GENETIC ARCHITECTURE, GENECOLOGY AND PHENOTYPIC PLASTICITY IN SEED AND SEEDLING TRAITS OF YELLOW-CEDAR (*Chamaecyparis nootkatensis* (D.Don) Spach)

by

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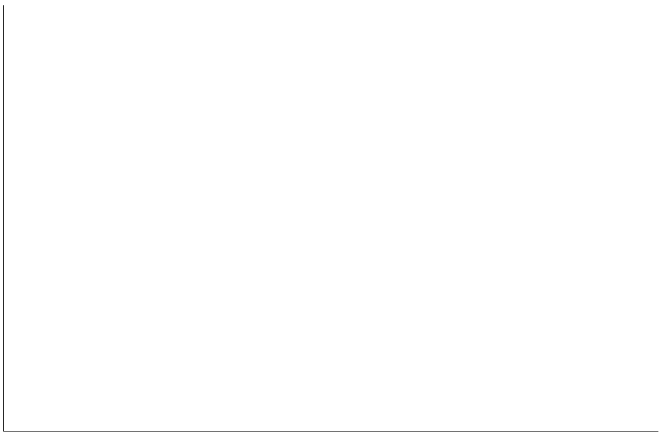

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ABSTRACT

The overall objective of the thesis was to estimate the amount and distribution of genetic and environmental variation, and correlations between genetic variability and seed source origin, of yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach). Variation was measured for traits of seed, and for morphological and physiological traits of seedlings grown in a common garden, and in differing greenhouse environments. The study focused on traits that sampled the developmental sequence of events that influence a population's adaptation to its environment. These included growth rate, phenology, drought resistance, cold acclimation, and dormancy.

Significant variability was evident at both the population and family within population level in most traits measured. Substantially more genetic variability (2 to 16 times) was found at the family within population level as opposed to the population level in all but two traits. Narrow-sense heritabilities varied from 0.16 for growth during third-year shoot initiation to 0.64 for first-year height in the nurserybed.

There was little evidence of adaptive variation for seed and germination traits, however, growth traits and cold-hardiness were moderately to strongly correlated with latitude and elevation of seed origin. Seedlings from more southerly and high elevation populations were taller, had greater diameter, grew later into the growing season, and were more susceptible to cold injury during acclimation and at maximum hardiness, than more northern populations. The above trends were not apparent if southern populations (Oregon) were excluded.

Environments had a large effect on growth and morphology of yellow-cedar. Shoot elongation was extremely plastic, responding to both decreased

photoperiod and water-stress through decreased shoot growth. Upon release of the stress treatments, growth increased to relative rates greater than the non-stressed trees. In all growth and morphology traits, there was minimal evidence for significant genotype by environment interactions at either the population or family within population level, with both photoperiod treatments and water regimes.

Genetic variation in gas exchange, water relation parameters, and morphological traits, in response to a drought, was evident with 2-year-old yellow-cedar seedlings among and within populations. Seedlings from Coquihalla, a xeric habitat, had less shoot and lateral branch extension, and less biomass allocated to branches and more to roots, as compared to mesic sources, under both well-watered and drought conditions. As well, these seedlings maintained greater rates of net photosynthesis and higher levels of stomatal conductance under both well-watered and droughty conditions.

Yellow-cedar populations at the extremes of environment for the species, i.e. southern and continental populations, have responded to environmental selection pressures by changes in gene frequencies. The changes most likely have been aided by reduced gene flow due to spatial isolation and poor sexual reproduction (Russell *et al.* 1990). At the same time, however, the species has maintained a substantial amount of both genetic variation and phenotypic plasticity within populations. Yellow-cedar seems to have evolved an intermediate mode of adaptation with less genetic differentiation associated with geography than Douglas-fir, Sitka spruce, and western hemlock, but more genetic differentiation than western white pine and western redcedar.

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CHAPTER 1: INTRODUCTION

1.1 OBJECTIVES

The overall objective of this study was to investigate the amount and distribution of genetic and environmental variation of yellow-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach) seed, and seedling morphological and physiological traits, and to elucidate any adaptive variation. The thesis involves two main studies, a common-garden genetics study and a greenhouse environment by genetics study.

The common-garden study extended over a 3-year period, and consisted of 33 populations and 171 open-pollinated families which sampled the botanical range of yellow-cedar. The first year involved measuring seed and germination traits prior to sowing, growth and phenology during the first growing season in a greenhouse, and cold-hardiness during the acclimation period following the growing season. The second and third year involved measuring growth, phenology and cold-hardiness of seedlings planted in a nurserybed. The objective of the common-garden study was to investigate the extent and pattern of genetic variability in seed and seedling traits among and within populations of yellow-cedar, and to correlate traits to seed source origin. The study focused on traits that sampled the developmental sequence of events that influence the adaptation of a population to the environment including growth rate, phenology, acclimation, and dormancy.

The second study (Chapter 3) was comprised of 1-year-old seedlings from 18 populations and 27 open-pollinated families grown in four different greenhouse environments represented as two photoperiods and two soil moisture regimes. The study involved measuring growth, phenology, biomass

accumulation, gas exchange, water relations and cold-hardiness over the second growing season. The objectives of the second study were to investigate the effects of different environments on morphological expression and physiological processes, determine the extent of genotype by environment interactions for morphological and physiological traits, and elucidate the presence of adaptive genetic variation in morphological expression and physiological processes in response to changing photoperiods, and moisture stress.

1.2 LITERATURE REVIEW

a) botanical range and ecology of yellow-cedar

The botanical distribution of yellow-cedar extends over 20° latitude from northern California to southeast Alaska. Within this latitudinal range, yellow-cedar occupies a unique geographic distribution. It occurs strictly at high elevations (over 1200 m) in both the Siskiyou Mountains in northern California and southwest Oregon, and the west side of the Cascade Mountains in Washington and Oregon. It occurs at high elevations in both the Olympic Mountains in Washington and in the Coastal Mountains in southern British Columbia. From approximately 51° N. latitude and northward, yellow-cedar occurs from sea level to timberline, however, it is restricted to a narrow longitudinal band along the coast. A number of isolated stands occur over 200 km inland from the most easterly coastal populations, one in central Oregon and at least two in southern British Columbia.

Throughout most of the range of yellow-cedar, the climate is very humid with relatively cool summers and mild winters (Krajina 1969). Winter temperatures rarely go below -20° C in both high elevation sites in the Oregon Cascades and in low elevation coastal Alaska sites.

Yellow-cedar has a wide ecological amplitude throughout its distribution. In the southern part of its range (south of Mt. Rainier, Washington), yellow-cedar is found on wet to dry sites, and occurs in generally open-habitats from bogs to rocky ridges (Antos and Zobel 1986). In British Columbia, yellow-cedar occurs on moderately dry to wet soils, and on nutrient very-poor soils to very-rich soils (Klinka 1991). The most productive sites in British Columbia occur on very-moist and nitrogen very-rich soils in montane, very-wet maritime climates where yellow-cedar competes with amabilis fir (*Abies amabilis* (Dougl.) Forbes) (Krajina 1969).

Yellow-cedar does not occur on many sites that it seems capable of occupying. On disturbed sites, amabalis fir comes in quicker and is more shade tolerant, thus replacing yellow-cedar in a closed canopy forest (Antos and Zobel 1986). On deep, well drained soils, other species such as redcedar (*Thuja plicata* Donn), western hemlock (*Tsuga heterophylla* (Raf.) Sarg), and Sitka spruce (*Picea sitchensis* (Bong.) Carr) outgrow yellow-cedar. The main limiting factor to the distribution of yellow-cedar seems to be its inability to compete because of slow initial growth, and not because of limited ecological amplitude (Antos and Zobel 1986).

b) phenotypic plasticity, specialization, and genetic architecture of Pacific Northwest conifers

Different conifer species in the Pacific Northwest region of North America have been shown to follow alternative strategies, specialization or phenotypic plasticity, in adapting to heterogeneous environments (Rehfeldt 1984). Specialization is a consequence of changes in gene frequencies of a population in response to environmental differences. The genotype is

expressed phenotypically and selection acts directly on the genotype (Rehfeldt 1984).

Phenotypic plasticity is the degree to which phenotypic expression of a genotype varies under different environmental conditions (Bradshaw 1965, Sultan 1987). It can be measured by the amount to which the expressions of individual characteristics of a genotype are changed by different environments (Bradshaw 1965). Phenotypic plasticity has been shown to exist for a wide variety of species, to be trait and environmental specific, to be under genetic control (for both direction and amount), and to be of adaptive value (Bradshaw 1965, Scheiner and Goodnight 1984, Sultan 1987, Macdonald and Chinnappa 1989).

Having both ample genetic variability and phenotypic plasticity within a population had historically been thought of as unlikely (Bradshaw 1965), since a population with a well developed plastic response has no need for genetic variation (Schlichting 1986). Thus, it was expected that an inverse relationship existed between plasticity and heterozygosity. Although negative correlations have been reported (Jain 1979, Silander 1985), the overwhelming evidence points to no relationship between plasticity and heterozygosity (Scheiner and Goodnight 1984, Bagchi and Iyama 1983, Schlichting and Levin 1984, Schlichting and Levin 1986, Macdonald and Chinnappa 1989).

There is substantial evidence that ecotypes have evolved through changes in gene frequencies brought about by selection. However, the ability of selection to shape ecotypes is constrained by: 1) the relationship of genotype to phenotype; 2) relative scales of the environment and the species; and, 3) migration versus selection pressure (Sultan 1987). Lewontin (1957)

states that selection will favour individual plasticity unless a set of narrowly adapted genotypes can survive a greater range of environments than any single genotype. Phenotypic flexibility of plants, along with the nature of their environment, can substantially buffer the effects of natural selection (Sultan 1987).

Possible reasons for plasticity not evolving in a character are: 1) canalization of highly conserved traits such as floral structures; 2) environmental change is too sudden and plants can only survive by already being adjusted to the sudden changes (such as frost or drought) through permanent genetic change; 3) the plastic response is not reversible when needed to be (i.e. shallow roots in flood, followed by a drought); 4) limits to plasticity may be imposed by the genome which is in turn limited by the organism's chemical system, and; 5) relationships among plasticities of various fitness traits (i.e. genetic correlations) (Bradshaw 1965, Sultan 1987, Schlichting 1989, Stearns 1989).

The above limits to plasticity possibly explain why plants do not evolve to a single genotype with an infinite array of plastic responses (Bradshaw 1965). Bradshaw states that "although plasticity plays an important role in adaptation, permanent adaptation by genetic change is more common".

Adaptation through specialization is displayed by coastal Douglas-fir (*Pseudostuga menziesii* (Mirb.) Franco var. *menziesii* (Griffin 1977, Campbell 1986, Loopstra and Adams 1989, Ying 1990), Sitka spruce (Roche 1969, Falkenhagen 1977, Lines 1987, Ying 1990), and western hemlock (Kuser and Ching 1980). These studies have focused on traits that describe the developmental sequence of events that influence a population's adaptation to

its environment (e.g. growth rhythms, growth rate, acclimation, and dormancy). In general, trees from seed collected in relatively mild and/or wet climates, grow faster, cease growth later, and are more cold susceptible than populations from colder and/or drier climates. Species showing weak or no correlations of fitness traits with macrogeography, a generalist strategy, include western white pine (*Pinus monticola* Dougl.) (Rehfeldt 1984), and western redcedar (Rehfeldt pers. comm.).

