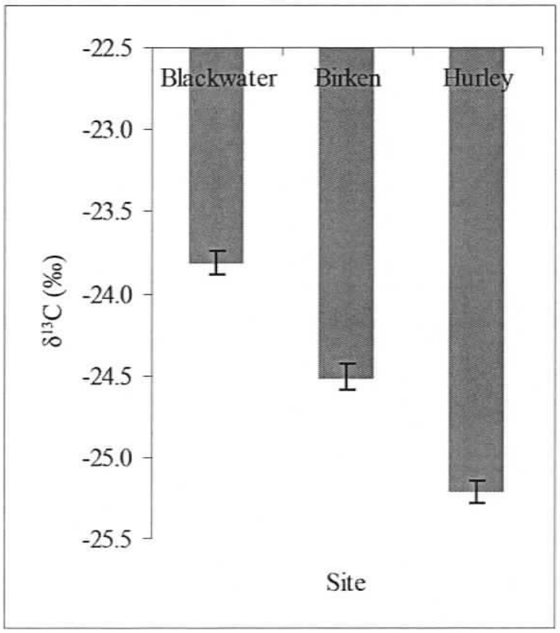

Figure 1. Differences in carbon fractionation ($\delta^{13}C$ (‰)) in 2008 in Douglas-fir seedlings planted in 2005 at 3 field sites in the coast mountains of British Columbia. Less negative $\delta^{13}C$ indicates greater water use efficiency over the growing season. Error bars are standard error.

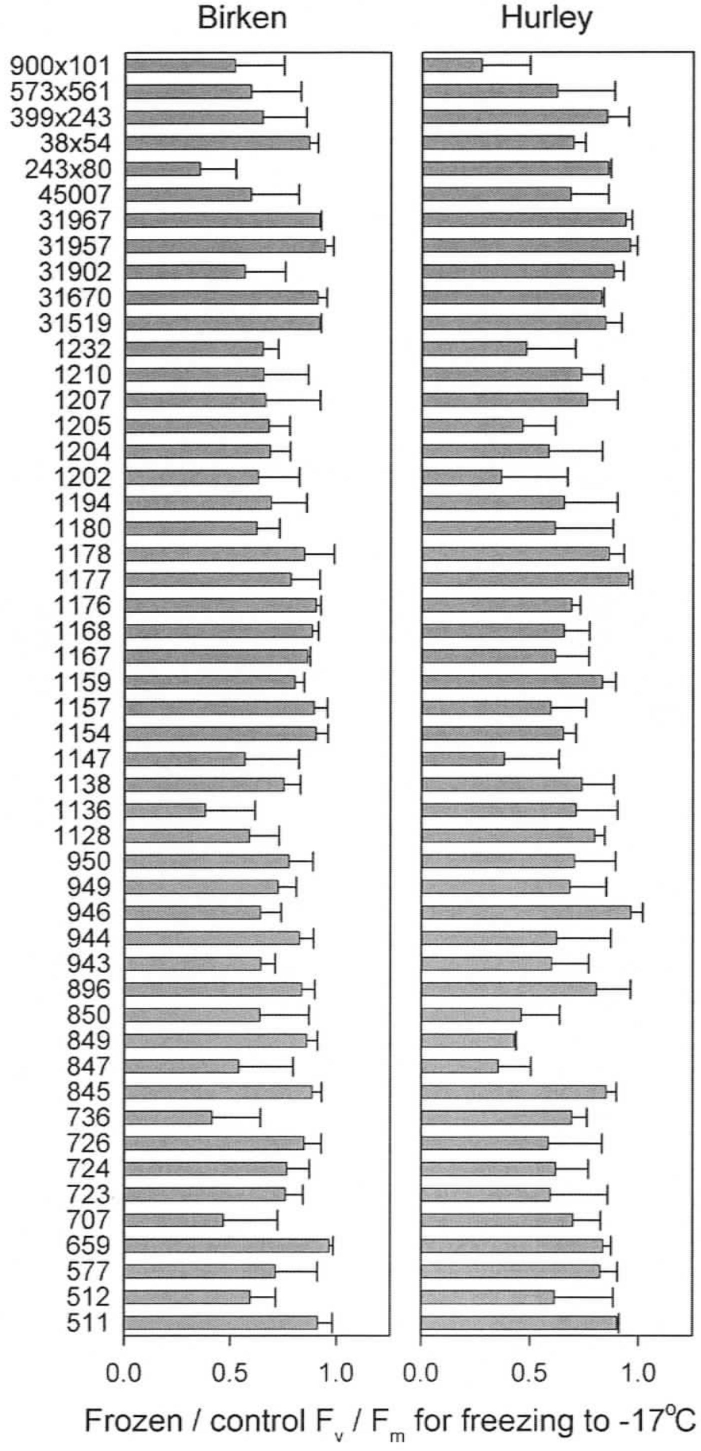

Figure 2. Cold hardiness of 50 Douglas-fir families planted at Birken and Hurley, frozen to -17°C and assessed using chlorophyll fluorescence in October 2008. Values are post-freezing F_v/F_m / pre-freezing F_v/F_m, with higher values indicating higher cold hardiness.

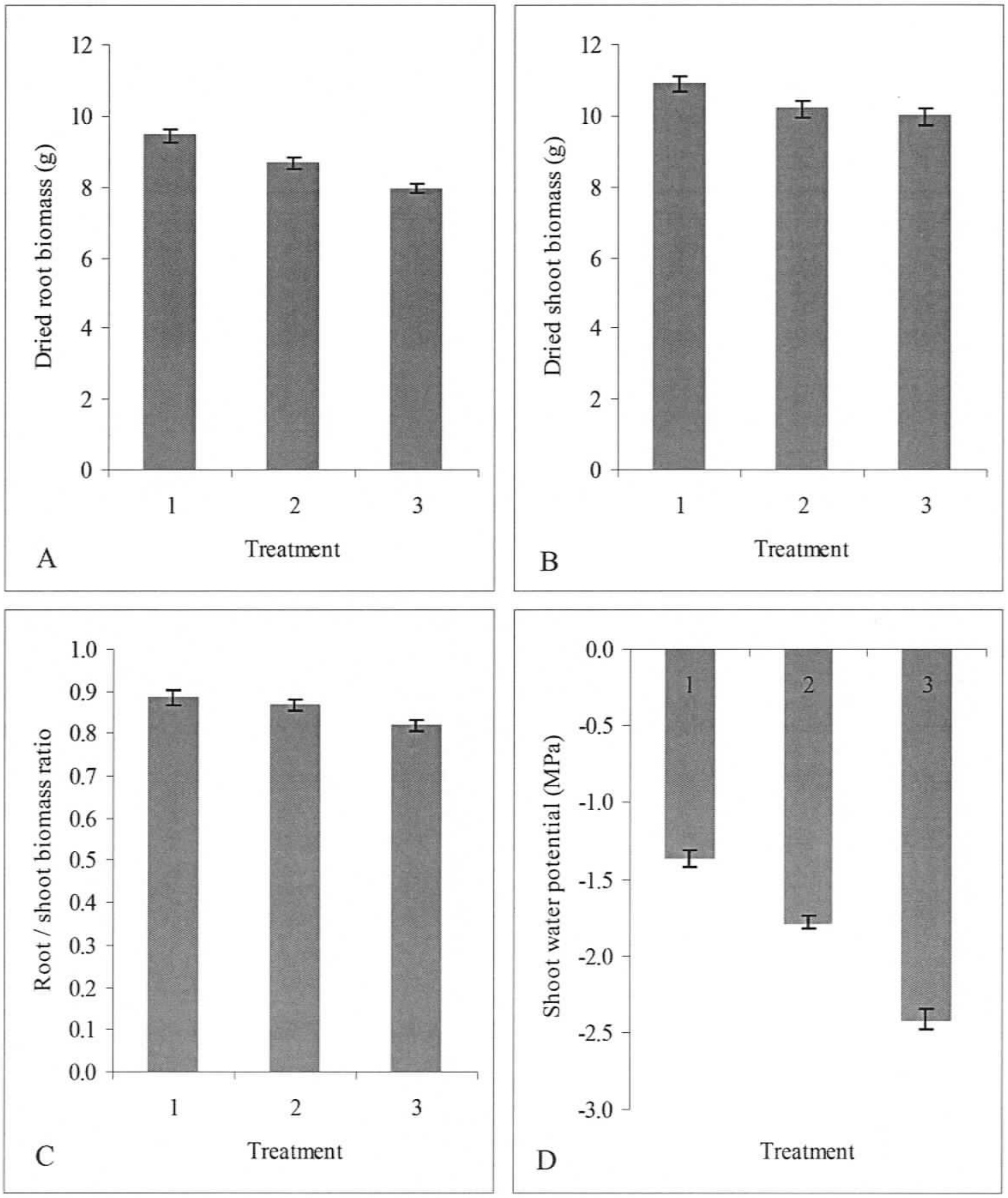

Figure 3. Differences in (**A**) root biomass (g), (**B**) shoot biomass (g), (**C**) root / shoot biomass ratio, and (**D**) midday shoot water potential (MPa) of potted Douglas-fir seedlings grown in 3 drought treatments at the University of Victoria in 2008, with Treatment 3 receiving the least water, and Treatment 1 the most water. Error bars are standard error.

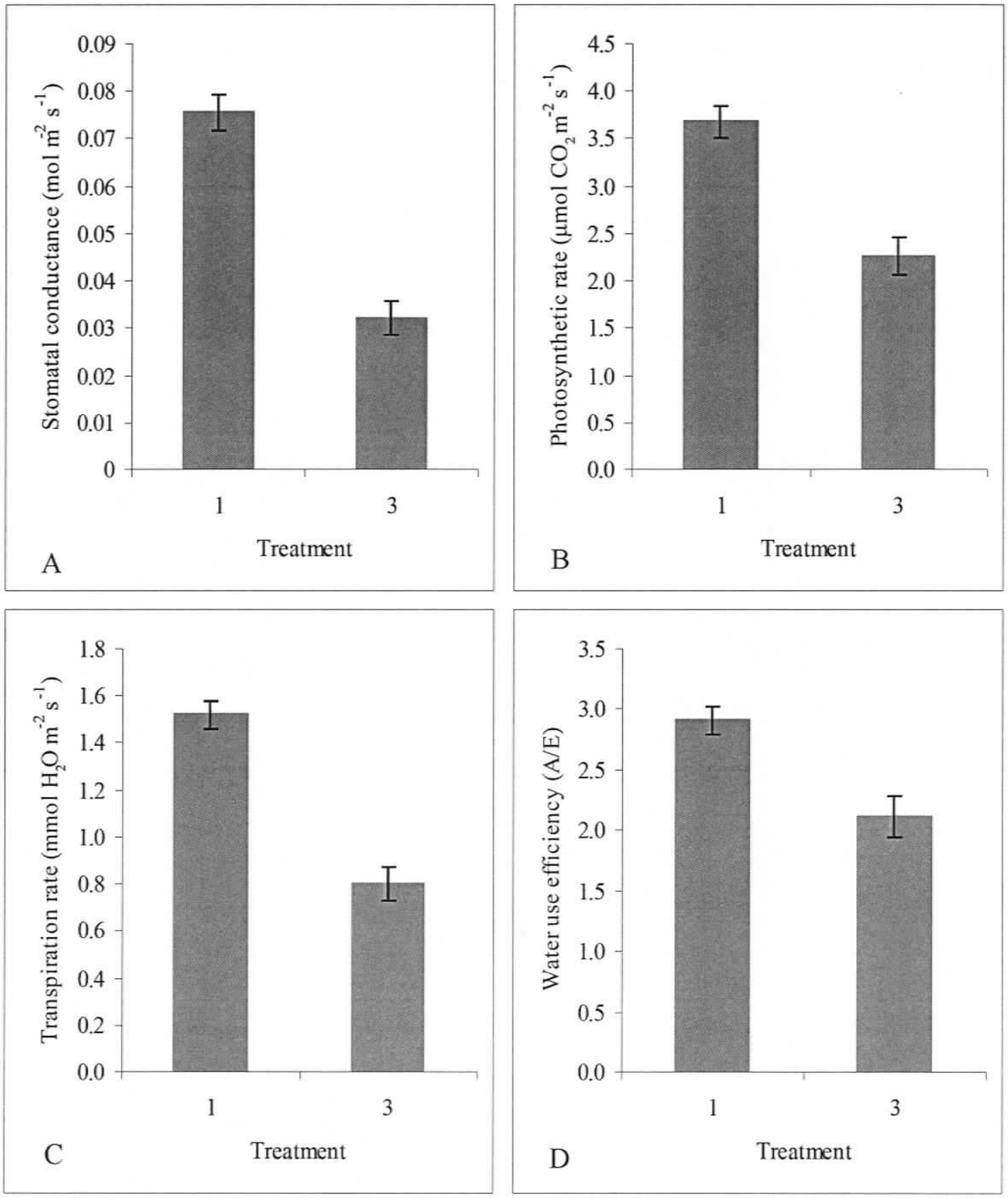

Figure 4. Differences in (**A**) stomatal conductance (gs) (mol m^{-2} s^{-1}), (**B**) photosynthetic rate (A) (µmol m^{-2} s^{-1}), (**C**) transpiration rate (E) (mmol m^{-2} s^{-1}) and (**D**) instantaneous water use efficiency (A/E) (µmol CO_2 taken up per mmol H_2O released) in potted Douglas-fir seedlings from 22 families grown under 3 drought treatments at the University of Victoria in 2008, with Treatment 3 receiving the least water, and Treatment 1 the most water. Error bars are standard error.