If a species tends towards being a generalist, it should, according to theory, exhibit more genetic variability within populations than among (Rehfeldt 1984, Sultan 1987). Depending on the geographic range of the species being studied, and the extent of sampling, range-wide provenance testing with family structure in conifers has usually shown that the variation among populations is substantially greater than the variation among families within populations (Namkoong *et al.* 1972, Namkoong and Conkle 1976, Fashler *et al.* 1985). This has especially been the case where a species has shown a high degree of specialization in response to heterogeneous environments (Campbell 1979, White *et al.* 1981, Loopstra and Adams 1989). However, in white pine, which has exhibited a generalist mode of adaptation (Rehfeldt 1984), genetic variation among families within populations was substantially larger than among populations (Rehfelt 1979a).

c) photoperiodic effects on seedling morphology and cold-hardiness

It has long been known that woody tree species of the northern temperate zone have a marked response to photoperiod with respect to shoot growth (Kramer 1936, Wareing 1956, Lavender 1980). Exposure to short days results in reduced shoot height extension either due to early budset or

reduced internode extension in bud-formed (determinate) species (e.g. Wareing 1956, Giertych and Farrar 1961, Heide 1974, Perry and Lotan 1978, Colombo *et al.* 1982, Arnott *et al.* 1988) and reduced shoot growth in non-bud forming (indeterminate) species (Grossnickle *et al.* 1988, Krasowski and Owens 1991, Arnott *et al.* 1992, Major *et al.* 1993). Exposure to long days results in the opposite effect (Wareing 1956).

Photoperiodic effects on dry weight allocation to roots and shoots of conifer seedlings are less clear. For determinate species, exposure of seedlings to artificial short days has been shown to decrease shoot dry weight and to have no significant impact on root dry weight (Heide 1974, Perry and Lotan 1978, Hawkins and Draper 1988), to decrease both shoot and root dry weight (Giertych and Farrar 1961), or to have no significant impact on either shoot or root dry weight allocation (Heide 1974, Burdett and Yamamoto 1986, Arnott *et al.* 1988). Those studies that reported significant decreases in shoot dry weight with short-day treatment also reported terminal bud set in response to the shorter photoperiod. For indeterminate species, studies have shown no significant impact on dry matter allocation (Grossnickle *et al.* 1988, Krasowski and Owens 1991, Arnott *et al.* 1992, Major *et al.* 1993).

Conflicting reports in the literature on the effects of photoperiod on dry weight allocation may be due to the timing and duration of treatment, the length of the photoperiods, and allometric relationships. Ledig and Perry (1965) demonstrated that changes in dry weight allocation in response to environmental stresses were confounded with correlations of individual dry weight components with total dry weight. If these correlations were removed through the use of allometric relationships, then only extreme environments resulted in changes in allocation of dry weight (Ledig and Perry 1965, Ledig

et al. 1970).

Temperate woody plants in nature respond to shortening photoperiods and decreasing temperatures during the later part of the growing season by decreasing shoot growth and setting buds. Thus, growth cessation is a prerequisite to cold-acclimation in woody plants (Levitt 1980a), and this is considered to be the first stage of cold-acclimation (Weiser 1970, Levitt 1980a). For determinate species, artificial short days applied during the growing season of seedlings in the nursery result in decreased shoot growth, early onset of dormancy, and increased cold-acclimation (e.g. van den Driessche 1970, Aronsson 1975, Christersson 1978, McCreary *et al.* 1978, D'Aoust and Cameron 1982, Colombo *et al.* 1989, Grossnickle *et al.* 1991, Bigras and D'Aoust 1992). A similar response has been reported for indeterminate species (Colombo and Raitenan 1991, Arnott *et al.* 1992, Folk *et al* 1993, Major *et al.* 1993).

d) genotype x photoperiod interaction effects on seedling morphology and cold-hardiness

The significance of population by photoperiod interactions for shoot growth and shoot rhythms with determinate conifer species is well documented in the literature, and many of these interactions have been attributed to adaptive responses to environmental selection pressures (Pauley and Perry 1954, Perry *et al.* 1965, Vaartaja 1959, Irgens-Moller 1957, Neinstadt and Olson 1961, Irgens-Moller 1962, Heide 1974, Pollard *et al.* 1975, Pollard and Ying 1979). Northern temperate tree populations from southern portions of a species range or from low elevations, when grown under short photoperiods, grow longer into the season, whereas northern or high elevation populations set bud earlier resulting in decreased shoot extension. Thus, populations of

species with extended latitudinal or elevational ranges have responded to environmental pressures (i.e. frost events) such that changes in gene frequencies have resulted in differential responses to photoperiod with respect to shoot growth extension and phenology.

There have been few studies for indeterminate species, although the lack of a genetic influence on photoperiodic response has been reported for redcedar (Vaartaja 1959) and for eastern white-cedar (*Thuja occidentalis* L.) (Vaartaja 1962).

Family by photoperiod interactions are not as well documented. In a study by Perry and Lotan (1978), 50 open-pollinated families from five populations of lodgepole pine (*Pinus contorta* var. *latifolia* Dougl.) were grown under four photoperiods during the first growing season. Significant family within population and photoperiod variation was evident in shoot growth, total dry weight, root and shoot dry weight, and shoot:root dry weight ratio. Family within population by photoperiod interaction was significant for all traits except root dry weight. The ratio of family within population variance to family within population by photoperiod variance ranged from 0.5 to 2.0.

In a study with 66 open-pollinated families from 11 populations of white spruce (*Picea glauca* (Moench) Voss) (Pollard and Ying 1979) sampled from a narrow range of latitude, significant family within population variation in response to declining photoperiod was found for shoot extension, whereas population effects were minimal.

e) moisture stress effects on seedling morphology and cold-hardiness

Moisture stress applied to seedlings during shoot growth has been reported to decrease shoot extension in determinate species (e.g. Lavender

et al. 1968, Cheung 1973, Nelson and Lavender 1978, Macey and Arnott 1986, Bongarten and Teskey 1987, Arnott *et al.* 1988, Joly *et al.* 1989), and indeterminate species (Harry 1987, Krasowski and Owens 1991, Arnott *et al.* 1992, Major *et al.* 1993) and to result in early budset for determinate species (Lavender *et al.* 1968, Cheung 1973, Young and Hanover 1978, Vance and Running 1985, Macey and Arnott 1986). Cell enlargement, thus shoot extension, is highly sensitive to water stress through the effects of drought on cell turgor (Hsiao 1973).

In general, studies of moisture stress effects on allocation of dry weight reported decreases in both shoot and root dry weight, with a larger decrease in shoot dry weight resulting in a decrease in shoot:root dry weight ratio (Perry *et al.* 1978, Blake *et al.* 1979, Seiler and Johnson 1988, Joly *et al.* 1989). With indeterminate species, studies have reported that moisture stress significantly decreased shoot dry weight, but had no significant impact on root dry weight (Krasowski and Owens 1991, Arnott *et al.* 1992, Major *et al.* 1993). The interpretation of these studies on dry weight allocation may be tenuous because of the correlation of shoot and branch dry weights with total dry weight (Ledig and Perry 1965, Bongarten and Teskey 1987).

If moisture stress is not too severe, or it is applied in conjunction with short days during the growing season of seedlings, it can increase cold-hardiness during the acclimation stage, for both determinate and indeterminate species (Timmis and Tanaka 1976, Blake *et al.* 1979, Lavender 1980, Major *et al.* 1993). This is attributed to an indirect effect of moisture stress resulting in decreased shoot extension and early budset (Glerum 1985). If moisture stress is too severe, cold-acclimation may be

affected through the disruption of physiological processes correlated with cold-hardiness (Blake *et al.* 1979, Glerum 1985).

f) genotype x moisture interaction effects on seedling morphology and cold-hardiness

In nursery studies, significant population by moisture interactions for growth and dry weight allocation have been reported for loblolly pine (*Pinus taeda* L.) (van Buijtenen 1966, Bongarten and Teskey 1987). However, most studies indicate minimal population by moisture interaction for both shoot extension and dry weight allocation for determinate species (Ledig *et al.* 1970, Perry *et al.* 1978, Seiler and Johnson 1988, Joly *et al.* 1989), and for shoot extension with indeterminate species (Harry 1987).

Significant family by moisture or family within population by moisture interactions in nursery studies were found for shoot growth extension and dry weight allocation in loblolly pine (Cannell *et al.* 1978, Waxler and van Buijtenen 1981), for root dry weight in lodgepole pine (Perry *et al.* 1978), and for budset and root dry weight in Douglas-fir (Joly *et al.* 1989). Except for the study by Cannell *et al.* (1978), all interactions were scale effects as opposed to rank changes.

No significant family by moisture or family within population by moisture interactions were reported for shoot extension, root collar diameter, and dry weight in Douglas-fir (Joly *et al.* 1989), for dry weight allocation in loblolly pine (Seiler and Johnson 1988), and for shoot weight in lodgepole pine (Perry *et al.* 1978). In the only study reported involving an indeterminate species, Harry (1987), found no population by moisture or family within population by moisture interactions for relative growth rate

measured periodically during the growing season of incense-cedar (*Libocedrus decurrens* Torr).

g) morphological adaptations to drought

There are numerous morphological adaptations to drought including decreased shoot growth, changes in phenology, allocation of relatively more carbohydrates to roots than shoots, thicker needles and leaves, and decreased number and length of branches (e.g. Ledig *et al.* 1970, Young and Hanover 1978, Harry 1987, Bongarten and Teskey 1987, Abrams *et al.* 1990, Joly *et al.* 1989, Kubiske and Abrams 1992).

Morphological adaptations are primarily drought avoidance mechanisms which can result in the maintenance of high internal water potential despite low soil water potential and high evaporative demands (Levitt 1980b). Decreased shoot and branch growth restricts transpirational surface area and more allocation of biomass to roots relative to shoots can increase absorption efficiency (Levitt 1980b, Joly *et al.* 1989).

Evidence of drought ecotypes for woody tree species, with respect to morphological adaptations, has been reported for many species including Scots pine (*Pinus sylvestris*) (Brown 1969), Douglas-fir (Ferrell and Woodward 1966, White 1987, Joly *et al.* 1989), loblolly pine (van Buijtenen 1966, Bongarten and Teskey 1987), lodgepole pine (Dykstra 1974), red maple (*Acer rubra* L.) (Townsend and Roberts 1973), red oak (*Quercus rubra* L.) (Kubiske and Abrams 1992), and green ash (*Fraxinus pennsylvanica* Marsh.) (Abrams *et al.* 1990).

h) physiological processes influencing drought resistance

Genetic differences in physiological processes, such as gas exchange and water relations, among and within populations may be important with respect to fitness. As stated by Kramer (1986): "...physiological processes are the machinery through which genetic potential and environment operate to determine the quantity and quality of growth". The adaptation of plants to water stress through the development of drought avoidance and tolerance mechanisms are important for survival and growth, since physiological processes are inhibited more often by water stress than by any other single factor (Kramer 1986).

Pacific Northwest conifers, including yellow-cedar, experience periods of drought when water deficits occur because of low soil water potential and high atmospheric demands during the growing season. The ability to tolerate or avoid injurious desiccation due to high atmospheric evaporative demand determines the potential for survival and growth during periods of drought (Hsiao *et al.* 1976).

There are many physiological and morphological factors that can result in better adaptation to drought including both avoidance and tolerance mechanisms. Drought avoidance is usually a result of drought-induced alterations in anatomical and morphological structures that decrease transpiration through increased resistance to water loss (Hsiao 1973, Levitt 1980b, Kramer 1983) (see Section 1.2 g)).

Drought tolerance, on the other hand, involves changes in physiological processes that result in the ability to function under decreased relative water content and lower water potential (Levitt 1980b, Blum 1988). Physiological properties that confer drought tolerance, including both

dehydration avoidance and dehydration tolerance, are osmotic adjustment, changes in cell wall physical properties, water use efficiency, and respiration rate.

The evolution of drought resistant ecotypes in forest tree species depends upon many factors including the presence of heritable genetic variation in physiological processes and morphological structures that infer drought resistance, and the presence of environmental selection pressures. There have been numerous genetic studies on gas exchange processes of forest trees at the population, family and clonal level. In most of the studies, genetic variability has been detected, some attributed to adaptive responses in gas exchange traits including total and net photosynthesis, stomatal conductance, and transpiration (e.g. Bourdeau 1963, Campbell and Rediske 1966, Ledig and Perry 1967, Fryer and Ledig 1972, Pelkonen and Luukkanen 1974, Whitehead *et al.* 1983, Mebrahtu and Hanover 1991). In all of these studies, gas exchange measurements were taken at one or a few times during a normal growing season, usually on seedlings in a nursery. There have been limited studies on the genetics of gas exchange processes and water relation parameters with respect to extended water stress during the active growing season. Drought may exert a stronger or different selection pressure than in a mesic environment. Given the presence of heritable genetic variation in the fundamental physiological processes that confer drought avoidance and tolerance, these selection pressures can result in populations differing in drought resistance capability and mechanisms.

Early studies on Douglas-fir seedlings from wet and dry habitats showed that xeric ecotypes had a greater reduction in transpiration rate, and stomata were more sensitive, during drought (Ferrell and Woodward 1966,

Zavitkovski and Ferrell 1968, Unterscheutz *et al*. 1974). Under well-watered conditions, there were no differences in transpiration rates. Similar results were reported for Scots pine under drought (Hellkvist 1970).

In loblolly pine, seedlings from xeric habitats exhibited increased transpiration rates and stomatal conductance under well-watered conditions, compared to mesic habitats (Bongarten and Teskey 1986). There was no discernable pattern among seed sources in response to drought. In another study with loblolly pine, an open-pollinated family from a xeric habitat had a lower transpiration rate under well-watered conditions, and less decline in transpiration rate during a drought, compared to seedlings from two mesic habitats (Seiler and Johnson 1988). There was no difference in net photosynthesis among the three seed sources under well-watered conditions or during the drought.

Clones of eastern cottonwood (*Populus deltoides* Bartr.) from dry sites had greater growth under both moist and dry soil conditions, and decreased stomatal sensitivity compared to clones from wet sites (Kelliher and Tauer 1980, McGee *et al* 1981). In a study with red maple, transpiration was greatest with seedlings from a very wet site under both well-watered and drought conditions as compared to seedlings from a wet site and two dry sites (Townsend and Roberts 1973). As well, growth was greater for the red maple seedlings from the very wet and wet sites compared to seedlings from the dry sites, at all water stress levels. Seedlings of green ash from a xeric habitat exhibited greater net photosynthesis and stomatal conductance throughout a drought compared to seedlings from a mesic habitat (Abrams *et al*. 1990).

For species that are drought avoiders, water savers (Levitt 1980b), stomata may close earlier in response to drought (i.e. more sensitive to water stress) with seedlings from xeric sites compared to mesic sites, thus decreasing growth, but reducing mortality. For species that are drought avoiders, water-spenders (Levitt 1980b), stomata remain open at lower levels of drought, thus allowing photosynthesis, and improving water use efficiency. From the above discussion, Douglas-fir and red maple from xeric habitats had greater stomatal sensitivity under increasing water stress, closing their stomata earlier and decreasing growth, compared to seedlings from mesic sites. Eastern cottonwood and green ash from xeric sites, on the other hand, had decreased stomatal sensitivity with increasing drought, with stomata remaining open at lower water potentials. In green ash, reduced stomatal sensitivity reflected the greater photosynthetic rate of xeric seedlings under stress, and in eastern cottonwood, resulted in greater growth than plants from mesic sites. Loblolly pine studies reported conflicting results with respect to stomatal sensitivity.

In studies comparing different species known to vary in drought tolerance, water use efficiency has been both higher or lower in more drought tolerant species or has shown no relationship to levels of drought tolerance (DeLucia and Heckathorn 1989, Ni and Pallardy 1991). In population studies, seedlings from more xeric sites have shown no difference in water use efficiency compared to seedlings from more mesic sites (Seiler and Johnson 1988, Abrams *et al.* 1990). However, in both of these studies, water use efficiency was averaged over the entire drought period.

A high water use efficiency may be advantageous if there is conserved soil moisture available in the future (DeLucia and Heckathorn 1989). On the

other hand, if the conserved water is readily lost by evaporation or transpiration by competing vegetation, a high water use efficiency may be disadvantageous.

Studies on gas exchange among families in response to drought are rare. Seedlings from eight full-sib families, of which only five had unrelated parents, from a drought resistant population of loblolly pine, showed no significant differences in stomatal conductance during a drought (Raley and Tauer 1986). In a study with radiata pine (*Pinus radiata* D.Don), three open-pollinated families differing in their response to weed competition, exhibited significant differences in gas exchange and water use efficiency in response to a drought (Sands *et al.* 1984). As well, the interaction of family by shoot water potential was statistically significant with one family having the greatest photosynthetic rate under well-watered conditions and the poorest when under high water stress.

Genetic studies on shoot water relation parameters of forest trees are not numerous, and most of the studies involve an extended drought on either bulk populations or one family per population. In the study cited earlier on green ash, seedlings from the most mesic source did not osmotically adjust after an extended drought, whereas all other populations did (Abrams *et al.* 1990). Tissue elasticity increased after the drought in seedlings from the most mesic site, and significantly decreased in seedlings from one of the drier sites. Seed source differences in tissue water relation parameters were only evident after the drought and not under well-watered conditions.

Significant differences in osmotic adjustment, tissue elasticity and relative water content during drought were evident among eight open-pollinated families of black walnut (*Juglans nigra* L.) from eight populations

selected along a longitudinal transect (Parker and Pallardy 1985). However, there was no correlation of drought resistance traits with moisture availability at seed source location. The two most xeric populations differed in their ability to osmotically adjust, with one population osmotically adjusting the most of all populations, and the other xeric population the least.

Choi (1992) investigated the genetic differences in water relation parameters among six open-pollinated families of shortleaf pine (*Pinus echinata* Mill) from at least five different geographic areas. Significant family differences were found for osmotic potential, symplastic water, and modulus of elasticity after a drydown, as well as for osmotic adjustment and change in elasticity between well-watered control seedlings and droughted seedlings. There was no attempt to correlate the genetic variation with site variation in moisture availability.

Studies by Bongarten and Teskey (1986) with six populations of loblolly pine and Joly and Zaerr (1987) with three populations of Douglas-fir showed no ecotypic variation in drought resistance with respect to water relation parameters.

Osmotic adjustment can maintain positive turgor at low water potentials allowing for stomata to remain open longer and thus assimilate carbon dioxide (Hsiao *et al.* 1976, Turner and Begg 1981). However, accumulation of solutes may have an adverse affect on photosynthetic capacity possibly due to inhibition of enzymatic activity (Turner and Begg 1981). As well, cell wall elasticity also affects the ability of a cell to maintain positive turgor under internal plant water stress. Elastic cell walls will lose less turgor pressure with a drop in relative water content, while inelastic cell walls

have a rapid loss of turgor pressure with a drop in relative water content before turgor loss point (Abrams 1988).

In the water relation studies cited above, population differences in water relation parameters were evident. However, only the study with green ash (Abrams *et al.* 1990) showed any evidence of adaptive value of variation in water relation parameters. Differences among families were shown in two studies (Parker and Pallardy 1985, Choi 1992), but these differences were confounded with seed source variation.

CHAPTER 2: COMMON-GARDEN STUDIES

2.1 OBJECTIVES

This chapter describes two common-garden genetic studies: first-year greenhouse trial and the nurserybed trial. The greenhouse trial involved measuring seed and germination traits prior to sowing, growth and phenology measurements during the first growing season, and cold-hardiness during acclimation, of seedlings originating from different populations and open-pollinated families within populations. The nurserybed trial involved measuring growth, phenology and cold-hardiness over two growing seasons of seedlings originating from different populations and families within populations. The two trials are considered separately with respect to statistical analyses because of differences in genetic sampling and experimental design.

The objectives of both trials were to investigate the extent and pattern of genetic variability in seed and seedling traits among and within populations of yellow-cedar that sampled the botanical range, and to correlate seed and seedling traits to seed source origin.

Cold-hardiness was measured after each of the three growing seasons, each time with different objectives. The first year involved investigating patterns of cold-hardiness acclimation on a small subset of populations. The objective of the second-year trials was to partition genetic variability among populations and among families within populations for cold-hardiness during acclimation and at maximum hardiness. Finally, the third year's objective was to investigate adaptive patterns among populations in cold-hardiness during acclimation and at maximum hardiness and correlations with growth and phenological traits.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Design

a) population sampling and cone collection

Yellow-cedar occurs in small, disjunct populations throughout most of its range. Cone crops are infrequent, the percentage of viable seed is low, and seed germination is poor. These factors mostly dictated the population sampling pattern. Since one of the objectives of the study was to describe the genecology of the species, it would be desirable to systematically sample the latitudinal, longitudinal and elevational range of the species, however, this was not feasible. In the southern part of its range, yellow-cedar occurs along a narrow, discontinuous band only at high elevations in the western Cascade Mountains. Populations in this area were sampled along a latitudinal gradient.

In southern British Columbia, where the species range is more extensive, populations were collected, where possible, to sample the range of biogeoclimatic subzones in which yellow-cedar occurs (Krajina *et al.* 1982). However, it was not possible to always sample along an elevational or longitudinal transect at the macrogeographic level because of cone availability or physical access.

From central British Columbia to Alaska, many of the areas are isolated, thus populations were collected where possible, mostly along a latitudinal gradient. Two populations were obtained from Alaska, however, only one had viable seed. Thus, the overall collection strategy stressed sampling the latitudinal range of yellow-cedar.

A total of 33 populations was used in this study (Table 1). At 26 of the locations, cones were collected from, and kept separate by individual

Table 1: Geographic location of yellow-cedar populations

Population[1]	N. Latitude	W. Longitude	Elevation (m)
Mitkof Island, AK	56° 49′	132° 57′	300
Kitimat Valley, B.C.	54° 07′	128° 43′	450
Porcher Island, B.C.	54° 00′	130° 20′	430
Queen Charlotte Islands, B.C.	53° 33′	132° 25′	400
King Island, B.C.	52° 22′	127° 22′	400
Clayton Falls, B.C.	52° 17′	126° 52′	732
Kwatna Inlet, B.C.	52° 08′	127° 23′	488
Holberg, V.I.	50° 42′	127° 40′	300
Coal Harbour, V.I.	50° 39′	127° 47′	500
Pemberton, B.C.	50° 32′	123° 29′	900
Pt. McNeill, V.I.	50° 32′	127° 15′	250
Beaver Cove, V.I.	50° 31′	126° 48′	731
Waukwass Creek, V.I.	50° 29′	127° 18′	750
Twin Peaks, V.I.	50° 29′	127° 15′	900
Kelsey Bay, V.I.	50° 22′	126° 03′	800
Bunster Hills, B.C.	50° 01′	124° 36′	800
Bullet Lake, V.I.	49° 56′	126° 21′	579
Squamish, B.C.	49° 47′	122° 55′	900
Mt. Washington, V.I.	49° 45′	125° 20′	1200

1. B.C. = British Columbia, Canada; V.I. = Vancouver Island, B.C.; WA = Washington, U.S.A.; OR = Oregon, U.S.A.; AK = Alaska, U.S.A.

Table 1: (con't)

Population[1]	N. Latitude	W. Longitude	Elevation (m)
Coquihalla, B.C.	49° 37′	121° 02′	1350
Talc Creek, B.C.	49° 30′	121° 40′	800
Statlu Creek, B.C.	49° 21′	122° 06′	975
Yellow Creek, B.C.	49° 12′	124° 41′	1000
Sparton Lake, V.I.	48° 56′	124° 12′	823
Valentine Mt., V.I.	48° 33′	123° 51′	760
Mt. Angeles, WA	47° 59′	123° 28′	1750
Johnson Creek, WA	47° 48′	121° 15′	1200
Huckleberry Ridge, WA	47° 03′	121° 37′	1350
Mt. Rainier, WA	46° 47′	121° 45′	1350
White Pass, WA	46° 38′	121° 25′	1350
Humbug Creek, OR	44° 48′	122° 09′	1200
County Creek, OR	44° 16′	122° 06′	1200
Jackass Mt., OR	43° 03′	122° 27′	1600

1. B.C. = British Columbia, Canada; V.I. = Vancouver Island, B.C.; WA = Washington, U.S.A.; OR = Oregon, U.S.A.; AK = Alaska, U.S.A.

trees. Cones were collected from 10 individual trees at each of 18 populations, and from three to seven trees at each of the eight remaining populations, for a total of 223 trees. A minimal distance of 250 meters between trees was imposed to limit relatedness. Up to one litre of cones was collected from the upper crown of most trees. Limited cone crops required that some cones had to be collected from lower in the crown. The limited availability of trees with cones, as well as the small number of trees in each stand, dictated the sampling strategy. Tree form ranged from large, single stem, straight trees to low-lying shrubs.