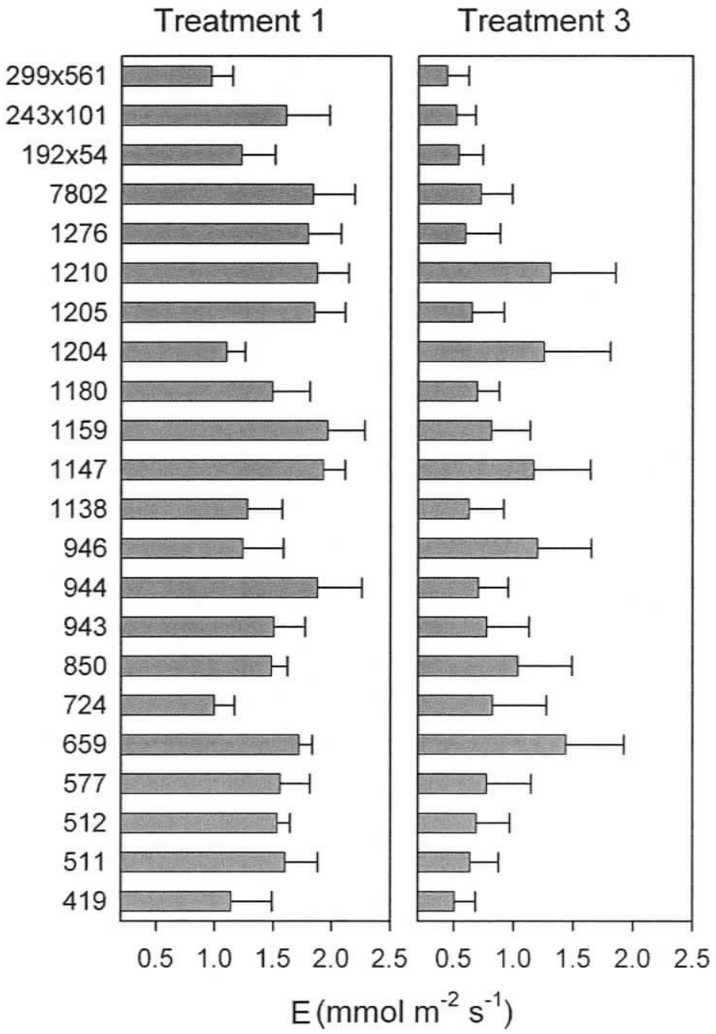

Figure 5. Transpiration rate (E) (mmol m^{-2} s^{-1}) of potted Douglas-fir seedlings from 22 families grown under 2 drought treatments, with Treatment 3 receiving the least water, and Treatment 1 the most water, at the University of Victoria in 2008. Error bars are standard error.

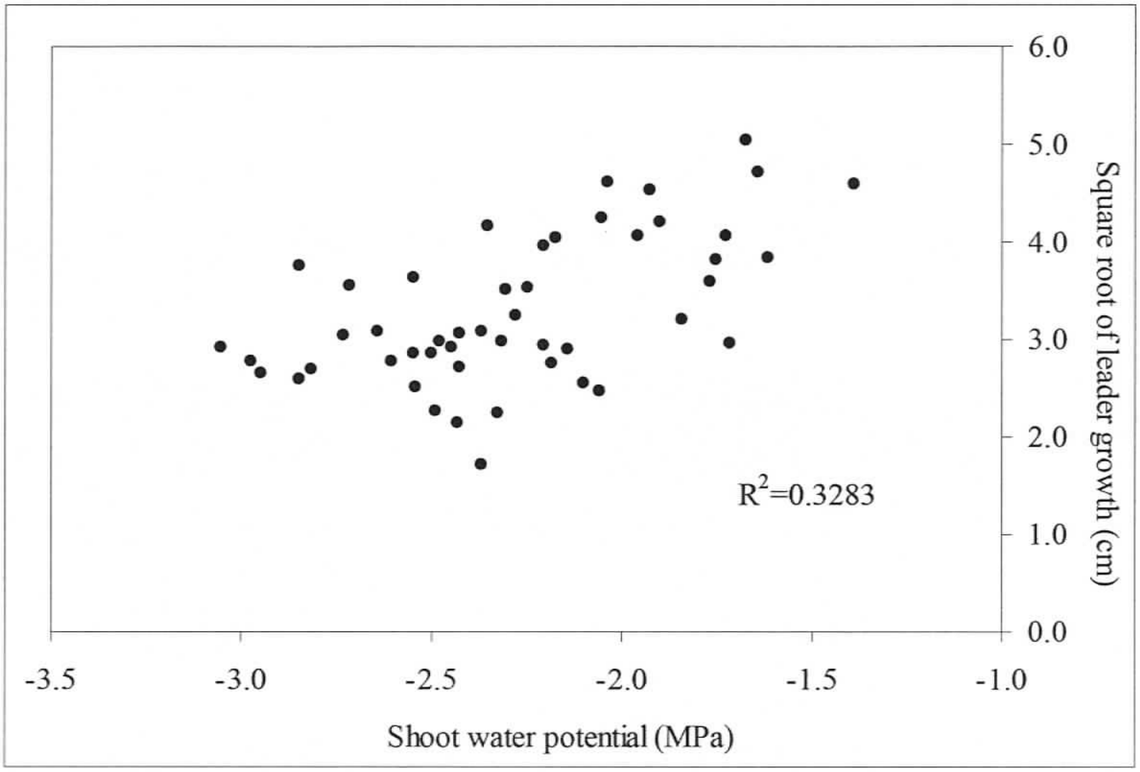

Figure 6. Correlation of the predawn shoot water potential (MPa) and the square root of leader growth (cm) of 50 Douglas-fir families growing at Blackwater in 2008.

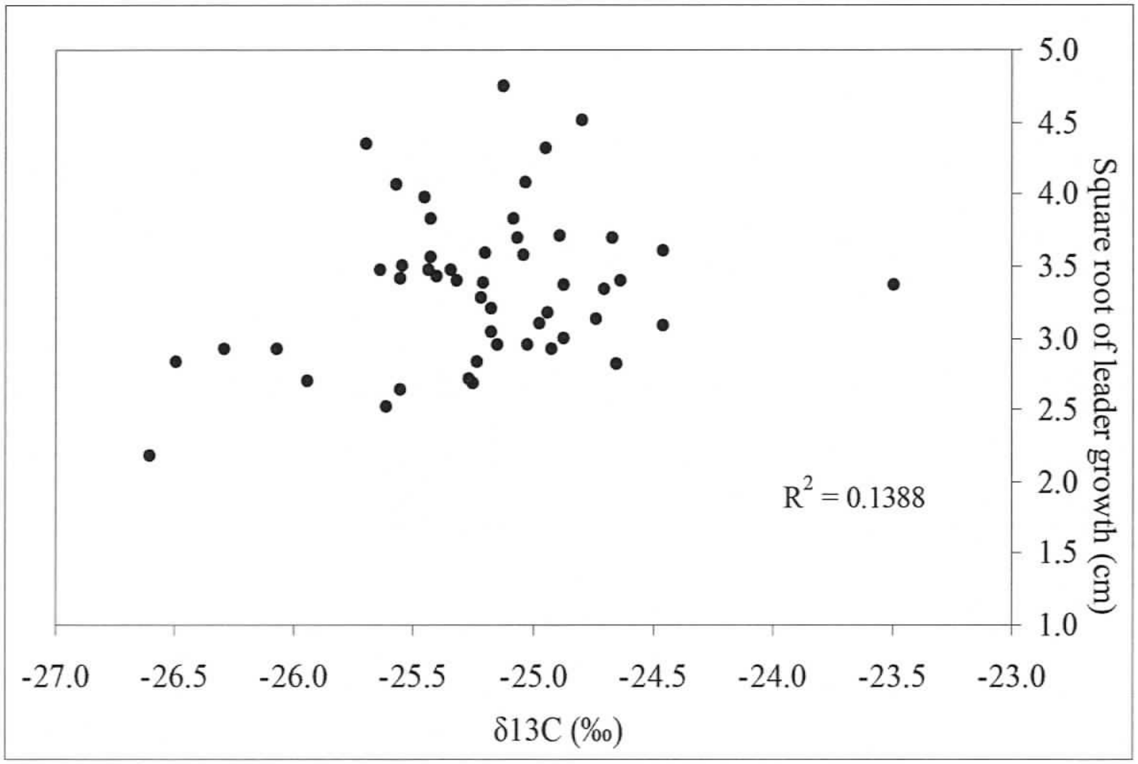

Figure 7. Correlation of carbon fractionation ($\delta^{13}C$ (‰)) and the square root of leader growth (cm) in 50 Douglas-fir families growing at Hurley in 2008. Less negative $\delta^{13}C$ indicates greater water use efficiency over the growing season.

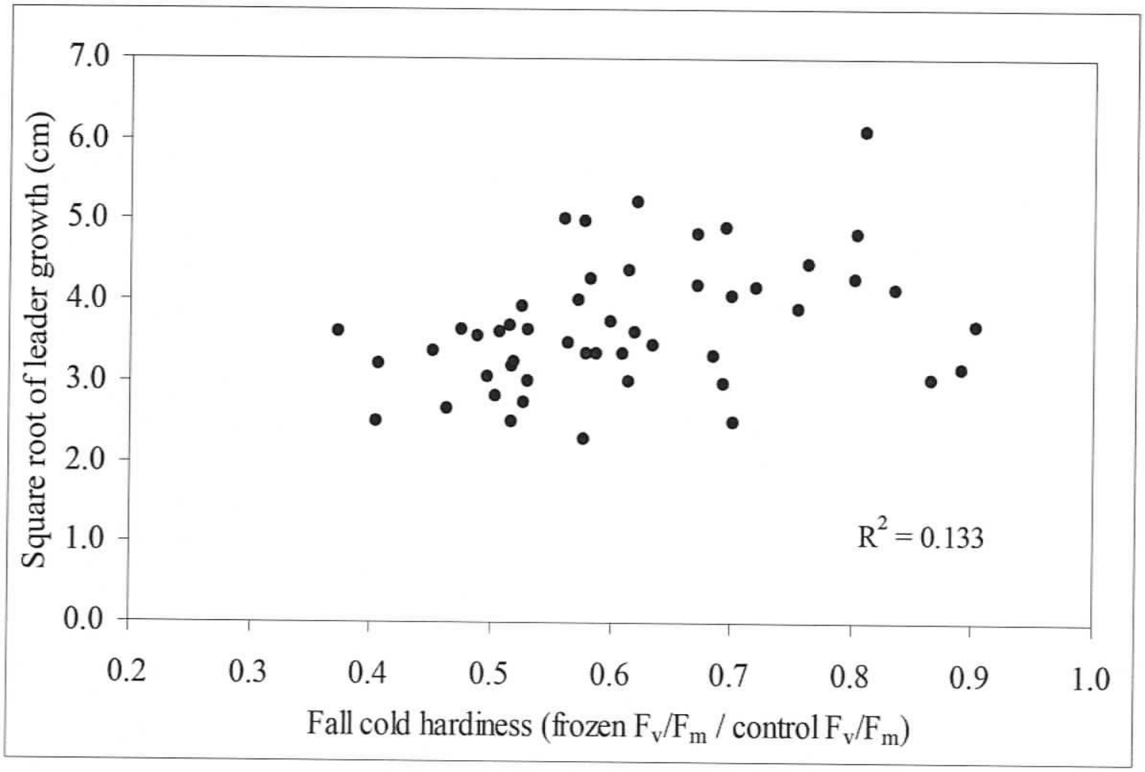

Figure 8. Correlation of fall cold hardiness (ratio of frozen to control F_v/F_m in a controlled freezing trial) and the square root of leader growth (cm) in 50 Douglas-fir families growing at Birken in 2008.

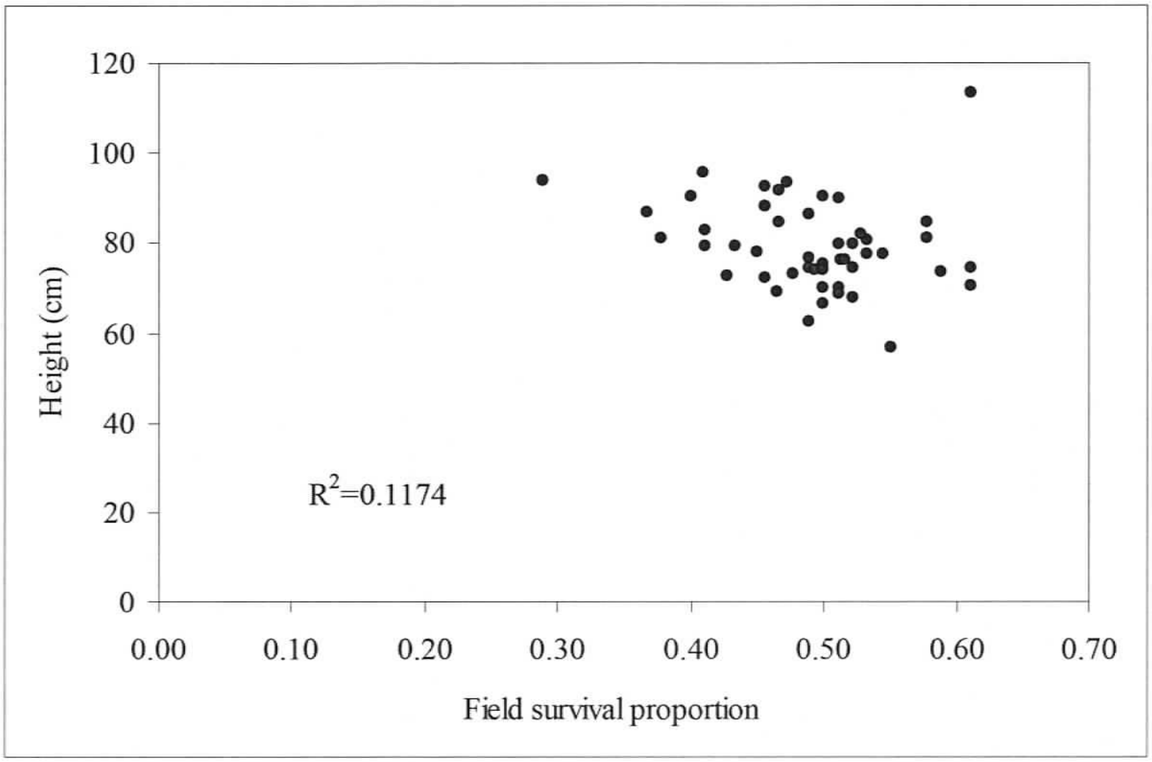

Figure 9. Correlation of field survival proportion and height (cm) in 50 Douglas-fir families growing at 3 field sites, measured in 2008. The outlier is family 944; when this family is excluded, R^2=0.2506.

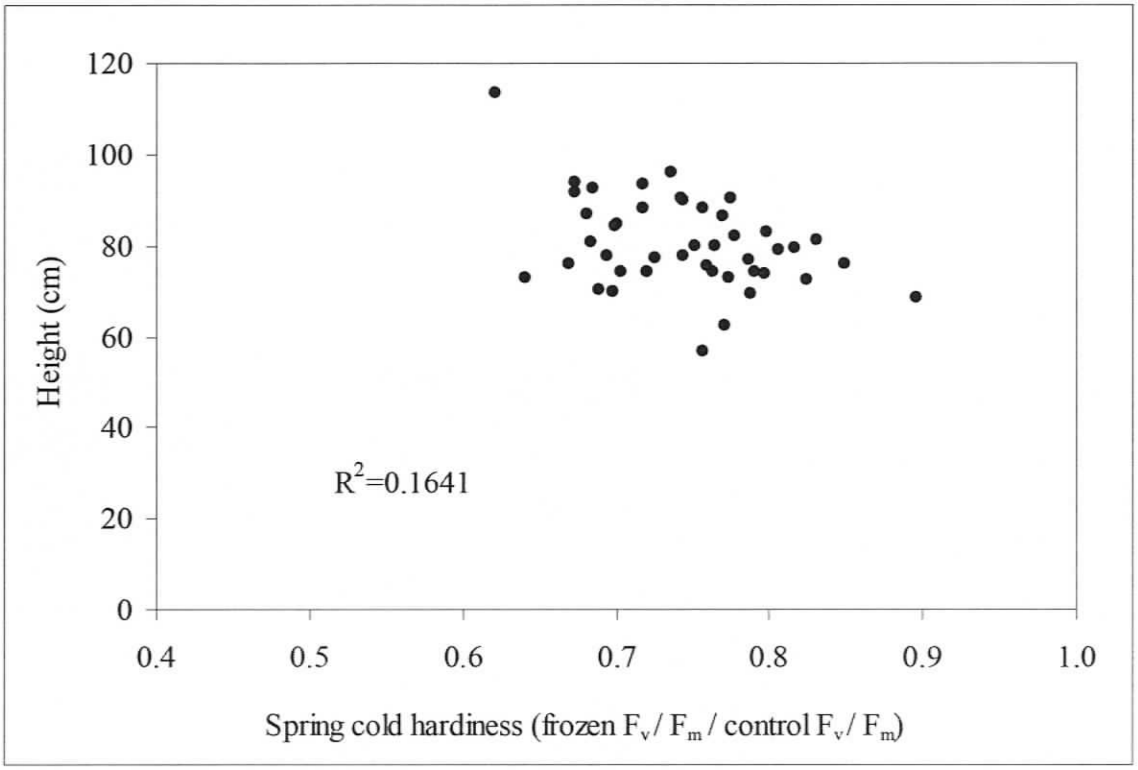

Figure 10. Correlation of spring cold hardiness (ratio of frozen to control F_v/F_m in a controlled freezing trial) and height (cm) in 50 Douglas-fir families growing at Blackwater in 2008.

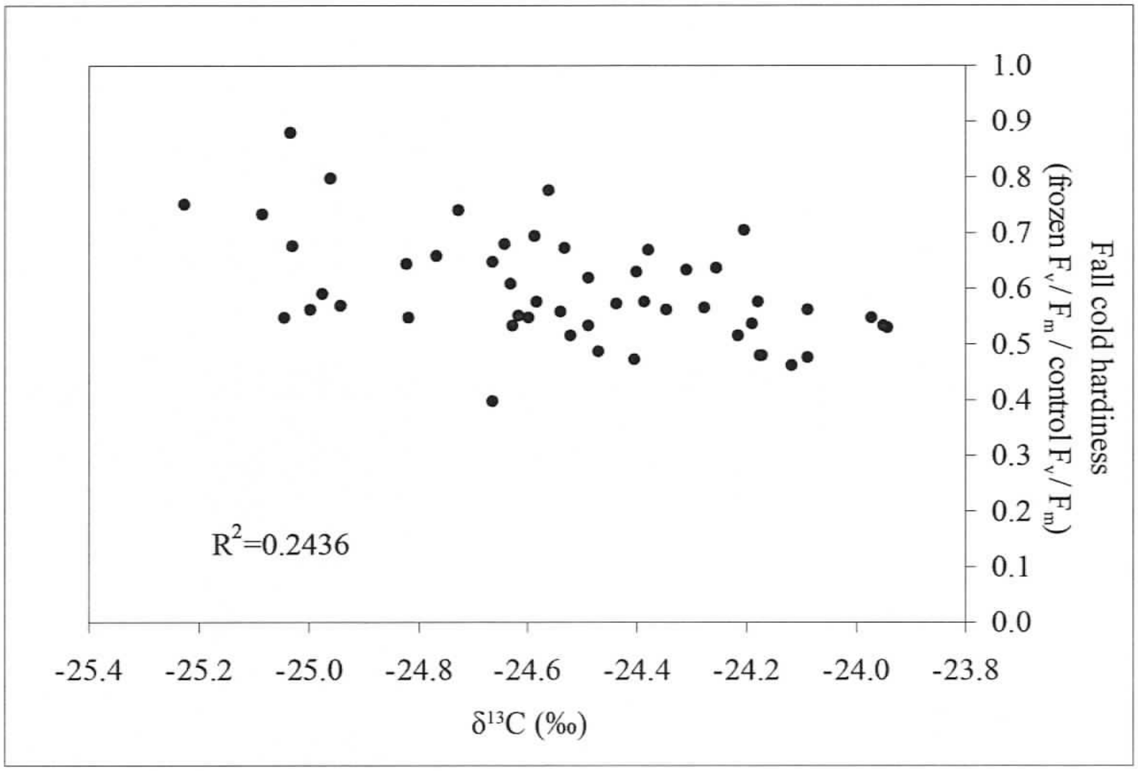

Figure 11. Correlation of carbon fractionation (δ^{13}C (‰)) and fall cold hardiness (ratio of frozen to control F_v/F_m in a controlled freezing trial) in 50 Douglas-fir families growing at Birken and Hurley in 2008. Less negative δ^{13}C indicates greater water use efficiency over the growing season.

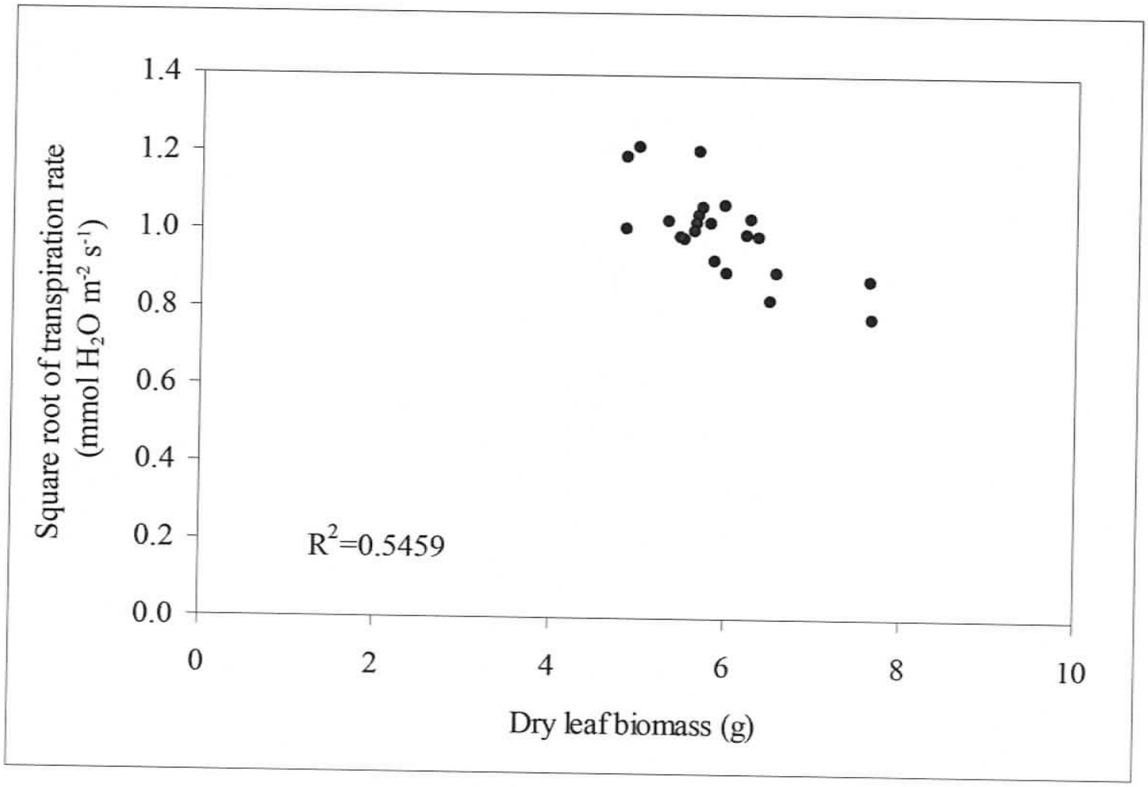

Figure 12. Correlation of leaf biomass (g) and the square root of transpiration rate (mmol m^{-2} s^{-1}) in seedlings from 22 Douglas-fir families growing in a pot trial under 3 drought treatments at the University of Victoria in 2008.

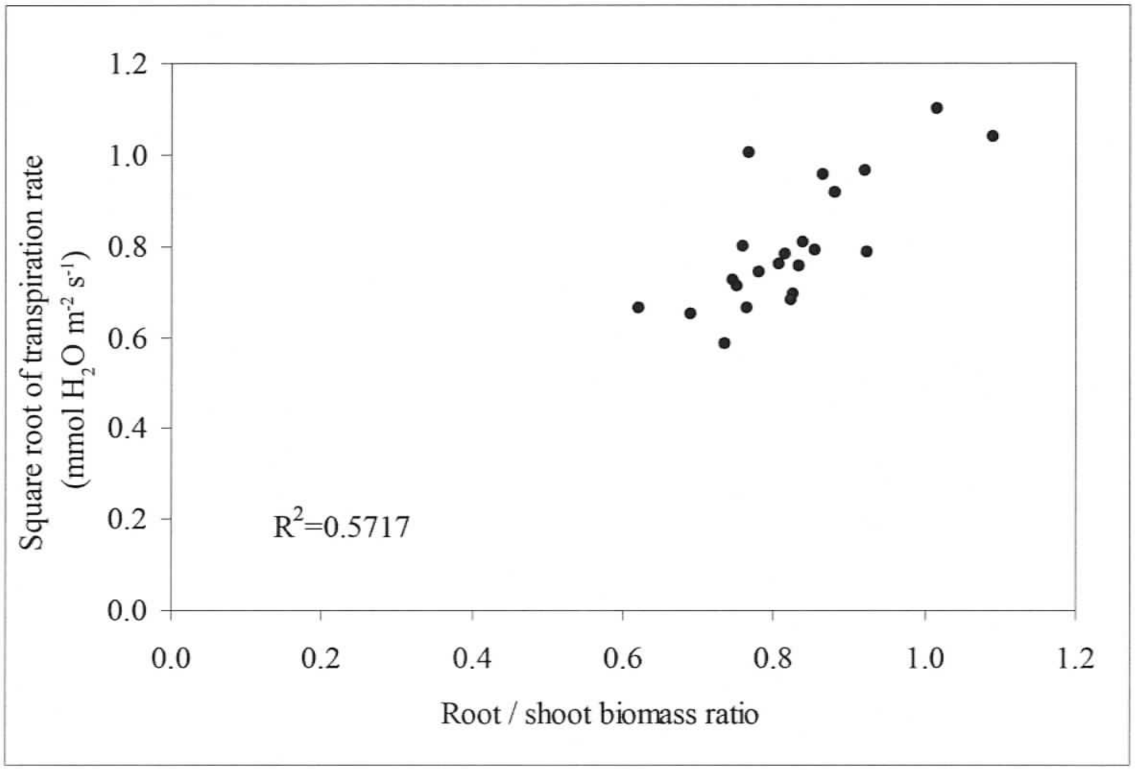

Figure 13. Correlation of root / shoot biomass ratio and the square root of transpiration rate (mmol m^{-2} s^{-1}) in seedlings from 22 Douglas-fir families growing in a pot trial under Treatment 3 (most water-limited) at the University of Victoria in 2008.