In no case were all the sampled trees from one population more than 200 metres apart in elevation.

b) seed handling, germination, and first-year greenhouse trial design

Cones were air-dried and tumbled to extract seed. The seed were stratified by soaking in water in plastic bags at room temperature for 48 hours then drained, kept in plastic bags at room temperature for one month, and then at 4° C for an additional three months. After stratification, seed were placed in petri dishes in a germinator with a day (16 hours) temperature of 18° C and night (8 hours) temperature of 15° C. Germinants were sown at 1-week intervals starting at day 1, day 7 and day 13, dibbled into plastic containers (93 cm^3 soil volume per cavity, 96 cavities per container) and placed in a fibreglass greenhouse with a photoperiod of 20 hours. A minimum temperature of 15° C was maintained during early seedling development. The greenhouse seedlings were arranged in a randomized design with three replications of 24 seedlings per family per population, or bulked population. Each plot was arranged in a 3x8 configuration. The containers were

rerandomized every 2 weeks during the greenhouse growing season to minimize greenhouse edge effects.

The seedlings were watered and fertilized with a balanced (N:P:K) soluble fertilizer including micronutrients. The seedlings were moved out of the greenhouse near the end of the growing season in September to acclimatize. They were placed under 50% shade-cloth for 4 weeks, then moved into an outdoor compound.

c) nurserybed trial design

One-year-old seedlings were planted into a nurserybed at Mesachie Lake, B.C. (48° 49′ N. Latitude, 124° 09′ W. Longitude, 175 m elevation) in March, 1990. All families were represented with 24 seedlings per family, and a minimum of 24 seedlings per bulk population (depending upon availability of seedlings). Seedlings were planted in a randomized complete block design with six blocks and 4-tree square plots per block, at 5 cm x 5 cm spacing. Approximately 4000 seedlings were planted with 33 populations, seven of which were bulk, and the other 26 populations represented by 171 families.

2.2.3 Traits Measured

2.2.3.1 First-year greenhouse trial

a) seed and germination traits

One-hundred seed per family were x-rayed for three random families per population from each of 24 populations and two random families from each of two populations, as well as 100 seed from each of five bulk populations (n=31 populations). Two bulk populations were inadvertently misplaced. Number of filled seed (fseed) was determined by visually counting the number of seed

which had white embryos which filled more than one-half of the embryo cavity (El Kassaby pers. comm.). The subsequent filled seed were weighed to determine the average weight of a filled seed (wfseed).

Germination was scored in each petri dish every 2 to 4 days for 20 days. A seed was considered germinated if the radicle had extruded 0.5 cm or more from the seed coat. For the above 76 families there were three replications (replication=petri dish) of 100 seed (n=300 seed per family) and six replications of 100 seed per bulk population (n=600 seed per population). Total germination (grml) and number of days to 50% germination of viable seed (rr50) were calculated. After all germinants were removed, the petri dishes were placed back into the darkened cooler until the following April, and then moved back into the germinator under the same environmental conditions as the previous year. Total germination after the second year was recorded (grm2).

b) growth and phenology traits

Starting 8 weeks after the first dibbling, periodic shoot heights were measured at 2-week intervals 12 times (ht0.1 to ht0.12). Root collar diameters (rcdl) were taken at the time of the last height measurement. All height measurements were taken from the cotyledons to the growing tip. All measurements were taken on 10 seedlings from each of the three plots per family from the same 76 randomly-chosen families as in the seed and germination measurements (n=30 seedlings per family and 90 seedlings per population), and from 10 seedlings from each of the three plots for each of the five bulk populations, plus the two bulk populations that were not x-rayed (n=30 seedlings per population). In order to minimize competition effects from surrounding families, the interior 8-tree row was measured in

each plot plus two seedlings from one of the edge rows. Every family (n=171) was not measured periodically during the first growing season in the greenhouse because of time constraints, however, every family had first-year total shoot heights measured at the time of outplanting in the nurserybed trial.

An additional 10 seedlings per plot were measured for final height and root collar diameter on a subset of the above families (20 families from 8 populations) to determine if there were any effects of repeated measuring on final shoot height and root collar diameter. Subsequent analysis of this data as compared to the repeated measures data showed that repeated measuring resulted in significantly reduced shoot height (17.9 cm compared to 19.1 cm) and greater diameter (3.00 mm compared to 2.91 mm). However, there was no significant family by measuring treatment interactions for either shoot height or root collar diameter.

Since yellow-cedar has an indeterminate growth pattern, measuring growth cessation at the end of the growing season involves describing both the number of seedlings growing during a particular time interval and the amount of growth during that specified interval. Shoot growth cessation traits, the average number of seedlings growing (ngrwc1; average based on 10 seedlings per genetic identity per replication) and total shoot growth (grwc1), were calculated for the period between ht0.8 and ht0.12.

c) cold-hardiness measurements

Cold-hardiness was tested at five different dates on a sample of 10 populations during acclimation starting in early November through to early

February, with the objective of describing patterns of cold-hardiness acclimation among populations. The electrolyte leakage technique, as described by van den Driessche (1976) and modified for yellow-cedar (Silim 1991), was used. At each of the five test dates, three seedlings from each of five families per population were used (n=15 seedlings per population). Two populations were represented by four families (n=12 seedlings per population). The main stem from the top one-third of a seedling was cut into 0.5 cm pieces after removing side branches, and placed into 20 ml vials. The 15 or 12 samples from each population were mixed together. One ml of distilled water was added to each vial along with two to five grains of silver iodide to prevent super-cooling. Three temperatures and a control were tested with five vials per population per temperature. Vials were placed in a programmable freezer and cooled to each test temperature at a rate of 5° C per hour and held for 1 hour. After the completion of the run, the vials were placed in a darkened cooler (+4° C). After the tissue thawed, 15 ml of distilled water was added to each vial and the vials were kept at room temperature for 20 hours. The first conductivity reading was taken at this time; the vials were then heated in a convection oven for 2 hours at 100° C, and a second conductivity reading taken after 20 hours.

Index of injury at each test date (inj1.1 to inj1.5) was calculated for each vial and temperature as follows:

$$[(EC_{froz}/EC_{klfr})-(EC_{ctrl}/EC_{klct})]/[1-(EC_{ctrl}/EC_{klct})] \qquad (2.1)$$

where,

EC_{ctrl}=electrolyte leakage due to cutting control tissue;

EC_{klct}=total electrolytes in control tissue;

EC_{froz}=electrolyte leakage due to freezing tissue;

EC_{klfr}=total electrolytes of frozen tissue.

2.2.3.2 Nurserybed trial

a) growth and phenology traits

Total shoot heights were measured at the time of planting (ht1), and after the first (ht2) and second (ht3) growing season in the field. Root collar diameter (rcd2) was measured after the first growing season in the field. All seedlings from 33 populations and 171 families were measured. Periodic growth during the second and third growing season (htgrw2, htgrw3, respectively) was calculated for each seedling.

Growth initiation and cessation during the third growing season were measured on a subset of the seedlings by placing stakes beside each tree to be measured, and marking on the stake the top of the main stem on successive dates. Fourteen populations representing the latitudinal spread of the collections were selected with three randomly chosen families for each of 12 populations, and two bulk populations. Two seedlings were measured from each of three blocks for each family (n=6 seedlings per family and 18 seedlings per population) and four trees from each of three blocks for the bulk populations (n=12 seedlings per population).

Growth initiation measurements were taken on 10 dates starting on April 12, 1991 with 2 to 3 days between measurements. Growth cessation measurements were taken weekly on five dates starting on September 9, 1991. For all measurement dates, the number of seedlings growing and the amount of growth, were recorded.

The following variables were derived from the third-year shoot growth initiation and cessation data:

1. ngrwi3, ngrwc3 = average number seedlings growing during a specific measurement period during growth initiation at the beginning of the

third growing season, and during growth cessation at the end of the third growing season, respectively, and;

2. grwi3, grwc3 = total growth during the growth initiation period at the beginning of the third growing season, and during growth cessation period at the end of the third growing season, respectively.

b) cold-hardiness measurements: second year

Cold-hardiness was tested on a sample of the populations and families within populations from the nurserybed test, during acclimation (early November) and during assumed maximum hardiness (mid-January), with the objective of partitioning variation within and among populations. Given the large number of genetic identities, only two test temperatures (and a control) were used. Pretesting of a subsample of the populations was done prior to each test date in order to approximate the current LT_{50} for the main cold-hardiness tests. Given this information, two temperatures were chosen that would come close to the LT_{50} for all populations. The electrolyte leakage technique, as described in Section 2.2.3.1 c), was used. At both test dates, the main stem from the top one-third of a seedling was cut into 0.5 cm pieces after removing side branches, and placed into 20 ml vials.

The November test involved six seedlings per family, three randomly chosen families per population, and 20 populations. One population was represented by two families. The six samples from each family were mixed together. Two temperatures (-12° C, -15° C) and a control were tested with four vials per family per temperature, as per the technique described above.

The January test of maximum hardiness involved five to six seedlings per family, three families per population, and 14 populations, which were a subset of the 20 populations tested in November. Two populations were

represented by two families. Identities of individual progeny of parent trees were maintained. Two temperatures (-24° C, -27° C) and a control were tested with four vials per seedling per family per temperature, as per the technique described above.

Index of injury was calculated for each vial as previously defined, for both the November test (injacc2) and the January test (injmax2).

c) cold-hardiness measurements: third year

Cold-hardiness was measured during the acclimation period in October 1991, and during assumed maximum hardiness in December 1991, on all populations (n=33), with the objective of assessing adaptive variability among seed sources. A mixture of a maximum of five families per population and four seedlings per family (n=20 seedlings per population), or 24 seedlings per bulk population was tested, using the electrolyte leakage technique as described above. Tissue for each test was cut from one to two upper lateral branches from the current year's growth from each seedling. Two test temperatures (-9° C and -12° C for the October test; -18° C and -21° C for the December test) and a control were used, with five vials per population per temperature.

Index of injury was calculated for each vial as previously defined, for both the October test (injacc3) and the December test (injmax3). Pretesting was done for each test date as described in Section 2.3.2.2 b).

Table 2 summarizes the measured and derived seed, germination, growth, phenological, and cold-hardiness traits used for the studies described in this chapter, along with their description, and abbreviations used in subsequent text, tables, and figures.

Table 2: Description of measured and derived traits used in the yellow-cedar genetic architecture and genecology study (symbols used to describe trait in text in parenthesis)

1. Seed and Germination	
Filled seed (fseed)	percent seed with white embryos filling more than one-half embryo cavity
Weight of filled seed (wfseed)	average weight of one filled seed, in grams
Completeness of germination (grm1, grm2)	percent germination of filled seed after the first and second year, respectively
Speed of germination (rr50)	number of days to 50% germination of viable seed
2. 1st Year Greenhouse Trial	
Periodic heights (ht0.1-ht0.12)	height of seedling measured from the cotyledons over 12 2-week growing periods during the first growing season, in cm, respectively
Final root collar diameter of periodically-measured seedlings (rcd1)	final diameter measured at the root collar, in mm
Inflection point (inflec)	time to inflection point of predicted heights over the first growing season based on a logistic function (see page 34), in weeks since first measurement
Growth cessation (ngrwc1, grwc1)	number of trees growing (% of total trees measured) and their amount of growth (cm) during an 8-week period of height growth cessation at the end of the first growing season, respectively
Injury to cold (inj1.1-inj1.5)	percent index of injury to cold during first-year acclimation for five testing dates, respectively
Lethal temperature (LT_{50}.1-LT_{50}.5)	temperature where index of injury equals 50% during first year acclimation for five testing dates, respectively, in °C

Table 2: (con't)

3. Nurserybed Trial	
Total height (ht1, ht2, ht3)	measured from ground to tip of leader branch in cm for year 1, 2, and 3, respectively
Height growth (htgrw2, htgrw3)	periodic growth in cm during the second and third growing seasons, respectively
Diameter (rcd2)	root collar diameter in mm for year 2
Growth initiation (ngrwi3, grwi3)	number of trees growing (% of total trees measured) and their amount of growth (cm) during the 5-week period of height growth initiation at the start of the third growing season
Growth cessation (ngrwc3, grwc3)	number of trees growing (% of total trees measured) and their amount of growing (cm) during the 5-week period of height growth cessation at the end of the third growing season
Injury to cold (injacc2, injacc3, injmax2, injmax3)	index of injury (percent) to cold during second and third-year acclimation, and second and third-year maximum hardiness, respectively

2.2.4 Statistical Analyses

2.2.4.1 First-year greenhouse trial

a) genetic architecture

A generalized logistic equation was fitted to individual seedlings for periodic height over the growing season to determine if the time to maximum growth rate differed among populations and families within populations. The generalized logistic equation used was:

$$y=a/(d+e^{b-cx}) \qquad (2.2)$$

where,

y = seedling height (cm);

x = time from first measurement (weeks);

a,b,c,d = equation parameters.