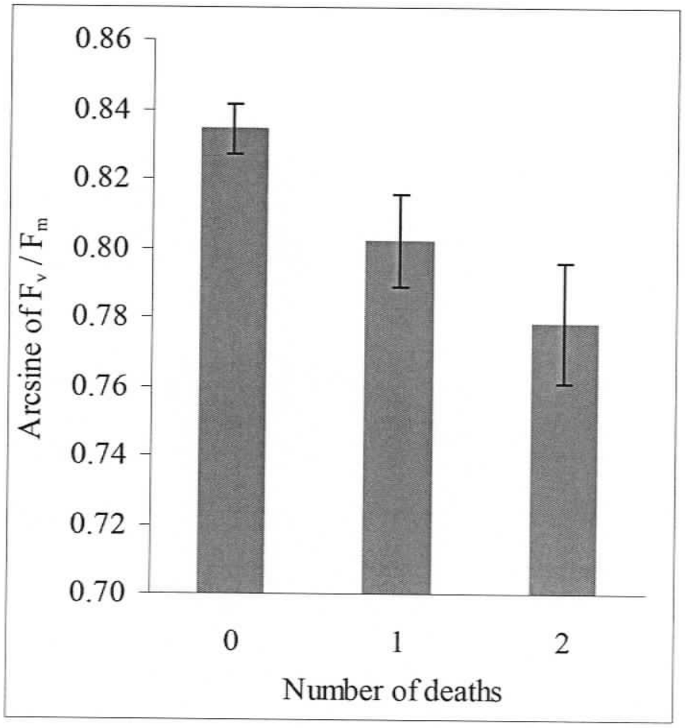

Figure 14. Seedlings from families which had 1 or 2 individuals die had lower summer chlorophyll fluorescence (F_v/F_m) than families in which all individuals survived. Seedlings from 22 Douglas-fir families were grown in a pot trial under 3 drought treatments at the University of Victoria in 2008. Mortality was assessed in November 2008. Error bars are standard error.

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Appendices

Appendix A: Additional ANOVAs

Analysis of variance using transformed data

Table 10. ANOVA table of the square root of 2008 leader growth increment (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at 3 field sites.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	49	74.56	1.52	1.073	0.3528	
Site	2	13.06	6.53	4.605	0.01072	*
Family:Site	97	143.96	1.48	1.0466	0.38035	
Residuals	300	425.42	1.42			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 11. ANOVA table of spring cold hardiness (arcsine of frozen/control Fv/Fm) of seedlings of 98 half-sib submaritime Douglas-fir families grown at Blackwater, assessed using controlled freezing trials and chlorophyll fluorescence.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	97	3.514	0.036	1.0466	0.3668	
Freeze.temp	1	60.169	60.169	1738.17	<2e-16	***
Family:Freeze temp	97	3.273	0.034	0.9749	0.5505	
Residuals	784	27.139	0.035			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 12. ANOVA table of fall cold hardiness (arcsine of frozen/control Fv/Fm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at 2 field sites Birken and Hurley, assessed using controlled freezing trials and chlorophyll fluorescence.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	49	5.4534	0.1113	2.6181	1.44E-07	***
Freeze.temp	1	28.7803	28.7803	677.046	< 2.2e-16	***
Site	1	0.1849	0.1849	4.3488	0.03767	*
Family:Freeze temp	49	1.5934	0.0325	0.765	0.87506	
Family:Site	49	3.2813	0.067	1.5753	0.01062	*
Freeze temp:Site	1	0.0156	0.0156	0.3669	0.54503	
Family:Freeze temp:Site	49	1.4961	0.0305	0.7183	0.92255	
Residuals	400	17.0035	0.0425			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 13. ANOVA table of instantaneous water use efficiency (A/sqrt E) of potted seedlings of 22 half-sib submaritime Douglas-fir families grown under 3 drought treatments, sampled twice in summer 2008. Treatment is made significant due to square root transformation of E.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	21	34.326	1.635	1.6671	0.102552	
Treatment	1	32.999	32.999	33.6566	3.13E-06	***
Block	2	0.393	0.197	0.2006	0.819399	
Month	1	52.882	52.882	53.937	5.37E-08	***
Family*Treatment	21	28.994	1.381	1.4082	0.196484	
Family*Block	42	71.906	1.712	1.7462	0.061469	.
Treatment*Block	2	4.65	2.325	2.3715	0.111826	
Family*Month	21	12.044	0.574	0.585	0.895619	
Treatment*Month	1	166.788	166.788	170.1142	2.04E-13	***
Block*Month	2	1.114	0.557	0.5683	0.572886	
Family*Treatment*Block	41	33.106	0.807	0.8236	0.719245	
Family*Treatment*Month	20	27.123	1.356	1.3832	0.210781	
Family*Block*Month	8	32.766	4.096	4.1774	0.002176	**
Treatment*Block*Month	1	0.108	0.108	0.1104	0.742117	
Residuals	28	27.453	0.98			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of variance by field site

Table 14. ANOVA table of fall cold hardiness (frozen/control Fv/Fm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Hurley, assessed using controlled freezing trials and chlorophyll fluorescence.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	5.149	0.105	0.7835	0.8467
Residuals	250	33.53	0.134		

Table 15. ANOVA table of fall cold hardiness (frozen/control Fv/Fm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Birken, assessed using controlled freezing trials and chlorophyll fluorescence.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	6.883	0.14	1.105	0.3066
Residuals	250	31.78	0.127		

Table 16. ANOVA table of height (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Birken.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	39169	799	0.972	0.5345
Residuals	100	82244	822		

Table 17. ANOVA table of 2008 leader growth increment (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Birken.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	6666.7	136.1	1.154	0.2704
Residuals	100	11789.5	117.9		

Table 18. ANOVA table of root collar diameter (mm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Birken.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	1711.5	34.9	0.7388	0.8795
Residuals	100	4727.6	47.3		

Table 19. ANOVA table of carbon fractionation $\delta^{13}C$ (‰) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Birken.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	39.373	0.804	0.9125	0.6328
Residuals	98	86.296	0.881		

Table 20. ANOVA table of height (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Blackwater.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	48	41608	867	1.0134	0.467
Residuals	100	85536	855		

Table 21. ANOVA table of 2008 leader growth increment (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Blackwater.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	48	4592.7	95.7	1.0046	0.481
Residuals	100	9524.2	95.2		

Table 22. ANOVA table of root collar diameter (mm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Blackwater.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	48	1955.7	40.7	0.9613	0.5513
Residuals	100	4238.5	42.4		

Table 23. ANOVA table of carbon fractionation $\delta^{13}C$ (‰) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Blackwater.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	48	32.235	0.672	0.9042	0.6449
Residuals	97	72.046	0.743		

Table 24. ANOVA table of height (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Hurley.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	49	41343	844	1.4345	0.06514	.
Residuals	100	58818	588			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 25. ANOVA table of 2008 leader growth increment (cm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Hurley.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Family	49	2150.2	43.9	1.0168	0.4619
Residuals	100	4315.7	43.2		

Table 26. ANOVA table of root collar diameter (mm) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Hurley.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	49	2523.7	51.5	1.5787	0.02776	*
Residuals	100	3262.4	32.6			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 27. ANOVA table of carbon fractionation δ^{13}C (‰) of seedlings of 50 half-sib submaritime Douglas-fir families grown at Hurley.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Family	49	41.399	0.845	1.5791	0.02769	*
Residuals	100	53.503	0.535			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix B: Pot study experimental design

Figure 15. Experimental design for pot study which applied 3 drought treatments to 318 seedlings from 22 submaritime Douglas-fir families.

	Treat. 1	Treat. 2	Treat. 3	n=
Block 1	35	36	35	106
Block 2	36	35	35	106
Block 3	36	35	35	106
n=	107	106	105	318

Family	Block 1	Block 2	Block 3	n=
419	4	6	5	15
511	5	5	5	15
512	5	5	5	15
577	4	6	5	15
659	4	4	4	12
724	4	4	5	13
850	6	4	5	15
943	5	5	5	15
944	5	5	5	15
946	6	4	5	15
1138	5	5	5	15
1147	5	5	5	15
1159	4	5	5	14
1180	5	5	5	15
1204	5	5	5	15
1205	5	5	5	15
1210	5	5	4	14
1276	5	5	5	15
7802	4	4	4	12
192x54	5	5	5	15
243x101	4	5	4	13
299x561	6	4	5	15
n=	106	106	106	318

Family	Treat. 1	Treat. 2	Treat. 3	n=
419	5	5	5	15
511	5	5	5	15
512	5	5	5	15
577	5	5	5	15
659	4	4	4	12
724	5	4	4	13
850	5	5	5	15
943	5	5	5	15
944	5	5	5	15
946	5	5	5	15
1138	5	5	5	15
1147	5	5	5	15
1159	4	5	5	14
1180	5	5	5	15
1204	5	5	5	15
1205	5	5	5	15
1210	5	5	4	14
1276	5	5	5	15
7802	4	4	4	12
192x54	5	5	5	15
243x101	5	4	4	13
299x561	5	5	5	15
n=	107	106	105	318

Appendix C: Trait correlations

Table 28. Pearson product moment correlation coefficients, r, for family means of physiological variables in pot and field trials. This indicates the extent of a linear relationship between 2 data sets, and ranges between -1 and 1.

Key to correlation table.

	Family means
V1	Spring cold hardiness (sqrt) at Blackwater
V2	Fall cold hardiness (sqrt) at Birken and Hurley
V3	Fall cold hardiness (sqrt) at Hurley
V4	Fall cold hardiness (sqrt) at Birken
V5	Field height at 3 sites
V6	Field height (without family 944)
V7	Field leader growth increment (sqrt)
V8	Field root collar diameter (RCD)
V9	Blackwater height
V10	Blackwater RCD
V11	Blackwater leader increment (sqrt)
V12	Birken height
V13	Birken RCD
V14	Birken leader increment (sqrt)
V15	Hurley height
V16	Hurley RCD
V17	Hurley leader increment (sqrt)
V18	Blackwater Delta PDB 07
V19	All 3 sites Delta PDB 08
V20	Blackwater Delta PDB 08
V21	Birken Delta PDB 08
V22	Hurley Delta PDB 08
V23	Blackwater shoot water potential
V24	Field survival 3yr
V25	Female parent elevation
V26	Pot initial height
V27	Pot leader growth
V28	T1 leader growth
V29	T2 leader growth
V30	T3 leader growth
V31	Leaf weight
V32	T1 leaf weight
V33	T2 leaf weight
V34	T3 leaf weight
V35	Wood weight
V36	Shoot weight
V37	Root weight
V38	T1 Root weight
V39	T2 Root weight
V40	T3 Root weight
V41	R/S ratio

V42	T1 R/S ratio
V43	T2 R/S ratio
V44	T3 R/S ratio
V45	mean jdbb
V46	median jdbb
V47	Pot water potential
V48	T1 water potential
V49	T3 water potential
V50	Fv/m (arcsine)
V51	T1 Fv/m
V52	T2 Fv/m
V53	T3 Fv/m
V54	T1 Fv/m arcsine
V55	T3 Fv/m arcsine
V56	A
V57	T1 A
V58	T3 A
V59	E (sqrt)
V60	T1 E (sqrt)
V61	T3 E (sqrt)
V62	gs (sqrt)
V63	T1 gs (sqrt)
V64	T3 gs (sqrt)
V65	A/E(sqrt)
V66	T1 A/E(sqrt)
V67	T3 A/E(sqrt)
V68	Pot trial survival proportion

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13
V1	1.00	0.17	0.23	0.00	0.11	0.11	0.17	0.19	0.13	0.26	0.09	0.21	0.02
V2	0.17	1.00	0.71	0.63	-0.08	-0.08	-0.13	-0.17	-0.36	-0.26	-0.26	0.38	0.04
V3	0.23	0.71	1.00	-0.09	-0.08	-0.08	-0.16	-0.20	-0.34	-0.34	-0.24	0.19	0.03
V4	0.00	0.63	-0.09	1.00	-0.02	-0.02	0.00	-0.03	-0.13	0.01	-0.11	0.32	0.03
V5	0.11	-0.08	-0.08	-0.02	1.00	1.00	0.93	0.92	0.54	0.58	0.67	0.36	0.57
V6	0.11	-0.08	-0.08	-0.02	1.00	1.00	0.93	0.92	0.54	0.58	0.67	0.36	0.57
V7	0.17	-0.13	-0.16	0.00	0.93	0.93	1.00	0.94	0.65	0.72	0.77	0.17	0.37
V8	0.19	-0.17	-0.20	-0.03	0.92	0.92	0.94	1.00	0.58	0.68	0.69	0.15	0.52
V9	0.13	-0.36	-0.34	-0.13	0.54	0.54	0.65	0.58	1.00	0.94	0.90	-0.11	0.36
V10	0.26	-0.26	-0.34	0.01	0.58	0.58	0.72	0.68	0.94	1.00	0.91	0.01	0.37
V11	0.09	-0.26	-0.24	-0.11	0.67	0.67	0.77	0.69	0.90	0.91	1.00	-0.03	0.26
V12	0.21	0.38	0.19	0.32	0.36	0.36	0.17	0.15	-0.11	0.01	-0.03	1.00	0.49
V13	0.02	0.04	0.03	0.03	0.57	0.57	0.37	0.52	0.36	0.37	0.26	0.49	1.00
V14	0.22	0.38	0.01	0.53	0.45	0.45	0.46	0.37	0.01	0.17	0.06	0.72	0.23
V15	-0.11	-0.01	0.11	-0.13	0.51	0.51	0.44	0.51	-0.24	-0.21	-0.02	-0.13	0.03
V16	-0.01	-0.04	0.04	-0.09	0.46	0.46	0.46	0.51	-0.29	-0.19	-0.04	-0.05	-0.12
V17	0.08	-0.21	-0.03	-0.26	0.65	0.65	0.64	0.71	0.13	0.12	0.20	-0.21	0.22
V18	0.14	0.34	0.02	0.46	0.29	0.29	0.28	0.40	0.00	0.12	-0.14	0.30	0.48
V19	-0.25	-0.40	-0.55	0.03	0.65	0.65	0.58	0.52	0.37	0.32	0.45	0.17	0.19
V20	0.30	-0.08	-0.03	-0.08	0.30	0.30	0.34	0.29	0.72	0.73	0.67	0.04	0.24
V21	-0.12	-0.48	-0.54	-0.08	0.41	0.41	0.27	0.31	0.14	0.14	0.14	0.50	0.46
V22	-0.33	-0.24	-0.33	0.02	0.49	0.49	0.45	0.39	0.02	0.01	0.19	0.03	-0.10

V23	0.30	-0.36	-0.48	0.02	0.49	0.49	0.66	0.65	0.65	0.68	0.63	-0.25	0.11
V24	-0.28	-0.05	-0.12	0.06	-0.59	-0.59	-0.59	-0.49	-0.57	-0.59	-0.67	-0.24	-0.27
V25	-0.26	0.29	-0.01	0.42	-0.22	-0.22	-0.30	-0.30	-0.21	-0.28	-0.21	0.06	-0.01
V26	-0.19	0.06	0.09	-0.01	-0.25	-0.25	-0.16	-0.33	0.13	-0.06	-0.18	-0.18	-0.06
V27	0.13	-0.03	0.07	-0.12	0.29	0.29	0.13	0.16	0.29	0.16	0.08	0.19	0.60
V28	-0.02	-0.13	0.01	-0.19	0.56	0.56	0.37	0.46	0.37	0.30	0.26	0.28	0.81
V29	0.14	0.04	-0.12	0.19	0.28	0.28	0.12	0.14	0.33	0.28	0.20	0.34	0.56
V30	0.18	0.02	0.25	-0.25	-0.04	-0.04	-0.12	-0.14	0.04	-0.13	-0.21	-0.08	0.21
V31	-0.29	-0.05	-0.17	0.12	0.03	0.03	0.14	0.16	0.03	-0.04	-0.16	-0.30	0.16
V32	-0.34	-0.20	-0.03	-0.25	0.06	0.06	0.10	0.14	-0.04	-0.13	-0.15	-0.28	0.19
V33	-0.13	0.18	-0.33	0.62	-0.04	-0.04	0.12	0.10	0.25	0.25	0.07	-0.18	0.13
V34	-0.25	-0.05	-0.09	0.02	0.04	0.04	0.09	0.10	-0.15	-0.22	-0.33	-0.26	0.06
V35	-0.17	0.08	0.09	0.02	-0.38	-0.38	-0.26	-0.36	-0.02	-0.16	-0.31	-0.26	-0.08
V36	-0.25	0.02	-0.05	0.08	-0.20	-0.20	-0.07	-0.11	0.00	-0.11	-0.26	-0.31	0.04
V37	-0.18	0.22	0.05	0.25	-0.21	-0.21	-0.12	-0.21	-0.15	-0.15	-0.29	0.06	-0.13
V38	-0.31	-0.01	0.12	-0.16	-0.06	-0.06	-0.01	-0.15	-0.06	-0.15	-0.14	0.07	0.00
V39	-0.18	0.31	-0.05	0.49	-0.45	-0.45	-0.38	-0.39	-0.29	-0.26	-0.42	-0.05	-0.29
V40	-0.11	0.12	-0.24	0.44	0.16	0.16	0.17	0.09	0.15	0.22	0.11	0.28	0.06
V41	0.17	0.23	0.09	0.23	0.09	0.09	0.00	0.00	-0.13	0.03	0.07	0.46	-0.10
V42	0.18	0.20	-0.12	0.41	0.20	0.20	0.17	0.08	-0.06	0.10	0.16	0.48	-0.22
V43	-0.12	0.03	0.14	-0.11	-0.20	-0.20	-0.33	-0.27	-0.43	-0.38	-0.31	0.17	-0.22
V44	0.38	0.35	0.18	0.29	0.25	0.25	0.21	0.21	0.21	0.38	0.36	0.52	0.18
V45	0.05	0.12	-0.28	0.48	0.18	0.18	0.10	0.16	0.32	0.44	0.37	0.42	0.36
V46	-0.12	0.01	-0.35	0.40	0.26	0.26	0.22	0.29	0.50	0.57	0.49	0.26	0.52
V47	0.15	-0.18	0.23	-0.52	-0.15	-0.15	-0.13	-0.21	-0.01	-0.15	0.04	-0.34	-0.33
V48	-0.02	0.33	0.72	-0.33	-0.10	-0.10	-0.17	-0.22	-0.32	-0.39	-0.18	0.01	-0.06
V49	0.19	-0.54	-0.40	-0.32	-0.09	-0.09	-0.08	-0.10	0.15	0.05	0.08	-0.38	-0.34
V50	-0.25	0.06	-0.31	0.44	0.07	0.07	-0.04	-0.01	0.03	0.13	0.03	0.60	0.37
V51	-0.10	-0.12	-0.54	0.43	0.27	0.27	0.28	0.32	0.20	0.32	0.18	0.21	0.19
V52	-0.17	-0.52	-0.66	0.00	-0.21	-0.21	-0.03	-0.02	0.41	0.39	0.16	-0.31	-0.03
V53	-0.23	0.10	-0.08	0.24	-0.09	-0.09	-0.20	-0.19	0.01	0.03	0.00	0.56	0.36
V54	-0.03	-0.16	-0.56	0.40	0.22	0.22	0.23	0.28	0.16	0.29	0.15	0.17	0.11
V55	-0.27	0.21	0.06	0.23	-0.09	-0.09	-0.22	-0.23	-0.06	-0.04	-0.06	0.61	0.38
V56	0.48	0.09	-0.30	0.47	0.13	0.13	0.14	0.09	0.12	0.28	0.13	0.50	-0.01
V57	0.13	-0.10	-0.42	0.32	0.44	0.44	0.43	0.45	0.22	0.39	0.27	0.38	0.20
V58	0.54	0.32	0.06	0.38	-0.35	-0.35	-0.36	-0.44	-0.18	-0.11	-0.20	0.37	-0.22
V59	0.37	0.15	-0.20	0.44	0.21	0.21	0.24	0.19	0.36	0.49	0.39	0.33	0.04
V60	-0.02	0.00	-0.17	0.19	0.46	0.46	0.41	0.43	0.19	0.31	0.25	0.31	0.24
V61	0.46	0.25	-0.08	0.44	-0.12	-0.12	-0.10	-0.14	0.20	0.28	0.20	0.26	-0.06
V62	0.42	0.12	-0.19	0.38	0.21	0.21	0.25	0.19	0.31	0.45	0.36	0.34	-0.02
V63	0.10	-0.03	-0.19	0.17	0.51	0.51	0.50	0.48	0.29	0.43	0.39	0.39	0.23
V64	0.50	0.31	-0.01	0.45	-0.24	-0.24	-0.24	-0.28	0.02	0.10	0.01	0.21	-0.21
V65	0.58	-0.01	-0.26	0.27	0.05	0.05	0.11	0.07	-0.03	0.15	-0.01	0.36	-0.16
V66	0.13	-0.14	-0.57	0.43	0.40	0.40	0.43	0.46	0.28	0.45	0.28	0.36	0.24
V67	0.60	0.17	0.26	-0.04	-0.37	-0.37	-0.33	-0.41	-0.39	-0.33	-0.37	0.16	-0.43
V68	-0.20	-0.02	-0.23	0.23	0.64	0.64	0.51	0.52	0.42	0.46	0.47	0.62	0.71

	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26
V1	0.22	-0.11	-0.01	0.08	0.14	-0.25	0.30	-0.12	-0.33	0.30	-0.28	-0.26	-0.19
V2	0.38	-0.01	-0.04	-0.21	0.34	-0.40	-0.08	-0.48	-0.24	-0.36	-0.05	0.29	0.06
V3	0.01	0.11	0.04	-0.03	0.02	-0.55	-0.03	-0.54	-0.33	-0.48	-0.12	-0.01	0.09
V4	0.53	-0.13	-0.09	-0.26	0.46	0.03	-0.08	-0.08	0.02	0.02	0.06	0.42	-0.01

V5	0.45	0.51	0.46	0.65	0.29	0.65	0.30	0.41	0.49	0.49	-0.59	-0.22	-0.25
V6	0.45	0.51	0.46	0.65	0.29	0.65	0.30	0.41	0.49	0.49	-0.59	-0.22	-0.25
V7	0.46	0.44	0.46	0.64	0.28	0.58	0.34	0.27	0.45	0.66	-0.59	-0.30	-0.16
V8	0.37	0.51	0.51	0.71	0.40	0.52	0.29	0.31	0.39	0.65	-0.49	-0.30	-0.33
V9	0.01	-0.24	-0.29	0.13	0.00	0.37	0.72	0.14	0.02	0.65	-0.57	-0.21	0.13
V10	0.17	-0.21	-0.19	0.12	0.12	0.32	0.73	0.14	0.01	0.68	-0.59	-0.28	-0.06
V11	0.06	-0.02	-0.04	0.20	-0.14	0.45	0.67	0.14	0.19	0.63	-0.67	-0.21	-0.18
V12	0.72	-0.13	-0.05	-0.21	0.30	0.17	0.04	0.50	0.03	-0.25	-0.24	0.06	-0.18
V13	0.23	0.03	-0.12	0.22	0.48	0.19	0.24	0.46	-0.10	0.11	-0.27	-0.01	-0.06
V14	1.00	0.06	0.22	0.06	0.58	0.25	-0.03	0.31	0.24	0.06	-0.20	-0.15	0.07
V15	0.06	1.00	0.94	0.88	0.16	0.36	-0.37	0.03	0.60	0.17	-0.02	-0.10	-0.32
V16	0.22	0.94	1.00	0.81	0.19	0.34	-0.43	0.08	0.61	0.20	0.02	-0.18	-0.38
V17	0.06	0.88	0.81	1.00	0.30	0.38	-0.16	0.11	0.47	0.47	-0.15	-0.20	-0.14
V18	0.58	0.16	0.19	0.30	1.00	0.00	-0.06	-0.01	0.03	0.05	0.04	0.06	0.15
V19	0.25	0.36	0.34	0.38	0.00	1.00	0.26	0.57	0.86	0.30	-0.42	0.05	-0.22
V20	-0.03	-0.37	-0.43	-0.16	-0.06	0.26	1.00	-0.12	0.05	0.21	-0.76	-0.15	-0.02
V21	0.31	0.03	0.08	0.11	-0.01	0.57	-0.12	1.00	0.29	0.18	0.00	-0.04	-0.25
V22	0.24	0.60	0.61	0.47	0.03	0.86	0.05	0.29	1.00	0.10	-0.30	0.03	-0.20
V23	0.06	0.17	0.20	0.47	0.05	0.30	0.21	0.18	0.10	1.00	-0.28	-0.18	-0.05
V24	-0.20	-0.02	0.02	-0.15	0.04	-0.42	-0.76	0.00	-0.30	-0.28	1.00	-0.02	0.07
V25	-0.15	-0.10	-0.18	-0.20	0.06	0.05	-0.15	-0.04	0.03	-0.18	-0.02	1.00	-0.01
V26	0.07	-0.32	-0.38	-0.14	0.15	-0.22	-0.02	-0.25	-0.20	-0.05	0.07	-0.01	1.00
V27	0.00	-0.04	-0.23	0.15	0.06	0.00	0.09	0.23	-0.27	0.26	-0.06	-0.22	0.35
V28	0.06	0.15	-0.03	0.34	0.23	0.24	0.22	0.34	0.01	0.27	-0.30	-0.18	0.16
V29	0.08	-0.21	-0.35	-0.09	-0.01	0.10	0.16	0.34	-0.26	0.34	-0.12	-0.03	0.12
V30	-0.11	-0.03	-0.19	0.13	-0.04	-0.28	-0.12	-0.04	-0.38	0.07	0.22	-0.34	0.55
V31	0.10	0.23	0.19	0.42	0.55	-0.06	-0.33	-0.07	0.02	0.17	0.17	0.14	0.51
V32	-0.07	0.32	0.25	0.46	0.35	-0.03	-0.29	0.00	0.07	-0.08	0.07	0.07	0.32
V33	0.20	-0.17	-0.19	0.02	0.52	-0.07	-0.09	-0.10	-0.17	0.38	0.09	0.42	0.39
V34	0.17	0.38	0.34	0.49	0.50	-0.02	-0.41	-0.11	0.16	0.12	0.26	-0.15	0.60
V35	-0.01	-0.28	-0.31	-0.12	0.21	-0.46	-0.23	-0.33	-0.40	-0.02	0.25	0.00	0.91
V36	0.05	-0.03	-0.08	0.16	0.42	-0.29	-0.31	-0.22	-0.21	0.08	0.23	0.08	0.78
V37	0.38	-0.16	-0.10	-0.15	0.46	-0.05	0.04	-0.29	0.14	-0.40	-0.06	0.04	0.55
V38	0.20	-0.06	-0.04	-0.01	0.12	-0.07	-0.13	-0.05	0.02	-0.31	-0.14	0.00	0.57
V39	0.18	-0.26	-0.19	-0.35	0.46	-0.08	0.01	-0.38	0.12	-0.43	0.18	0.38	0.29
V40	0.51	-0.16	-0.12	-0.21	0.23	0.41	0.41	0.05	0.43	-0.02	-0.31	-0.21	0.23
V41	0.30	-0.09	0.01	-0.32	-0.01	0.30	0.38	0.04	0.34	-0.41	-0.27	0.01	-0.53
V42	0.49	-0.03	0.11	-0.25	-0.06	0.44	0.28	0.12	0.44	-0.15	-0.32	-0.03	-0.45
V43	-0.04	0.04	0.09	-0.22	-0.03	0.16	0.09	-0.06	0.36	-0.68	0.07	0.06	-0.35
V44	0.35	-0.26	-0.19	-0.32	0.05	0.15	0.59	0.07	0.05	-0.14	-0.46	-0.03	-0.51
V45	0.15	-0.40	-0.40	-0.41	0.12	0.28	0.54	0.19	0.01	0.05	-0.41	0.33	-0.38
V46	0.09	-0.38	-0.43	-0.28	0.20	0.31	0.56	0.23	0.00	0.14	-0.43	0.35	-0.25
V47	-0.55	0.08	0.04	0.12	-0.55	-0.11	0.07	-0.27	-0.04	0.08	-0.34	0.26	0.06
V48	-0.26	0.20	0.12	0.07	-0.28	-0.46	-0.27	-0.34	-0.31	-0.31	-0.12	0.17	0.08
V49	-0.45	0.01	-0.01	0.09	-0.39	0.38	0.32	-0.01	0.33	0.23	-0.27	0.14	-0.08
V50	0.42	-0.39	-0.33	-0.47	0.18	0.33	0.22	0.52	0.19	-0.18	-0.11	0.14	-0.12
V51	0.41	-0.01	0.05	0.00	0.32	0.54	0.31	0.28	0.51	0.29	-0.25	-0.11	-0.05
V52	-0.07	-0.48	-0.40	-0.22	0.21	0.06	0.25	0.04	-0.08	0.20	0.06	-0.07	0.33
V53	0.22	-0.53	-0.50	-0.58	-0.03	0.02	0.03	0.53	-0.18	-0.28	0.09	0.27	-0.09
V54	0.36	-0.01	0.07	-0.02	0.28	0.51	0.30	0.24	0.49	0.29	-0.22	-0.12	-0.10
V55	0.25	-0.51	-0.50	-0.59	0.03	-0.02	0.04	0.45	-0.18	-0.45	0.02	0.31	-0.04
V56	0.54	-0.31	-0.16	-0.32	0.05	0.27	0.31	0.30	0.11	0.23	-0.20	-0.19	-0.22