SAS Proc NLIN (SAS Inst. 1985) was used to fit the nonlinear regression to the data. Since this procedure is iterative, choosing starting values for the four parameters was necessary. Equation 2.2 was linearized as follows:

$$\log_e (a/y - d) = b-cx. \qquad (2.3)$$

For each individual seedling, a was set to ht0.12 and d to 1 and linear regression analysis performed using SAS Proc REG (SAS Inst. 1985) to estimate starting values for b and c (intercept and slope). Nonlinear regression was then used to fit the logistic equation for each individual seedling using starting values of a=ht0.12, d=1, and b=intercept and c=slope from the above linear regression analysis. The coefficient of determination (R^2) was checked for each curve to ensure a reasonable fit was achieved (R^2>.90). The inflection point (inflec) defined as b/c, measured in weeks from first measurement (ht0.1), was used for determining time to maximum growth rate.

Analyses of variance were performed on the 26 populations with family structure, using SAS Proc GLM, Type I sums of squares (SAS Instit. 1985) for inflec, ht0.12, rcd1 and grwc1 according to the following model:

$$Y_{ijkl} = u + P_i + F_j(P_i) + R_k(F_j(P_i)) + e_{(ijk)l} \quad (2.4)$$

where,

u = overall mean;

P_i = effect of the ith population;

$F_j(P_i)$ = effect of the jth family within the ith population;

$R_k(F_j(P_i))$ = effect of the kth replication within the jth family within the ith population

$e_{(ijk)l}$ = random error associated with measurement of individual seedlings.

All main effects were considered random. Estimation of variance components was performed using SAS Varcomp (SAS Instit. 1985) according to expected mean squares, presented for a completely balanced model, in Table 3. Narrow-sense heritabilities, the ratio of additive genetic variance to total phenotypic variance, were calculated according to:

$$h^2_{ns} = 3\sigma^2_{f(p)}/(\sigma^2_{f(p)} + \sigma^2_{r(f(p))} + \sigma^2_e) \quad (2.5)$$

where,

$\sigma^2_{f(p)}$ = family(population) variance component;

$\sigma^2_{r(f(p))}$ = replication(family(population)) variance component;

σ^2_e = error variance component.

Table 3: ANOVA model for yellow-cedar seed and germination traits, and first-year greenhouse trial growth and phenology traits

Source[1]	df[2,3]	E(MS)[4]
P	p-1	$\sigma^2_e + n\sigma^2_{r(f/p)} + nr\sigma^2_{f(p)} + nrf\sigma^2_p$
F(P)	(f-1)p	$\sigma^2_e + n\sigma^2_{r(f/p)} + nr\sigma^2_{f(p)}$
R(F/P)	(r-1)fp	$\sigma^2_e + n\sigma^2_{r(f/p)}$
error	rfp(n-1)	σ^2_e

1. P = population, F = family, R = replication (all effects random)

2. n, r, f, p = number of trees per replication within family within population, number of families within populations, and number of populations, respectively

3. grm1, grm2, rr50 : n = 1 (based on 100 seed), f = 2-3, p = 31, r = 3-6
   ht0.12, rcd1, grwc1 : n = 10, f = 2-3, p = 31, r = 3

4. σ^2_p, $\sigma^2_{f(p)}$, $\sigma^2_{r(f/p)}$, σ^2_e = variance components due to effect of population, family within population, replication within family within population, and random error, respectively

A coefficient of three was used to represent the genetic correlation among offspring of open-pollinated parents in order to reflect the increased probability of inbreeding and selfing (Squillace 1974). Standard errors of heritabilities were calculated according to Becker (1984).

Analyses of variance were also calculated for grm1, grm2, and rr50 similar to the above model, but using plot means.

Cold-hardiness data were analyzed separately for each test date (inj1.1 to inj1.5) using SAS Proc GLM, Type III sums of squares (SAS Instit. 1985) according to the following model:

$$Y_{ijk} = u + P_i + T_j + PT_{ij} + e_{ijk} \qquad (2.6)$$

u = overall mean;

P_i = effect of the ith population;

T_j = effect of the jth temperature;

PT_{ij} = effect of the interaction of the ith population and the jth temperature;

e_{ijk} = random error associated with measurement of individual vials.

Temperatures were considered fixed and populations random. Expected mean squares are presented for a completely balanced model in Table 4. Appropriate F-tests in the case of data imbalance were constructed by Satterthwaite's approximation (Milliken and Johnson 1984).

Temperature resulting in 50% index of injury ($LT_{50.1}$ to $LT_{50.5}$) was estimated for each population at each test date using linear regression (SAS Proc REG, SAS Instit. 1985).

Table 4: ANOVA model for yellow-cedar first-year greenhouse cold-hardiness study

Source[1]	df[2,3]	E(MS)[4]
T	t-1	$\sigma^2_e + n\sigma^2_{tp} + np\emptyset_t$
P	p-1	$\sigma^2_e + nt\sigma^2_p$
T*P	(t-1)(p-1)	$\sigma^2_e + n\sigma^2_{tp}$
error	t p (n-1)	σ^2_e

1. T = temperature, P = population, (T = fixed; P = random)

2. n, t, p = number of vials per population per temperature, number of temperatures, and number of populations, respectively

3. inj1.1: n = 5, t = 2, p = 10
   inj1.2-inj1.5: n = 5, t = 3, p = 10

4. $\emptyset_t$, σ^2_p, σ^2_{tp}, σ^2_e = variance components due to effect of temperature, population, interaction of temperature and population, and random error, respectively

b) trait correlations

Simple linear correlation coefficients were estimated for all measured traits at both the family and population level among seed, germination, growth, and phenology traits, using all 31 populations for seed and germination traits, and 33 populations for growth and phenology.

c) genecology

Genecological analysis for first-year greenhouse study followed two stages: reduction of the variation among correlated traits to fewer dimensions using principal components, and; regression analysis, in which mean factor scores from principal components were fitted to geographic variables describing population location.

Population means for first-year growth and phenology traits were used for the correlation matrices for principal component analysis (SAS Proc FACTOR, SAS Inst. 1985). Factor scores for each population were obtained for each principal component (PC) which accounted for 20% or more of the total variation by the equation:

$$Y_{in} = a_{i1}x_1 + a_{i2}x2_2 + \ldots + a_{ik}x_k \qquad (2.7)$$

where,

Y_{in} = factor score for the ith PC and nth population

$a_{ik}x_k$ = weighted variables, where a_{ik} is the coefficients for the ith PC and kth original variable, and x_k is the original variable.

Mean factor scores for each population from each significant PC were fitted to two location variables, latitude and elevation. The preliminary model in regression analyses included first and second order and first order interactions of location variables. Descriptive models for each set of factor scores were chosen based on a combination of optimizing Mallow's C_p statistic, minimizing residual variance, and maximizing R^2, using stepwise regression analysis (SAS Proc REG, SAS Inst. 1985).

2.2.4.2. Nurserybed trial

a) genetic architecture

Analyses of variance were performed using SAS Proc GLM, Type III sums of squares (SAS Inst. 1985) for ht1, ht2, ht3, di2, htgrw2, htgrw3, grwi3, and grwc3 according to the following model:

$$Y_{ijkl} = u + P_i + B_j + P_iB_j + F_k(P_i) + F_k(P_i)B_j + e_{(ijk)l} \qquad (2.8)$$

where,

u = overall mean;

P_i = effect of the ith population;

B_j = effect of the jth block;

P_iB_j = effect of the interaction of the ith population with the jth block;

$F_j(P_i)$ = effect of the kth family within the ith population;

$F_k(P_i)B_j$ = effect of the interaction of the kth family within the ith population with the jth block.

$e_{(ijk)l}$ = random error associated with measurement of individual seedlings.

Only the 26 populations with family structure were included. All main effects were considered random. Estimation of variance components was performed using SAS Varcomp (SAS Inst. 1985) according to expected mean squares, presented for a completely balanced model, in Table 5. Narrow-sense heritabilities were calculated according to:

$$h^2_{ns} = 3\sigma^2_{f(p)}/(\sigma^2_{f(p)} + \sigma^2_{f(p)b} + \sigma^2_e) \qquad (2.9)$$

Table 5: ANOVA model for yellow-cedar nurserybed trial growth and phenology traits

Source[1]	df[2,3]	E(MS)[4]
P	p-1	$\sigma^2_e + n\sigma^2_{f(p)b} + nf\sigma^2_{pb} + nb\sigma^2_{f(p)} + nfb\sigma^2_p$
B	b-1	$\sigma^2_e + n\sigma^2_{f(p)b} + nf\sigma^2_{pb} + nfp\sigma^2_b$
P*B	(p-1)(b-1)	$\sigma^2_e + n\sigma^2_{f(p)b} + nf\sigma^2_{pb}$
F(P)	(f-1)p	$\sigma^2_e + n\sigma^2_{f(p)b} + nb\sigma^2_{f(p)}$
F(P)*B	(f-1)p (b-1)	$\sigma^2_e + n\sigma^2_{f(p)b}$
error	pbf(n-1)	σ^2_e

1. P = population, B = block, F = family, (all effects random)

2. n, f, p, b = number of trees per family per block, number of families per population, number of populations, and number of blocks, respectively

3. ht1, ht2, ht3, rcd2, htgrw2, htgrw3 : n = 2-4, f = 2-10, p = 31, b = 6
   grwi3, grwc3 : n = 2, f = 2-5, p = 12, b = 3

4. σ^2_p, σ^2_b, σ^2_{bp}, $\sigma^2_{f(p)}$, $\sigma^2_{f(p)b}$, σ^2_e = variance components due to effect of population, block, interaction of population and block, family within population, interaction of family within population and block, and random error, respectively

where,

$\sigma^2_{f(p)}$ = family(population) variance component;

$\sigma^2_{f(p)b}$ = interaction of family(population) and block variance component;

σ^2_e = error variance component.

A coefficient of three was used to represent the genetic correlation among offspring of open-pollinated parents in order to reflect the increased probability of inbreeding and selfing (Squillace 1974). Standard errors of heritabilities were calculated according to Becker (1984).

Analyses of variance were performed for index of injury from the November test (injacc2) and from the December test (injmax2) using SAS Proc GLM Type III sums of squares (SAS Inst. 1985) according to the following model:

$$Y_{ijkl} = u + T_i + P_j + F_k(P_j) + T_iP_j + T_iF_k(P)_j + e_{(ijk)l} \quad (2.10)$$

where,

u = mean;

T_i = effect of the ith temperature;

P_j = effect of the jth population;

$F_k(P_j)$ = effect of the kth family within the jth population;

T_iP_j = effect of the interaction of the ith temperature by the jth population;

$T_iF_k(P)_j$ = effect of the interaction of the ith temperature by the kth family within the jth population;

$e_{(ijk)l}$ = random error associated with measurement of individual vials.

Temperature was considered fixed and populations and families within populations, random. Appropriate F-tests were constructed using Satterthwaite's approximation (SAS Inst. 1985).

Estimation of variance components was performed using SAS Varcomp (SAS Inst. 1985) according to expected mean squares, presented for a completely balanced model, in Table 6. Narrow-sense heritabilities were calculated as:

$$h^2_{ns} = 3\sigma^2_{f(p)} / (\sigma^2_{f(p)} + \sigma^2_{t*f(p)} + \sigma^2_e) \qquad (2.11)$$

where,

$\sigma^2_{f(p)}$ = family(population) variance component;

$\sigma^2_{tf(p)}$ = interaction of temperature and family(population) variance component;

σ^2_e = error variance component.

b) trait correlations

Simple linear correlation coefficients were estimated for all measured traits at both the family and population level among growth, phenology and cold-hardiness traits.

Index of injury at -12° C for injacc2, and at -24° C for injmax2 were used for calculating population and family within population means for trait correlations.

Population means for index of injury at -12° C for injacc3, and at -18° C for injmax3 were calculated and used for correlations with growth and phenology traits, and with geographic descriptors of population origin.

Table 6: ANOVA model for second-year nurserybed cold-hardiness study

Source [1]	df[2,3]	E(MS)[4]
T	t-1	$\sigma^2_e + n\sigma^2_{tf(p)} + nf\sigma^2_{tp} + nfp\emptyset_t$
P	p-1	$\sigma^2_e + nt\sigma^2_{f(p)} + ntf\sigma^2_p$
T*P	(t-1)(p-1)	$\sigma^2_e + n\sigma^2_{tf(p)} + nf\sigma^2_{tp}$
F(P)	(f-1)(p)	$\sigma^2_e + nt\sigma^2_{f(p)}$
T*F(P)	(t-1)(f-1)(p)	$\sigma^2_e + n\sigma^2_{tf(p)}$
error	tpf(n-1)	

1. T = temperature, P = population, F = family, (T = fixed; P, F(P) = random)

2. t = number of temperatures, p = number of populations, f = number of families per population, n = number of vials per family per population per temperature

3. November test: t = 2, p = 20, f = 2-3, n = 4, error df = 352
   January test: t = 2, p = 14, f = 2-3, n = 5-6, error df = 390

4. $\emptyset_t$, σ^2_p, σ^2_{tp}, $\sigma^2_{f(p)}$, $\sigma^2_{tf(p)}$, σ^2_e = variance components due to effect of temperature, population, interaction of temperature with population, family within population, interaction of temperature with family within population, and random error, respectively

c) genecology

Genecological analyses were performed as outlined in Section 2.2.4.1.c). Growth and phenology data from the nurserybed trial, and third-year cold-hardiness traits were separated into two groups for genecological statistical analyses according to genetic structure (common populations and families within populations) and to biological interpretability. Group 1 included growth and cold-hardiness traits from all 33 populations with the objective of exploring the relationships between growth, cold-hardiness, and

seed source descriptors. Traits included were those that described vegetative growth during the field portion of the trial (third-year height (ht3), second-year diameter (di2), and annual height growth during the 2 years in the nurserybed (htgrw2, htgrw3) and cold-hardiness (index of injury) during acclimation (injacc3) and at maximum hardiness (injmax3), after the third growing season. All 33 populations were used.

The objective of Group 2 was to investigate the relationships between phenology, cold-hardiness, and seed source descriptors. Traits used were number of seedlings growing and their total amount of growth during third-year growth initiation (ngrwi3, grwi3) and cessation (ngrwc3, grwc3) and cold-hardiness during acclimation (injacc3) and during assumed maximum hardiness (injmax3), after the third-year growing season. Fourteen populations were used in the analysis.

Population means were used in the correlation matrices for principal component analyses. Factor scores from all factors that accounted for more than 20% of the variation were used for regressing on first and second order independent traits and first order interactions. Descriptive models for each set of factor scores were chosen based on a combination of optimizing Mallow's C_p statistic, minimizing residual variance, and maximizing R^2, using stepwise regression analysis (SAS Inst. 1985).

2.3 RESULTS AND DISCUSSION

2.3.1 Genetic Architecture

a) seed and germination

Collection, stratification, and germination of the seed was completed without any major problems. Germination percentage, after adjusting for number of filled seeds, averaged 61.5% for the first year and 29.8% for the

second year (Table 7). Seed and germination traits including percent filled seed (fseed), weight of filled seed (wfseed), speed of germination (rr50), and completeness of germination over the first and second years (grm1, grm2) varied relatively more as compared to growth and phenology traits, based on the coefficients of variation (Table 7).

Variation in germination traits was statistically significant at the family within population level only (Table 8). Differences among populations accounted for 0.0% to 12.7% of total variation for rr50 and grm1, respectively, whereas, differences among families within populations accounted for 63.6% (rr50) to 68.3% (grm2) (Table 8). Figure 1 illustrates family variation among populations in rr50. Considerable family variation in completeness of germination and rate of emergence has been shown in coastal Douglas-fir (St. Clair and Adams 1991, El Kassaby *et al.* 1992), Scots pine (Mikola 1984), and Virginia pine (Bramlett *et al.* 1983).

b) growth and phenology

The seedlings grew well in the greenhouse over the course of the first-year averaging 16.3 cm in height and 3.00 mm in diameter (Table 7) and during the course of the nurserybed trial, averaging around 70 cm in height after 3 years (Table 9). Survival was 100% in the nurserybed and all seedlings were healthy. Substantial variation was evident for all traits measured (Tables 7 and 9). Growth and phenology traits such as amount of shoot growth during first-year growth cessation (grwc1), height growth during the second and third year in the nurserybed (htgrw2, htgrw3), and number of seedlings growing during the period of vegetative growth cessation after the third

Table 7: Descriptive statistics for yellow-cedar seed, germination and first-year greenhouse trial growth and phenology traits

Trait[1]	No. of Populations	Mean	C.V.	Population Range
fseed (%)	31	68.8	30.2	37.6 - 97.5
wfseed (mg)	31	5.25	17.5	3.98 - 6.60
grml (%)	31	61.5	33.7	28.7 - 88.6
grm2 (%)	31	29.8	64.7	10.2 - 66.8
rr50 (days)	31	7.20	30.7	5.55 - 9.67
ht0.12 (cm)	33	16.3	18.6	13.9 - 19.8
rcdl (mm)	33	3.0	15.2	2.67 - 3.23
ngrwcl (%)	33	89.7	4.91	84.5 - 97.2
grwcl (cm)	33	2.82	28.2	2.23 - 3.39
inflec (wks)	33	9.88	10.2	9.24 - 10.46

1. See Table 2 for explanation of trait abbreviations

Table 8: Variance components (% of total), statistical significance of differences among means, heritabilities (h^2_{ns}) and standard errors of heritability (s.e.) for yellow-cedar first-year greenhouse trial germination, growth and phenology traits

Trait[1]	% of total variance components				h^2_{ns}	(s.e.)
	Pop	Fam (Pop)	Rep (Fam)	Error		
rr50	0.0[2]	63.6 ***	36.4	-	-	-
grm1	12.7	64.4 ***	22.9	-	-	-
grm2	3.7	69.3 ***	27.1	-	-	-
ht0.12	2.2	22.4 ***	1.5 ***	73.8	0.69	0.17
inflec	2.3	12.1 ***	1.3 ***	84.3	0.37	0.11
rcd1	2.2	9.8 ***	1.0	87.0	0.30	0.10
grwc1	0.6	19.1 ***	1.5 ***	78.8	0.58	(0.15)

1. See Table 2 for explanation of trait abbreviations
2. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

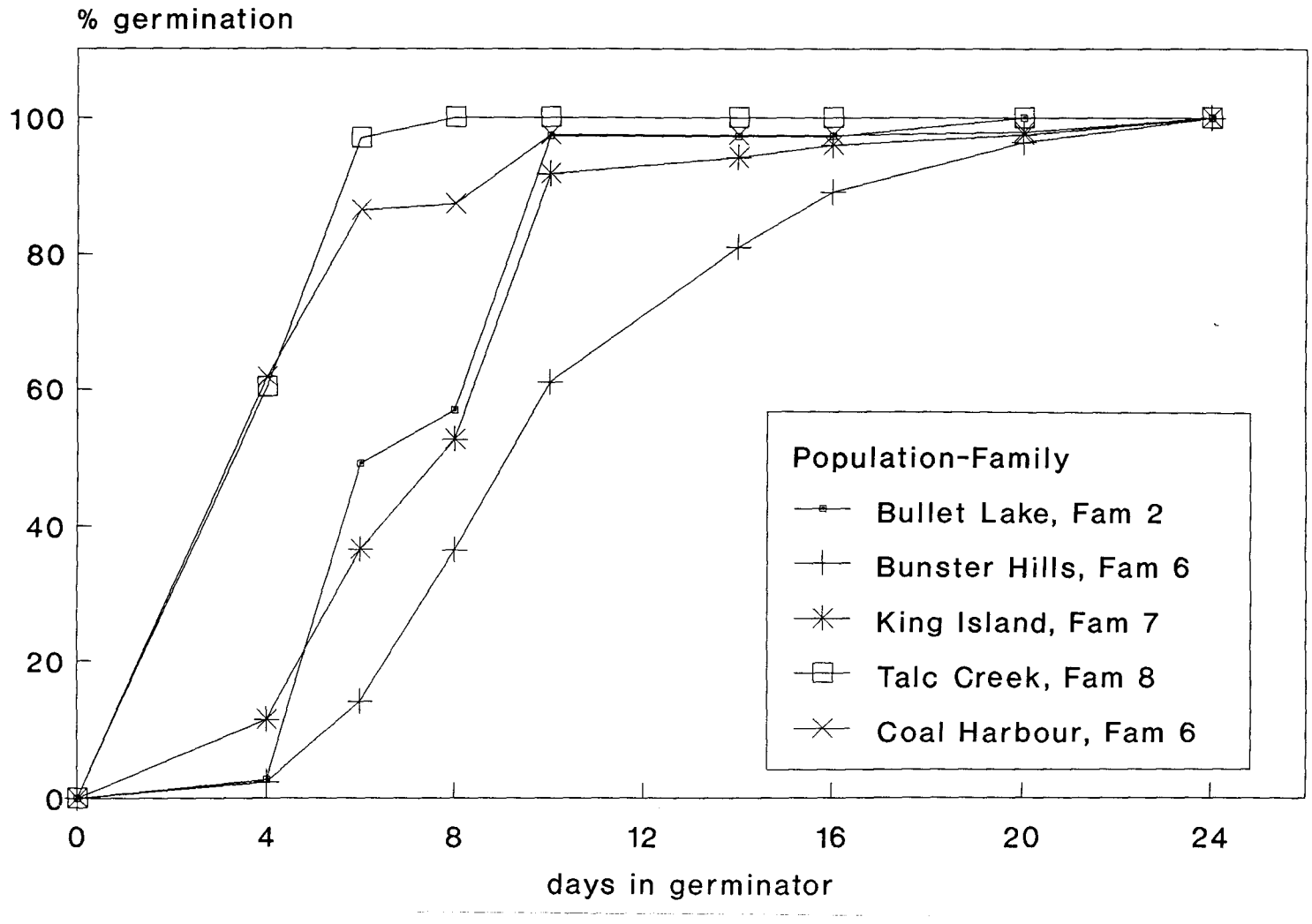


Figure 1: Germination curves based on total viable seed for five yellow-cedar open-pollinated families

Table 9: Descriptive statistics for yellow-cedar nurserybed trial seedling growth, phenology, and cold-hardiness traits

Trait[1]	No. of Populations	No. of Families per Population	Mean	C.V.	Population Range
htl (cm)	33	2-10	18.8	18.7	16.5 - 21.1
ht2 (cm)	33	2-10	39.1	20.0	30.1 - 43.3
ht3 (cm)	33	2-10	70.3	19.2	61.4 - 75.3
rcd2 (mm)	33	2-10	5.86	17.3	5.41 - 6.72
htgrw2(cm)	33	2-10	20.3	31.8	13.2 - 22.5
htgrw3 (cm)	33	2-10	31.2	26.5	21.6 - 34.0
ngrwi3 (%)	14	3	36.1	12.9	24.9 - 42.2
grwi3 (cm)	14	3	2.78	10.8	2.45 - 3.15
ngrwc3 (%)	14	3	37.8	30.6	14.4 - 55.6
grwc3 (cm)	14	3	2.20	14.7	1.52 - 2.70
injacc2 (%)	20	3	40.3	24.9	34.7 - 46.0
injmax2 (%)	14	2-3	34.8	16.7	29.0 - 40.5
injacc3 (%)	33	2-5	40.9	16.8	26.3 - 55.6
injmax3 (%)	33	2-5	40.1	16.6	29.6 - 52.0

1. See Table 2 for explanation of trait abbreviations

growing season (ngrwc3) varied widely, whereas traits such as root collar diameter after the first and second growing seasons (rcd1, rcd2), number of seedlings growing during cessation in the first year (ngrwc1), and growth during initiation and cessation after the third year (grwi3, grwc3) varied relatively less (Tables 7 and 9).