V57	0.57	0.06	0.17	0.06	0.27	0.51	0.28	0.35	0.43	0.32	-0.21	-0.44	-0.19
V58	0.18	-0.54	-0.44	-0.60	-0.23	-0.22	0.10	0.07	-0.36	-0.14	-0.01	0.21	-0.15
V59	0.38	-0.33	-0.26	-0.31	0.03	0.31	0.57	0.14	0.14	0.26	-0.31	-0.13	-0.23
V60	0.47	0.17	0.20	0.12	0.21	0.46	0.30	0.23	0.46	0.16	-0.18	-0.55	-0.11
V61	0.10	-0.54	-0.49	-0.53	-0.15	0.01	0.45	0.05	-0.20	0.06	-0.20	0.26	-0.30
V62	0.41	-0.29	-0.18	-0.27	0.01	0.34	0.62	0.12	0.25	0.24	-0.46	-0.14	-0.23
V63	0.52	0.09	0.16	0.07	0.13	0.51	0.43	0.31	0.48	0.25	-0.41	-0.48	-0.18
V64	0.08	-0.48	-0.43	-0.53	-0.14	-0.01	0.43	-0.11	-0.13	-0.08	-0.22	0.30	-0.26
V65	0.48	-0.16	0.04	-0.14	0.04	0.06	0.10	0.17	0.00	0.29	-0.18	-0.30	-0.12
V66	0.57	-0.02	0.09	0.03	0.33	0.48	0.24	0.41	0.34	0.43	-0.21	-0.31	-0.16
V67	0.07	-0.18	-0.04	-0.25	-0.27	-0.41	-0.14	-0.19	-0.34	-0.14	0.00	-0.12	-0.01
V68	0.47	-0.05	-0.07	0.04	0.30	0.54	0.24	0.69	0.26	0.15	-0.31	0.14	-0.19

	V27	V28	V29	V30	V31	V32	V33	V34	V35	V36	V37	V38	V39
V1	0.13	-0.02	0.14	0.18	-0.29	-0.34	-0.13	-0.25	-0.17	-0.25	-0.18	-0.31	-0.18
V2	-0.03	-0.13	0.04	0.02	-0.05	-0.20	0.18	-0.05	0.08	0.02	0.22	-0.01	0.31
V3	0.07	0.01	-0.12	0.25	-0.17	-0.03	-0.33	-0.09	0.09	-0.05	0.05	0.12	-0.05
V4	-0.12	-0.19	0.19	-0.25	0.12	-0.25	0.62	0.02	0.02	0.08	0.25	-0.16	0.49
V5	0.29	0.56	0.28	-0.04	0.03	0.06	-0.04	0.04	-0.38	-0.20	-0.21	-0.06	-0.45
V6	0.29	0.56	0.28	-0.04	0.03	0.06	-0.04	0.04	-0.38	-0.20	-0.21	-0.06	-0.45
V7	0.13	0.37	0.12	-0.12	0.14	0.10	0.12	0.09	-0.26	-0.07	-0.12	-0.01	-0.38
V8	0.16	0.46	0.14	-0.14	0.16	0.14	0.10	0.10	-0.36	-0.11	-0.21	-0.15	-0.39
V9	0.29	0.37	0.33	0.04	0.03	-0.04	0.25	-0.15	-0.02	0.00	-0.15	-0.06	-0.29
V10	0.16	0.30	0.28	-0.13	-0.04	-0.13	0.25	-0.22	-0.16	-0.11	-0.15	-0.15	-0.26
V11	0.08	0.26	0.20	-0.21	-0.16	-0.15	0.07	-0.33	-0.31	-0.26	-0.29	-0.14	-0.42
V12	0.19	0.28	0.34	-0.08	-0.30	-0.28	-0.18	-0.26	-0.26	-0.31	0.06	0.07	-0.05
V13	0.60	0.81	0.56	0.21	0.16	0.19	0.13	0.06	-0.08	0.04	-0.13	0.00	-0.29
V14	0.00	0.06	0.08	-0.11	0.10	-0.07	0.20	0.17	-0.01	0.05	0.38	0.20	0.18
V15	-0.04	0.15	-0.21	-0.03	0.23	0.32	-0.17	0.38	-0.28	-0.03	-0.16	-0.06	-0.26
V16	-0.23	-0.03	-0.35	-0.19	0.19	0.25	-0.19	0.34	-0.31	-0.08	-0.10	-0.04	-0.19
V17	0.15	0.34	-0.09	0.13	0.42	0.46	0.02	0.49	-0.12	0.16	-0.15	-0.01	-0.35
V18	0.06	0.23	-0.01	-0.04	0.55	0.35	0.52	0.50	0.21	0.42	0.46	0.12	0.46
V19	0.00	0.24	0.10	-0.28	-0.06	-0.03	-0.07	-0.02	-0.46	-0.29	-0.05	-0.07	-0.08
V20	0.09	0.22	0.16	-0.12	-0.33	-0.29	-0.09	-0.41	-0.23	-0.31	0.04	-0.13	0.01
V21	0.23	0.34	0.34	-0.04	-0.07	0.00	-0.10	-0.11	-0.33	-0.22	-0.29	-0.05	-0.38
V22	-0.27	0.01	-0.26	-0.38	0.02	0.07	-0.17	0.16	-0.40	-0.21	0.14	0.02	0.12
V23	0.26	0.27	0.34	0.07	0.17	-0.08	0.38	0.12	-0.02	0.08	-0.40	-0.31	-0.43
V24	-0.06	-0.30	-0.12	0.22	0.17	0.07	0.09	0.26	0.25	0.23	-0.06	-0.14	0.18
V25	-0.22	-0.18	-0.03	-0.34	0.14	0.07	0.42	-0.15	0.00	0.08	0.04	0.00	0.38
V26	0.35	0.16	0.12	0.55	0.51	0.32	0.39	0.60	0.91	0.78	0.55	0.57	0.29
V27	1.00	0.85	0.88	0.83	0.04	-0.09	0.01	0.24	0.29	0.18	-0.29	-0.07	-0.49
V28	0.85	1.00	0.73	0.49	0.11	0.08	-0.04	0.26	0.11	0.12	-0.21	0.01	-0.45
V29	0.88	0.73	1.00	0.55	-0.17	-0.38	0.12	-0.05	0.08	-0.05	-0.40	-0.27	-0.45
V30	0.83	0.49	0.55	1.00	0.12	0.02	-0.06	0.37	0.49	0.34	-0.14	0.05	-0.37
V31	0.04	0.11	-0.17	0.12	1.00	0.86	0.70	0.84	0.66	0.91	0.52	0.58	0.27
V32	-0.09	0.08	-0.38	0.02	0.86	1.00	0.34	0.67	0.45	0.72	0.47	0.70	0.12
V33	0.01	-0.04	0.12	-0.06	0.70	0.34	1.00	0.42	0.50	0.66	0.31	0.16	0.38
V34	0.24	0.26	-0.05	0.37	0.84	0.67	0.42	1.00	0.70	0.84	0.51	0.50	0.22
V35	0.29	0.11	0.08	0.49	0.66	0.45	0.50	0.70	1.00	0.91	0.53	0.59	0.27
V36	0.18	0.12	-0.05	0.34	0.91	0.72	0.66	0.84	0.91	1.00	0.57	0.64	0.30
V37	-0.29	-0.21	-0.40	-0.14	0.52	0.47	0.31	0.51	0.53	0.57	1.00	0.74	0.76
V38	-0.07	0.01	-0.27	0.05	0.58	0.70	0.16	0.50	0.59	0.64	0.74	1.00	0.21

V39	-0.49	-0.45	-0.45	-0.37	0.27	0.12	0.38	0.22	0.27	0.30	0.76	0.21	1.00
V40	0.06	0.14	0.17	-0.10	-0.05	-0.24	0.08	0.16	0.05	0.00	0.51	0.11	0.41
V41	-0.47	-0.33	-0.26	-0.55	-0.65	-0.51	-0.45	-0.62	-0.70	-0.74	0.10	-0.27	0.31
V42	-0.39	-0.32	-0.08	-0.51	-0.55	-0.55	-0.27	-0.47	-0.59	-0.63	0.13	-0.17	0.23
V43	-0.51	-0.34	-0.50	-0.43	-0.44	-0.19	-0.58	-0.33	-0.48	-0.51	0.21	-0.11	0.46
V44	-0.26	-0.16	-0.02	-0.43	-0.62	-0.54	-0.24	-0.74	-0.66	-0.71	-0.10	-0.37	0.08
V45	-0.04	0.14	0.35	-0.49	-0.34	-0.41	0.17	-0.53	-0.41	-0.42	-0.05	-0.27	0.17
V46	-0.02	0.21	0.29	-0.46	-0.03	-0.08	0.38	-0.36	-0.27	-0.17	0.02	-0.09	0.14
V47	-0.04	-0.04	-0.13	0.04	-0.10	0.09	-0.26	-0.13	0.07	-0.01	-0.19	0.16	-0.27
V48	0.06	0.06	-0.10	0.17	0.04	0.26	-0.25	0.01	0.20	0.13	-0.06	0.40	-0.32
V49	-0.08	-0.07	-0.04	-0.09	-0.21	-0.14	-0.21	-0.13	-0.18	-0.21	-0.10	-0.16	0.03
V50	0.02	0.18	0.27	-0.33	-0.23	-0.32	0.05	-0.24	-0.22	-0.25	0.13	-0.10	0.26
V51	0.04	0.25	0.23	-0.28	0.02	-0.21	0.20	0.17	-0.12	-0.05	0.23	-0.14	0.28
V52	-0.16	-0.09	-0.13	-0.21	0.42	0.31	0.47	0.24	0.38	0.44	0.46	0.30	0.42
V53	0.03	0.05	0.23	-0.17	-0.28	-0.26	0.03	-0.45	-0.17	-0.25	-0.15	-0.12	0.02
V54	0.01	0.20	0.21	-0.30	-0.04	-0.27	0.14	0.13	-0.15	-0.11	0.21	-0.19	0.30
V55	-0.02	0.03	0.14	-0.20	-0.22	-0.15	0.00	-0.41	-0.13	-0.19	0.02	0.06	0.11
V56	0.08	-0.03	0.38	-0.10	-0.50	-0.72	-0.06	-0.35	-0.33	-0.45	-0.07	-0.40	0.06
V57	0.16	0.28	0.33	-0.12	-0.22	-0.41	-0.07	0.04	-0.28	-0.28	0.05	-0.26	0.03
V58	-0.05	-0.37	0.22	0.01	-0.51	-0.59	-0.05	-0.57	-0.19	-0.38	-0.13	-0.27	0.06
V59	0.02	-0.04	0.30	-0.16	-0.57	-0.78	-0.03	-0.51	-0.43	-0.55	-0.17	-0.59	0.09
V60	0.24	0.36	0.28	0.03	-0.26	-0.39	-0.22	0.07	-0.26	-0.29	0.02	-0.27	-0.03
V61	-0.12	-0.31	0.21	-0.20	-0.58	-0.70	0.06	-0.75	-0.43	-0.55	-0.26	-0.55	0.10
V62	-0.07	-0.07	0.18	-0.26	-0.55	-0.71	-0.09	-0.46	-0.43	-0.53	-0.06	-0.46	0.13
V63	0.14	0.31	0.24	-0.12	-0.33	-0.43	-0.24	-0.08	-0.35	-0.38	-0.01	-0.24	-0.09
V64	-0.21	-0.40	0.09	-0.22	-0.55	-0.65	0.00	-0.65	-0.37	-0.51	-0.07	-0.44	0.28
V65	0.06	-0.04	0.25	-0.03	-0.23	-0.41	-0.05	-0.05	-0.06	-0.16	0.08	-0.07	-0.02
V66	0.15	0.27	0.37	-0.18	-0.04	-0.29	0.18	0.10	-0.18	-0.12	0.09	-0.17	0.05
V67	-0.05	-0.30	-0.02	0.15	-0.26	-0.22	-0.27	-0.13	0.10	-0.08	0.07	0.15	-0.06
V68	0.21	0.45	0.36	-0.21	-0.06	-0.07	0.13	-0.22	-0.35	-0.23	-0.16	-0.10	-0.17