There were no statistically significant differences among populations for first-year growth and phenology traits measured in the greenhouse (Table 8). Population variation accounted for 0.6% (grwc1) to 2.3% (inflec) of the total variance. Total shoot height at the time of planting in the nurserybed (ht1) and after the first growing season (ht2), and root collar diameter after year two (rcd2) were all significant at the population level, whereas total shoot height at the end of the third year (ht3), height growth after the second and third growing season in the field (htgrw2, htgrw3), and growth during shoot initiation at the start of the third growing season were not significant (Table 10). Population variation for growth and phenology traits in the nurserybed accounted for 0.0% (htgrw3) to 9.1% (grwc3) of the total variation (Table 10).

There was significant family variation in all traits measured in both studies, except growth during shoot initiation and cessation during the third growing season (Tables 8 and 10). Family variation accounted for 9.8% (rcd1) to 22.4% (ht0.12) of the total variance for first-year traits, and from 5.3% (grwi3) to 20.3% (ht1) for the nurserybed study. In most cases, percentage of total variance attributed to families within populations was 2 to 16 times greater than that attributed to populations, except for rcd2 which was similar, and for grwc3 in which variation attributed to populations was 1.4 times greater.

Table 10: Variance components (% of total), statistical significance of differences among means, and heritabilities ($h^2_{n.s.}$) and standard error of heritability (s.e.) for yellow-cedar nurserybed trial growth and phenology traits

Trait[1]	% of total variance components					$h^2_{n.s.}$	(s.e.)
	Pop	Pop * Blk	Fam (Pop)	Fam (Pop) * Blk	Error		
ht1	4.7 ***[2]	0.0	20.3 ***	17.8 ***	57.0	0.64	(.098)
ht2	3.6 ***	1.4	13.8 ***	13.2 ***	68.0	0.44	(.073)
ht3	1.0	1.5	10.0 ***	21.8 ***	65.6	0.31	(.066)
rcd2	5.1 ***	0.0	5.3 ***	17.0 ***	72.5	0.17	(.046)
htgrw2	0.9 *	1.9 *	10.0 ***	13.5 ***	73.6	0.31	(.058)
htgrw3	0.0	2.4 *	7.7 ***	25.4 ***	64.5	0.24	(.059)
grwi3	2.8	0.0	5.3	8.5	82.3	0.16	(.20)
grwc3	6.8	2.8	6.6	7.4	83.8	0.20	(.21)

1. See Table 2 for explanation of trait abbreviations
2. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

In the nurserybed trial, there were significant block by family within population interactions for all growth traits, except height growth during shoot initiation and cessation. Thus, although there were significant family within population main effects, these are not strictly interpretable. Given that there was a significant block by family within popoulation interaction for ht1 (i.e. seedlings had just been randomized and planted, and height measurements taken prior to growth), significant block interactions are a result of test design (i.e. large number of blocks and small plot size).

The distribution of genetic variability in seedling traits of yellow-cedar appears to be intermediate between specialists such as Douglas-fir (Griffin and Ching 1977, Rehfeldt 1984, Fashler *et al.* 1985) in which greater variability can be attributed to among populations than within, and generalists such as western white pine (Rehfeldt 1979, 1984) in which most of the variation occurs within populations, when comparing seed from range-wide sources.

Narrow-sense heritabilities varied from 0.30 (rcd1) to 0.69 (ht0.12) for first year traits in the greenhouse, and from 0.16 (grwi3) to 0.64 (ht1) in the nurserybed (Tables 8 and 10). Similar levels and patterns of heritability for early growth traits of Pacific Northwest conifers have been reported elsewhere (Namkoong and Conkle 1976, Franklin 1979, Fashler *et al.* 1985).

Heritabilities decreased by 0.33 from first-year height to third-year height in the nurserybed. Decreasing heritabilities in early growth traits have been reported elsewhere (Namkoong and Conkle 1976, Franklin 1979, Fashler *et al.* 1985). This decrease has been attributed to either an increase in environmental variances (Franklin 1979), an increase in variation due to population effects (Namkoong and Conkle 1976, Fashler *et al.* 1985), or

to a decrease in additive genetic variation (Gill 1987). The major influence in the reduction of heritability for height from year one to year three in this study was due to a reduction in additive genetic variation. The decrease in additive genetic variation could be attributable to the onset of competition since seedlings were planted at close spacings in the nurserybed, (Foster 1986), or to photoperiodic effects due to displacement of seedlings from their native environment (Morgenstern pers. comm.).

c) cold-hardiness

Significant differences among populations, temperatures and the interaction of temperature and populations were evident from cold-hardiness testing during acclimation after the first growing season (Table 11). A typical curvilinear response of increasing cold-hardiness with increasing degree-hours below 5° C was apparent (Figure 2), and has been reported elsewhere for yellow-cedar (Silim 1991, Arnott *et al.* 1992), and for western redcedar, another indeterminate species associated with yellow-cedar at lower elevations (Silim 1991, Folk *et al.* 1993). Populations hardened at different rates during the acclimation period and reached different LT_{50}'s at maximum hardiness in January (Table 12 and Figure 2), accounting for the significant temperature by population interaction.

There were significant differences among temperatures, family(populations) and temperature by family(populations) for index of injury for the November 1991 cold-hardiness test after the first growing season in the nurserybed (Table 13). Significant genetic variability was evident at both the population and family within population level at maximum hardiness (January 1991 test) (Table 13). Narrow-sense heritabilities were

Table 11: Mean squares and significance levels for index of injury of 1-year-old yellow-cedar tested five times over the cold acclimation period

	Mean squares for sources of variation			
Date	Temp	Prov	T*P	Error
11/06/89[1]	36342 ***[2]	856.19 *	194.55 **	58.85
04/30/89	17431 ***	397.51 *	126.51 ***	39.61
12/14/89	9490.0 ***	645.69 ***	45.32	28.40
01/02/90	12416 ***	652.71 ***	51.95 **	22.53
01/15/90	11979 ***	272.27 *	79.89 ***	21.05

1. Degrees of freedom for test date 11/06/89: temperature = 1, population = 9, t*p = 9, error = 80
2. Probability levels = * $p<.05$; ** $p<.01$; *** $p<.001$

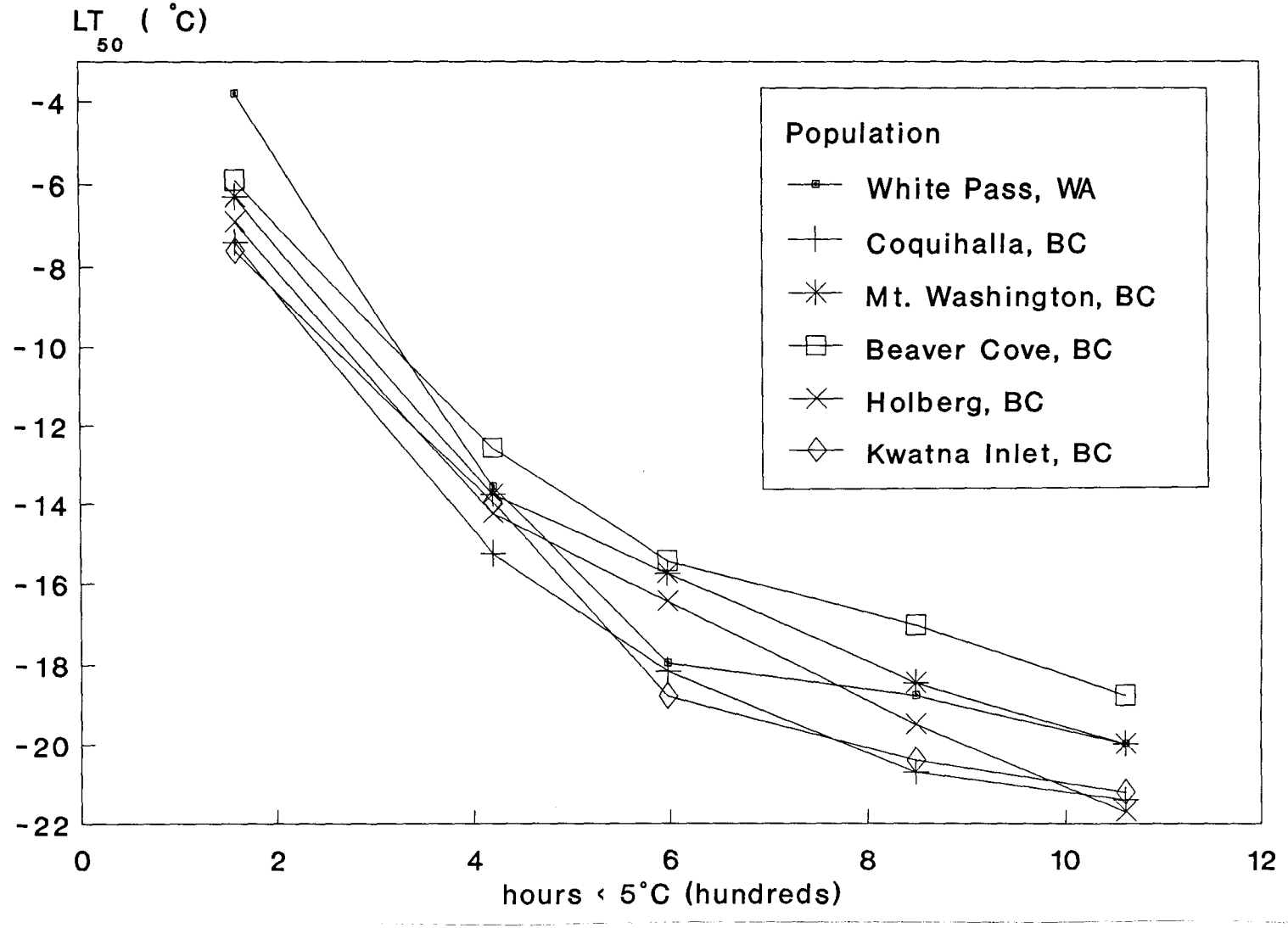


Figure 2: Relationship between LT_{50} and accumulated hours below 5°C for five cold-hardiness testing dates during the acclimation period for six populations of 1-year-old yellow-cedar seedlings

Table 12: LT_{50} values for 1-year-old yellow-cedar from 10 populations for five test dates over the cold acclimation period, and associated number of accumulated degree-hours below 5°C and 0°C at Cowichan Lake Research Station

Population[1]	Lat	Elev (m)	LT_{50}				
			11/06/89	11/30/89	12/14/89	01/02/90	01/15/90
Kitimat, B.C.	54° 07′	450	-7.1	-15.4	-17.5	-20.3	-20.8
Kwatna Inlet, B.C.	52° 08′	488	-7.6	-14.0	-18.8	-20.4	-21.2
Holberg, V.I.	50° 42′	300	-6.9	-14.3	-16.5	-19.5	-21.7
Beaver Cove, V.I.	50° 31′	731	-5.9	-12.6	-15.5	-17.1	-18.8
Squamish, B.C.	49° 47′	900	-6.7	-15.1	-14.8	-18.3	-19.6
Mt. Washington, V.I.	49° 45′	1200	-6.3	-13.8	-15.8	-18.5	-20.0
Coquihalla, B.C.	49° 37′	1350	-7.4	-15.3	-18.2	-20.7	-21.4
Sparton Lake, V.I.	48° 56′	823	-7.2	-14.7	-17.2	-18.9	-21.1
Mt. Angeles, WA	47° 59′	1750	-5.5	-14.3	-16.3	-19.9	-21.2
White Pass, WA	46° 38′	1350	-3.8	-13.6	-18.0	-18.8	-20.0
number hours	≤ 5°C		159	420	597	849	1061
number hours	≤ 0°C		17	55	100	131	173

1. B.C. = British Columbia, Canada; V.I. = Vancouver Island, B.C.; WA = Washington, U.S.A.

Table 13: Mean squares, variance components (% of total), statistical significance of differences among means, heritabilities ($h^2_{n.s.}$) and standard errors of heritability (s.e.) for index of injury of yellow-cedar 2-year-old seedlings to cold for two test dates

Source of variation[1]	November		January	
	Mean Squares	% Variation	Mean Squares	% Variation
T	13743 ***[2]	-	3910 ***	-
P	243.7	3.2	388.3 **	14.2
T*P	78.2	0.0	38.5	0.0
F(P)	234.1 *	12.2	108.3 **	10.7
T*F(P)	136.7 ***	18.0	35.0	0.0
Error	65.8	66.6	43.0	74.9
$h^2_{n.s.}$ (s.e.)	0.38 (0.24)		0.38 (0.16)	

1. T = temperature, P = population, F = family
2. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

0.38 for both index of injury during acclimation and at maximum hardiness (Table 13). Significant additive genetic variability in cold-hardiness during acclimation has been reported for other western conifers (Jonsson *et al.* 1986, Rehfeldt 1988). However, opposite to what has been found in this study, genetic variation during maximum hardiness in lodgepole pine decreased (Rehfeldt 1988).