	V40	V41	V42	V43	V44	V45	V46	V47	V48	V49	V50	V51	V52
V1	-0.11	0.17	0.18	-0.12	0.38	0.05	-0.12	0.15	-0.02	0.19	-0.25	-0.10	-0.17
V2	0.12	0.23	0.20	0.03	0.35	0.12	0.01	-0.18	0.33	-0.54	0.06	-0.12	-0.52
V3	-0.24	0.09	-0.12	0.14	0.18	-0.28	-0.35	0.23	0.72	-0.40	-0.31	-0.54	-0.66
V4	0.44	0.23	0.41	-0.11	0.29	0.48	0.40	-0.52	-0.33	-0.32	0.44	0.43	0.00
V5	0.16	0.09	0.20	-0.20	0.25	0.18	0.26	-0.15	-0.10	-0.09	0.07	0.27	-0.21
V6	0.16	0.09	0.20	-0.20	0.25	0.18	0.26	-0.15	-0.10	-0.09	0.07	0.27	-0.21
V7	0.17	0.00	0.17	-0.33	0.21	0.10	0.22	-0.13	-0.17	-0.08	-0.04	0.28	-0.03
V8	0.09	0.00	0.08	-0.27	0.21	0.16	0.29	-0.21	-0.22	-0.10	-0.01	0.32	-0.02
V9	0.15	-0.13	-0.06	-0.43	0.21	0.32	0.50	-0.01	-0.32	0.15	0.03	0.20	0.41
V10	0.22	0.03	0.10	-0.38	0.38	0.44	0.57	-0.15	-0.39	0.05	0.13	0.32	0.39
V11	0.11	0.07	0.16	-0.31	0.36	0.37	0.49	0.04	-0.18	0.08	0.03	0.18	0.16
V12	0.28	0.46	0.48	0.17	0.52	0.42	0.26	-0.34	0.01	-0.38	0.60	0.21	-0.31
V13	0.06	-0.10	-0.22	-0.22	0.18	0.36	0.52	-0.33	-0.06	-0.34	0.37	0.19	-0.03
V14	0.51	0.30	0.49	-0.04	0.35	0.15	0.09	-0.55	-0.26	-0.45	0.42	0.41	-0.07
V15	-0.16	-0.09	-0.03	0.04	-0.26	-0.40	-0.38	0.08	0.20	0.01	-0.39	-0.01	-0.48
V16	-0.12	0.01	0.11	0.09	-0.19	-0.40	-0.43	0.04	0.12	-0.01	-0.33	0.05	-0.40
V17	-0.21	-0.32	-0.25	-0.22	-0.32	-0.41	-0.28	0.12	0.07	0.09	-0.47	0.00	-0.22
V18	0.23	-0.01	-0.06	-0.03	0.05	0.12	0.20	-0.55	-0.28	-0.39	0.18	0.32	0.21
V19	0.41	0.30	0.44	0.16	0.15	0.28	0.31	-0.11	-0.46	0.38	0.33	0.54	0.06
V20	0.41	0.38	0.28	0.09	0.59	0.54	0.56	0.07	-0.27	0.32	0.22	0.31	0.25

V21	0.05	0.04	0.12	-0.06	0.07	0.19	0.23	-0.27	-0.34	-0.01	0.52	0.28	0.04
V22	0.43	0.34	0.44	0.36	0.05	0.01	0.00	-0.04	-0.31	0.33	0.19	0.51	-0.08
V23	-0.02	-0.41	-0.15	-0.68	-0.14	0.05	0.14	0.08	-0.31	0.23	-0.18	0.29	0.20
V24	-0.31	-0.27	-0.32	0.07	-0.46	-0.41	-0.43	-0.34	-0.12	-0.27	-0.11	-0.25	0.06
V25	-0.21	0.01	-0.03	0.06	-0.03	0.33	0.35	0.26	0.17	0.14	0.14	-0.11	-0.07
V26	0.23	-0.53	-0.45	-0.35	-0.51	-0.38	-0.25	0.06	0.08	-0.08	-0.12	-0.05	0.33
V27	0.06	-0.47	-0.39	-0.51	-0.26	-0.04	-0.02	-0.04	0.06	-0.08	0.02	0.04	-0.16
V28	0.14	-0.33	-0.32	-0.34	-0.16	0.14	0.21	-0.04	0.06	-0.07	0.18	0.25	-0.09
V29	0.17	-0.26	-0.08	-0.50	-0.02	0.35	0.29	-0.13	-0.10	-0.04	0.27	0.23	-0.13
V30	-0.10	-0.55	-0.51	-0.43	-0.43	-0.49	-0.46	0.04	0.17	-0.09	-0.33	-0.28	-0.21
V31	-0.05	-0.65	-0.55	-0.44	-0.62	-0.34	-0.03	-0.10	0.04	-0.21	-0.23	0.02	0.42
V32	-0.24	-0.51	-0.55	-0.19	-0.54	-0.41	-0.08	0.09	0.26	-0.14	-0.32	-0.21	0.31
V33	0.08	-0.45	-0.27	-0.58	-0.24	0.17	0.38	-0.26	-0.25	-0.21	0.05	0.20	0.47
V34	0.16	-0.62	-0.47	-0.33	-0.74	-0.53	-0.36	-0.13	0.01	-0.13	-0.24	0.17	0.24
V35	0.05	-0.70	-0.59	-0.48	-0.66	-0.41	-0.27	0.07	0.20	-0.18	-0.22	-0.12	0.38
V36	0.00	-0.74	-0.63	-0.51	-0.71	-0.42	-0.17	-0.01	0.13	-0.21	-0.25	-0.05	0.44
V37	0.51	0.10	0.13	0.21	-0.10	-0.05	0.02	-0.19	-0.06	-0.10	0.13	0.23	0.46
V38	0.11	-0.27	-0.17	-0.11	-0.37	-0.27	-0.09	0.16	0.40	-0.16	-0.10	-0.14	0.30
V39	0.41	0.31	0.23	0.46	0.08	0.17	0.14	-0.27	-0.32	0.03	0.26	0.28	0.42
V40	1.00	0.39	0.54	0.18	0.29	0.37	0.30	-0.46	-0.53	0.02	0.63	0.84	0.26
V41	0.39	1.00	0.86	0.78	0.83	0.50	0.28	-0.25	-0.28	0.09	0.45	0.26	-0.17
V42	0.54	0.86	1.00	0.50	0.68	0.53	0.26	-0.24	-0.33	0.15	0.42	0.42	-0.12
V43	0.18	0.78	0.50	1.00	0.41	0.14	-0.04	-0.08	-0.14	0.20	0.26	0.07	-0.09
V44	0.29	0.83	0.68	0.41	1.00	0.61	0.49	-0.30	-0.26	-0.13	0.47	0.20	-0.19
V45	0.37	0.50	0.53	0.14	0.61	1.00	0.90	-0.23	-0.34	0.10	0.64	0.49	0.22
V46	0.30	0.28	0.26	-0.04	0.49	0.90	1.00	-0.27	-0.34	-0.02	0.59	0.44	0.38
V47	-0.46	-0.25	-0.24	-0.08	-0.30	-0.23	-0.27	1.00	0.64	0.60	-0.55	-0.45	-0.19
V48	-0.53	-0.28	-0.33	-0.14	-0.26	-0.34	-0.34	0.64	1.00	-0.17	-0.49	-0.68	-0.52
V49	0.02	0.09	0.15	0.20	-0.13	0.10	-0.02	0.60	-0.17	1.00	-0.21	0.14	0.22
V50	0.63	0.45	0.42	0.26	0.47	0.64	0.59	-0.55	-0.49	-0.21	1.00	0.68	0.17
V51	0.84	0.26	0.42	0.07	0.20	0.49	0.44	-0.45	-0.68	0.14	0.68	1.00	0.34
V52	0.26	-0.17	-0.12	-0.09	-0.19	0.22	0.38	-0.19	-0.52	0.22	0.17	0.34	1.00
V53	0.08	0.25	0.10	0.11	0.42	0.41	0.44	-0.39	-0.19	-0.41	0.77	0.10	-0.01
V54	0.81	0.30	0.47	0.13	0.19	0.50	0.40	-0.39	-0.69	0.24	0.63	0.99	0.36
V55	0.11	0.31	0.12	0.19	0.44	0.40	0.44	-0.37	-0.06	-0.47	0.75	0.04	-0.04
V56	0.59	0.52	0.71	0.07	0.55	0.50	0.24	-0.37	-0.58	0.14	0.54	0.60	0.02
V57	0.77	0.38	0.58	0.09	0.33	0.41	0.25	-0.55	-0.66	0.03	0.56	0.86	0.16
V58	0.04	0.38	0.44	0.05	0.47	0.32	0.09	0.02	-0.08	0.11	0.22	-0.08	-0.18
V59	0.57	0.59	0.62	0.14	0.73	0.55	0.38	-0.40	-0.65	0.08	0.54	0.55	-0.01
V60	0.77	0.37	0.45	0.17	0.33	0.21	0.11	-0.58	-0.55	-0.13	0.51	0.75	-0.04
V61	0.13	0.53	0.50	0.11	0.73	0.58	0.43	-0.12	-0.37	0.14	0.34	0.10	-0.06
V62	0.65	0.63	0.68	0.19	0.73	0.53	0.35	-0.26	-0.58	0.19	0.54	0.63	0.01
V63	0.77	0.44	0.52	0.13	0.47	0.31	0.21	-0.44	-0.51	-0.05	0.58	0.78	-0.04
V64	0.22	0.60	0.58	0.25	0.67	0.54	0.33	-0.01	-0.30	0.30	0.27	0.13	-0.06
V65	0.43	0.22	0.54	-0.11	0.19	0.26	0.00	-0.07	-0.27	0.23	0.20	0.46	0.12
V66	0.73	0.24	0.51	-0.10	0.25	0.49	0.40	-0.54	-0.68	0.02	0.58	0.88	0.31
V67	-0.11	0.08	0.25	-0.01	-0.01	-0.13	-0.39	0.41	0.36	0.24	-0.29	-0.26	-0.18
V68	0.22	0.22	0.18	-0.05	0.43	0.50	0.61	-0.47	-0.32	-0.35	0.67	0.35	-0.02

	V53	V54	V55	V56	V57	V58	V59	V60	V61	V62	V63	V64	V65
V1	-0.23	-0.03	-0.27	0.48	0.13	0.54	0.37	-0.02	0.46	0.42	0.10	0.50	0.58
V2	0.10	-0.16	0.21	0.09	-0.10	0.32	0.15	0.00	0.25	0.12	-0.03	0.31	-0.01

V3	-0.08	-0.56	0.06	-0.30	-0.42	0.06	-0.20	-0.17	-0.08	-0.19	-0.19	-0.01	-0.26
V4	0.24	0.40	0.23	0.47	0.32	0.38	0.44	0.19	0.44	0.38	0.17	0.45	0.27
V5	-0.09	0.22	-0.09	0.13	0.44	-0.35	0.21	0.46	-0.12	0.21	0.51	-0.24	0.05
V6	-0.09	0.22	-0.09	0.13	0.44	-0.35	0.21	0.46	-0.12	0.21	0.51	-0.24	0.05
V7	-0.20	0.23	-0.22	0.14	0.43	-0.36	0.24	0.41	-0.10	0.25	0.50	-0.24	0.11
V8	-0.19	0.28	-0.23	0.09	0.45	-0.44	0.19	0.43	-0.14	0.19	0.48	-0.28	0.07
V9	0.01	0.16	-0.06	0.12	0.22	-0.18	0.36	0.19	0.20	0.31	0.29	0.02	-0.03
V10	0.03	0.29	-0.04	0.28	0.39	-0.11	0.49	0.31	0.28	0.45	0.43	0.10	0.15
V11	0.00	0.15	-0.06	0.13	0.27	-0.20	0.39	0.25	0.20	0.36	0.39	0.01	-0.01
V12	0.56	0.17	0.61	0.50	0.38	0.37	0.33	0.31	0.26	0.34	0.39	0.21	0.36
V13	0.36	0.11	0.38	-0.01	0.20	-0.22	0.04	0.24	-0.06	-0.02	0.23	-0.21	-0.16
V14	0.22	0.36	0.25	0.54	0.57	0.18	0.38	0.47	0.10	0.41	0.52	0.08	0.48
V15	-0.53	-0.01	-0.51	-0.31	0.06	-0.54	-0.33	0.17	-0.54	-0.29	0.09	-0.48	-0.16
V16	-0.50	0.07	-0.50	-0.16	0.17	-0.44	-0.26	0.20	-0.49	-0.18	0.16	-0.43	0.04
V17	-0.58	-0.02	-0.59	-0.32	0.06	-0.60	-0.31	0.12	-0.53	-0.27	0.07	-0.53	-0.14
V18	-0.03	0.28	0.03	0.05	0.27	-0.23	0.03	0.21	-0.15	0.01	0.13	-0.14	0.04
V19	0.02	0.51	-0.02	0.27	0.51	-0.22	0.31	0.46	0.01	0.34	0.51	-0.01	0.06
V20	0.03	0.30	0.04	0.31	0.28	0.10	0.57	0.30	0.45	0.62	0.43	0.43	0.10
V21	0.53	0.24	0.45	0.30	0.35	0.07	0.14	0.23	0.05	0.12	0.31	-0.11	0.17
V22	-0.18	0.49	-0.18	0.11	0.43	-0.36	0.14	0.46	-0.20	0.25	0.48	-0.13	0.00
V23	-0.28	0.29	-0.45	0.23	0.32	-0.14	0.26	0.16	0.06	0.24	0.25	-0.08	0.29
V24	0.09	-0.22	0.02	-0.20	-0.21	-0.01	-0.31	-0.18	-0.20	-0.46	-0.41	-0.22	-0.18
V25	0.27	-0.12	0.31	-0.19	-0.44	0.21	-0.13	-0.55	0.26	-0.14	-0.48	0.30	-0.30
V26	-0.09	-0.10	-0.04	-0.22	-0.19	-0.15	-0.23	-0.11	-0.30	-0.23	-0.18	-0.26	-0.12
V27	0.03	0.01	-0.02	0.08	0.16	-0.05	0.02	0.24	-0.12	-0.07	0.14	-0.21	0.06
V28	0.05	0.20	0.03	-0.03	0.28	-0.37	-0.04	0.36	-0.31	-0.07	0.31	-0.40	-0.04
V29	0.23	0.21	0.14	0.38	0.33	0.22	0.30	0.28	0.21	0.18	0.24	0.09	0.25
V30	-0.17	-0.30	-0.20	-0.10	-0.12	0.01	-0.16	0.03	-0.20	-0.26	-0.12	-0.22	-0.03
V31	-0.28	-0.04	-0.22	-0.50	-0.22	-0.51	-0.57	-0.26	-0.58	-0.55	-0.33	-0.55	-0.23
V32	-0.26	-0.27	-0.15	-0.72	-0.41	-0.59	-0.78	-0.39	-0.70	-0.71	-0.43	-0.65	-0.41
V33	0.03	0.14	0.00	-0.06	-0.07	-0.05	-0.03	-0.22	0.06	-0.09	-0.24	0.00	-0.05
V34	-0.45	0.13	-0.41	-0.35	0.04	-0.57	-0.51	0.07	-0.75	-0.46	-0.08	-0.65	-0.05
V35	-0.17	-0.15	-0.13	-0.33	-0.28	-0.19	-0.43	-0.26	-0.43	-0.43	-0.35	-0.37	-0.06
V36	-0.25	-0.11	-0.19	-0.45	-0.28	-0.38	-0.55	-0.29	-0.55	-0.53	-0.38	-0.51	-0.16
V37	-0.15	0.21	0.02	-0.07	0.05	-0.13	-0.17	0.02	-0.26	-0.06	-0.01	-0.07	0.08
V38	-0.12	-0.19	0.06	-0.40	-0.26	-0.27	-0.59	-0.27	-0.55	-0.46	-0.24	-0.44	-0.07
V39	0.02	0.30	0.11	0.06	0.03	0.06	0.09	-0.03	0.10	0.13	-0.09	0.28	-0.02
V40	0.08	0.81	0.11	0.59	0.77	0.04	0.57	0.77	0.13	0.65	0.77	0.22	0.43
V41	0.25	0.30	0.31	0.52	0.38	0.38	0.59	0.37	0.53	0.63	0.44	0.60	0.22
V42	0.10	0.47	0.12	0.71	0.58	0.44	0.62	0.45	0.50	0.68	0.52	0.58	0.54
V43	0.11	0.13	0.19	0.07	0.09	0.05	0.14	0.17	0.11	0.19	0.13	0.25	-0.11
V44	0.42	0.19	0.44	0.55	0.33	0.47	0.73	0.33	0.73	0.73	0.47	0.67	0.19
V45	0.41	0.50	0.40	0.50	0.41	0.32	0.55	0.21	0.58	0.53	0.31	0.54	0.26
V46	0.44	0.40	0.44	0.24	0.25	0.09	0.38	0.11	0.43	0.35	0.21	0.33	0.00
V47	-0.39	-0.39	-0.37	-0.37	-0.55	0.02	-0.40	-0.58	-0.12	-0.26	-0.44	-0.01	-0.07
V48	-0.19	-0.69	-0.06	-0.58	-0.66	-0.08	-0.65	-0.55	-0.37	-0.58	-0.51	-0.30	-0.27
V49	-0.41	0.24	-0.47	0.14	0.03	0.11	0.08	-0.13	0.14	0.19	-0.05	0.30	0.23
V50	0.77	0.63	0.75	0.54	0.56	0.22	0.54	0.51	0.34	0.54	0.58	0.27	0.20
V51	0.10	0.99	0.04	0.60	0.86	-0.08	0.55	0.75	0.10	0.63	0.78	0.13	0.46
V52	-0.01	0.36	-0.04	0.02	0.16	-0.18	-0.01	-0.04	-0.06	0.01	-0.04	-0.06	0.12
V53	1.00	0.03	0.97	0.24	0.03	0.35	0.33	0.06	0.43	0.24	0.15	0.24	-0.14
V54	0.03	1.00	-0.04	0.64	0.87	-0.04	0.56	0.73	0.12	0.64	0.76	0.18	0.53

V55	0.97	-0.04	1.00	0.15	-0.04	0.31	0.22	0.01	0.35	0.16	0.09	0.20	-0.20
V56	0.24	0.64	0.15	1.00	0.76	0.64	0.85	0.57	0.65	0.86	0.66	0.65	0.81
V57	0.03	0.87	-0.04	0.76	1.00	0.01	0.66	0.90	0.13	0.68	0.89	0.13	0.63
V58	0.35	-0.04	0.31	0.64	0.01	1.00	0.49	-0.17	0.82	0.48	-0.04	0.86	0.52
V59	0.33	0.56	0.22	0.85	0.66	0.49	1.00	0.61	0.77	0.95	0.70	0.69	0.43
V60	0.06	0.73	0.01	0.57	0.90	-0.17	0.61	1.00	0.01	0.60	0.94	-0.02	0.33
V61	0.43	0.12	0.35	0.65	0.13	0.82	0.77	0.01	1.00	0.71	0.15	0.94	0.29
V62	0.24	0.64	0.16	0.86	0.68	0.48	0.95	0.60	0.71	1.00	0.75	0.70	0.54
V63	0.15	0.76	0.09	0.66	0.89	-0.04	0.70	0.94	0.15	0.75	1.00	0.10	0.43
V64	0.24	0.18	0.20	0.65	0.13	0.86	0.69	-0.02	0.94	0.70	0.10	1.00	0.39
V65	-0.14	0.53	-0.20	0.81	0.63	0.52	0.43	0.33	0.29	0.54	0.43	0.39	1.00
V66	0.07	0.89	-0.01	0.75	0.95	0.06	0.59	0.75	0.16	0.62	0.78	0.14	0.69
V67	-0.28	-0.17	-0.25	0.31	-0.09	0.63	-0.07	-0.28	0.18	0.06	-0.19	0.36	0.67
V68	0.68	0.25	0.66	0.23	0.35	-0.07	0.37	0.36	0.20	0.29	0.44	-0.03	-0.13

	V66	V67	V68
V1	0.13	0.60	-0.20
V2	-0.14	0.17	-0.02
V3	-0.57	0.26	-0.23
V4	0.43	-0.04	0.23
V5	0.40	-0.37	0.64
V6	0.40	-0.37	0.64
V7	0.43	-0.33	0.51
V8	0.46	-0.41	0.52
V9	0.28	-0.39	0.42
V10	0.45	-0.33	0.46
V11	0.28	-0.37	0.47
V12	0.36	0.16	0.62
V13	0.24	-0.43	0.71
V14	0.57	0.07	0.47
V15	-0.02	-0.18	-0.05
V16	0.09	-0.04	-0.07
V17	0.03	-0.25	0.04
V18	0.33	-0.27	0.30
V19	0.48	-0.41	0.54
V20	0.24	-0.14	0.24
V21	0.41	-0.19	0.69
V22	0.34	-0.34	0.26
V23	0.43	-0.14	0.15
V24	-0.21	0.00	-0.31
V25	-0.31	-0.12	0.14
V26	-0.16	-0.01	-0.19
V27	0.15	-0.05	0.21
V28	0.27	-0.30	0.45
V29	0.37	-0.02	0.36
V30	-0.18	0.15	-0.21
V31	-0.04	-0.26	-0.06
V32	-0.29	-0.22	-0.07
V33	0.18	-0.27	0.13
V34	0.10	-0.13	-0.22
V35	-0.18	0.10	-0.35
V36	-0.12	-0.08	-0.23

V37	0.09	0.07	-0.16
V38	-0.17	0.15	-0.10
V39	0.05	-0.06	-0.17
V40	0.73	-0.11	0.22
V41	0.24	0.08	0.22
V42	0.51	0.25	0.18
V43	-0.10	-0.01	-0.05
V44	0.25	-0.01	0.43
V45	0.49	-0.13	0.50
V46	0.40	-0.39	0.61
V47	-0.54	0.41	-0.47
V48	-0.68	0.36	-0.32
V49	0.02	0.24	-0.35
V50	0.58	-0.29	0.67
V51	0.88	-0.26	0.35
V52	0.31	-0.18	-0.02
V53	0.07	-0.28	0.68
V54	0.89	-0.17	0.25
V55	-0.01	-0.25	0.66
V56	0.75	0.31	0.23
V57	0.95	-0.09	0.35
V58	0.06	0.63	-0.07
V59	0.59	-0.07	0.37
V60	0.75	-0.28	0.36
V61	0.16	0.18	0.20
V62	0.62	0.06	0.29
V63	0.78	-0.19	0.44
V64	0.14	0.36	-0.03
V65	0.69	0.67	-0.13
V66	1.00	-0.06	0.38
V67	-0.06	1.00	-0.59
V68	0.38	-0.59	1.00

Appendix D: Correlation matrix for selected field trial traits

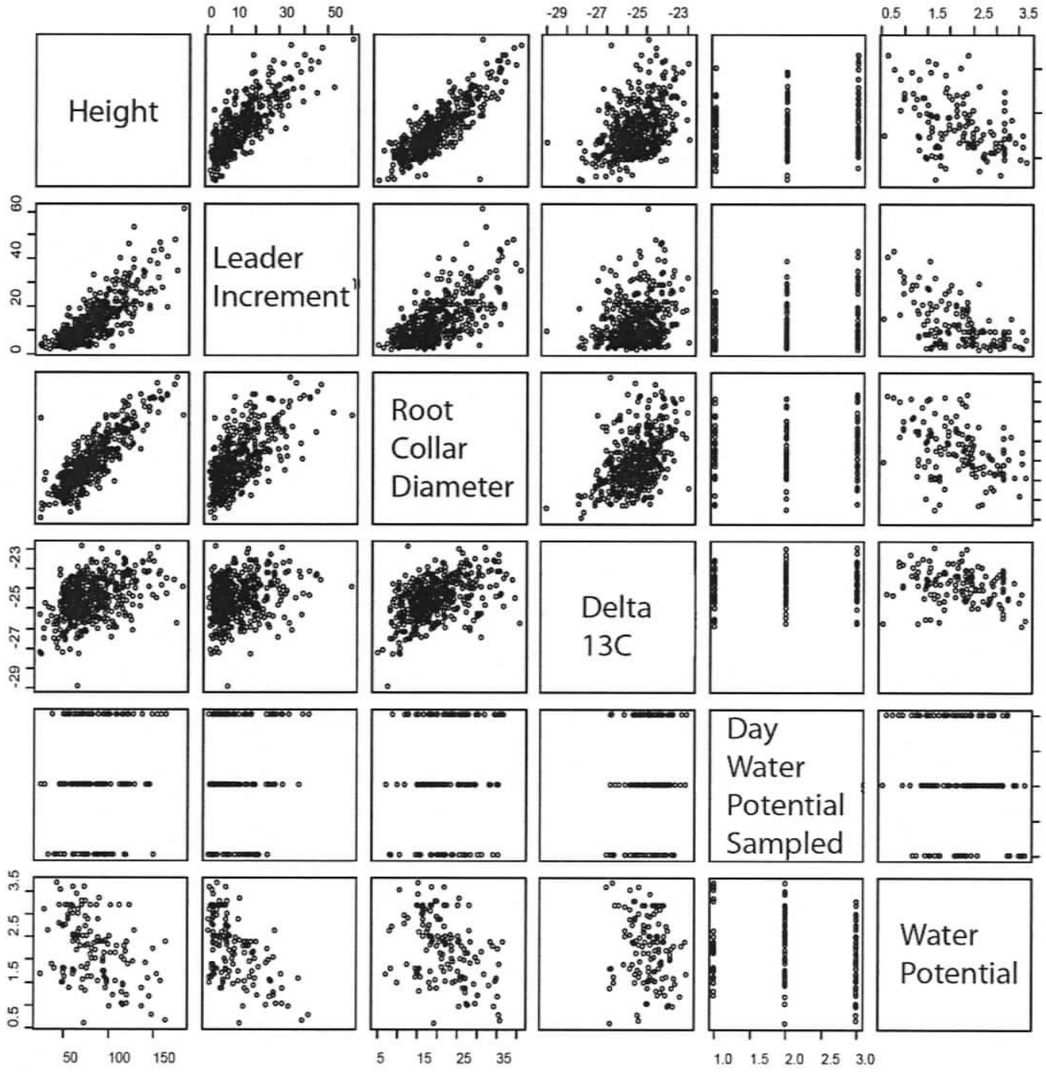

Figure 16. Correlation matrix using individual tree values for growth and hardiness traits in 50 Douglas-fir families growing at all 3 field sites in 2008.