2.3.2 Trait Correlations

a) first-year greenhouse trial

There were no statistically significant correlations among seed and germination traits except for a strong negative correlation between first- and second-year percent germination (family mean r=-.79) (Table 14). As well, there were no significant correlations of seed and germination traits with growth traits except between speed of germination and early height (Table 15). Weak to moderate negative correlations indicate that families that germinate quicker have a slight height advantage during the first 14 weeks (ht0.3 was measured 12 weeks after the first dibbling date) of growth. However, this advantage is negligible from week 14 (ht0.4) and on. Seed weight or completeness of germination had no measurable influence on height growth or diameter.

Table 14: Simple linear correlation coefficients (r) for family means within population (below diagonal) and population means (above diagonal) for yellow-cedar seed and germination traits, and geographic descriptors of seed origin[1]

	fseed[2]	wfseed	grm1	grm2	rr50
latitude				-.43	
elevation				.43	
fseed					
wfseed					
grm1				-.74	
grm2			-.79		
rr50					

1. r values with significance level >.05 have been omitted for ease of interpretation
2. See Table 2 for explanation of trait abbreviations

Table 15: Simple linear correlation coefficients (r) for family means within populations (below diagonal) and population means (above diagonal) for yellow-cedar germination and first-year greenhouse trial growth traits, and geographic descriptors of seed origin[1]

	rr50	ht0.1	ht0.2	ht0.3	ht0.4	ht0.5	ht0.6	ht0.7	ht0.8	ht0.9	ht0.10	ht0.11	ht0.12
lat								-.40		-.41	-.43	-.44	-.45
elev							.46	.48	.48	.52	.53	.54	.54
rr50[2]		-.34	-.49	-.34									
ht0.1	-.31		.65	.58	.63	.60	.59	.55	.52	.48	.45	.45	.44
ht0.2	-.29	.81		.97	.94	.91	.86	.80	.79	.75	.72	.72	.72
ht0.3	-.24	.75	.97		.98	.96	.92	.87	.84	.81	.78	.78	.78
ht0.4		.77	.94	.98		.98	.96	.92	.89	.86	.83	.83	.83
ht0.5		.73	.90	.95	.97		.99	.96	.94	.91	.88	.88	.88
ht0.6		.69	.84	.89	.93	.98		.99	.97	.95	.93	.92	.92
ht0.7		.61	.79	.83	.89	.95	.99		.99	.98	.96	.96	.96
ht0.8		.57	.74	.78	.84	.91	.96	.99		.99	.98	.98	.98
ht0.9		.54	.71	.74	.81	.88	.94	.97	.99		1.0	.99	.99
ht0.10		.53	.69	.71	.78	.85	.91	.96	.98	.99		1.0	1.0
ht0.11		.53	.68	.71	.77	.85	.91	.95	.98	.99	1.0		1.0
ht0.12		.53	.68	.71	.77	.85	.91	.95	.98	.99	1.0	1.0	
rcd1		.53	.54	.53	.52	.53	.51	.50	.45	.44	.44	.43	.43
ngrwc1											.30	.33	.33
grwc1					.33	.38	.44	.53	.57	.65	.71	.72	.73
inflec		-.58	-.67	-.73	-.68	-.62	-.51	-.39	-.27				

1. r values with significance level >.05 have been omitted for ease of interpretation
2. n(pop)=31, n(family)=71 for rr50
   n(pop)=33, n(family)=76 for all other traits

Table 15: (con't)

	rcd1	ngrwc1	grwc1	inflec
lat	-.57		-.58	
elev			.57	
rr50[2]				
ht0.1				-.50
ht0.2				-.74
ht0.3	.43			-.73
ht0.4	.46			-.68
ht0.5	.45		.43	-.61
ht0.6	.49		.50	-.50
ht0.7	.50		.58	-.42
ht0.8	.45		.61	-.35
ht0.9	.47		.69	
ht0.10	.47		.74	
ht0.11	.47		.75	
ht0.12	.48		.75	
rcd1			.46	
ngrwc1			.43	
grwc1		.47		
inflec	-.37	-.28	.36	

1. r values with significance level >.05 have been omitted for ease of interpretation
2. n(pop)=31, n(family)=71 for rr50
   n(pop)=33, n(family)=76 for all other traits

Seed weight of conifer species has been shown to have either no relationship with rate of emergence (Burgar 1964, Perry and Hafley 1981), a weak positive relationship (Mikola 1984, St. Clair and Adams 1991), or a moderately positive relationship (Dunlop and Barnett 1983).

The influence of seed weight on early growth traits among families or populations in other Pacific Northwest conifers varies from no correlation to a weak or moderate positive correlation (Kuser and Ching 1981, Lines 1987, Campbell *et al.* 1989, Loopstra and Adams 1989, St. Clair and Adams 1991).

Results similar to those shown here for correlations between rate of emergence and early shoot growth has been demonstrated for loblolly pine with positive correlations up to 11 weeks and no correlations with later growth (Dunlop and Barnett 1983). Other studies with conifers have shown no correlations between rate of emergence and first-year growth traits (Burgar 1964, Roche 1969, Griffin 1972, St. Clair and Adams 1991).

Height 8 weeks after dibbling (ht0.1) was moderately positively correlated with final height (family r=.53), whereas ht0.2 to ht0.4 were more strongly correlated (family r=.68 to .77), and all height measurements from ht0.5 were correlated with r>.85 to final height (Table 15). Periodic heights were all weakly to moderately correlated with final diameter, and in fact, family r values decreased with later height measurements.

The predicted inflection point (inflec) from the fitted logistic equation (b/c) had a strong negative correlation with early height (family r=-.73 with ht0.3) (Table 15 and Figure 3). Thus, early inflection point (measured in time from first measurement) is a good indicator of early fast growth. However, the inflection point is not a good indication of later

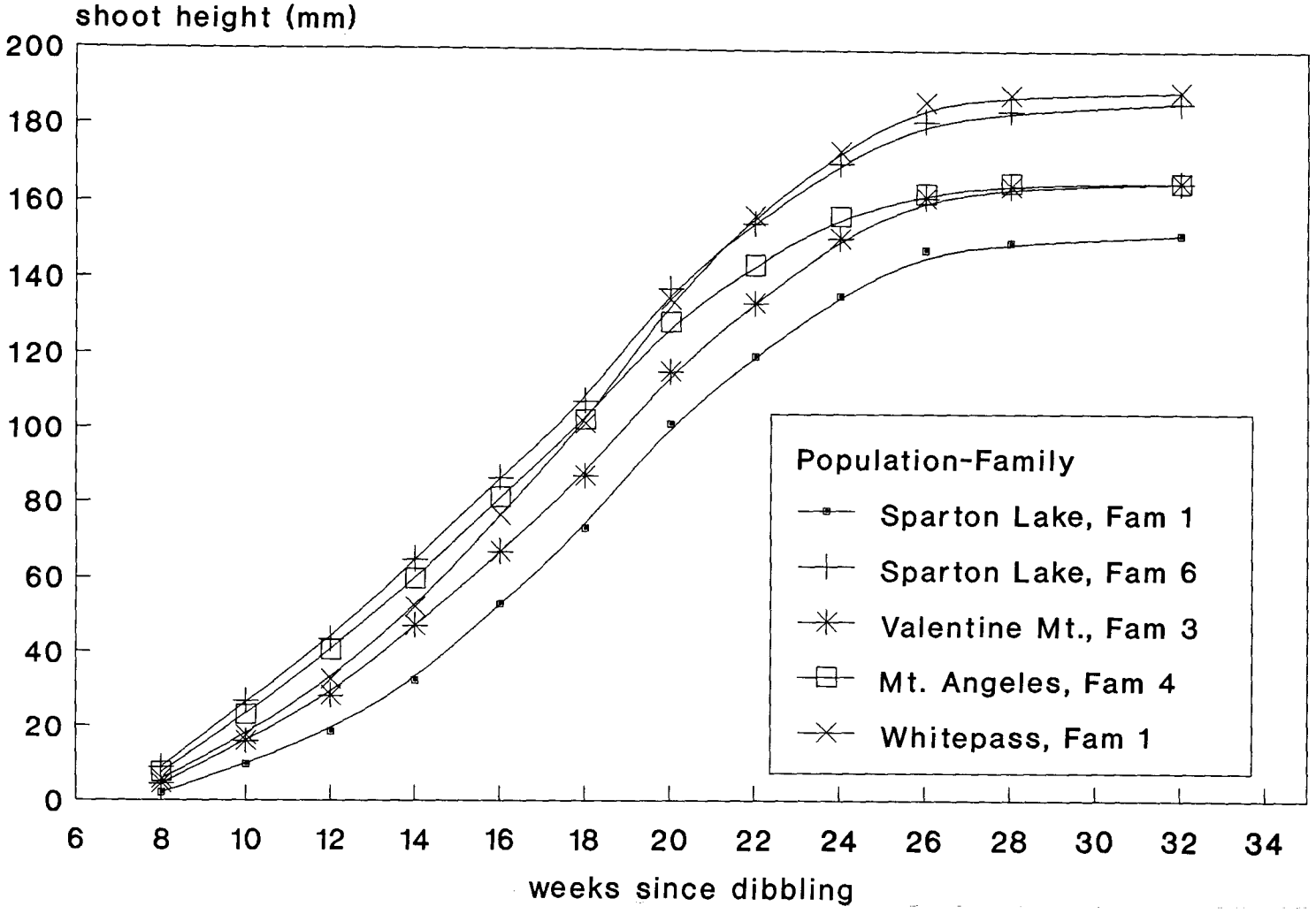


Figure 3: First-year seedling growth curves for five yellow-cedar open-pollinated families

growth as can be seen by the weak to lack of correlation of the inflection point with ht0.7 to ht0.12 (week 20 and later) (Table 15) and the crossing of height curves after week 20 in Figure 3.

b) nurserybed trial

First-year total height was strongly correlated with second-year total height (family mean r=0.77), however, only moderately correlated with third-year total height (family mean r=0.61) (Table 16). This was reflected in the moderate correlation between second-year height growth and third-year height growth (family mean r=0.51, population mean r=0.69). Both second-year total height, and second-year and third-year height growth were strongly correlated with third-year total height (family mean r=0.86, 0.79, and 0.86; population mean r=0.68, 0.84 and 0.75, respectively).

Second-year root collar diameter (rcd2) was moderately correlated at both the family and population mean level for all growth traits except with third-year height growth (Table 16). The number of seedlings growing during the early part of the third growth season (ngrwi3) was moderately correlated with all second and third-year height growth traits (family mean r= .41 to .66), (Table 16). The actual amount of growth during the early season (grwi3) was weakly to moderately correlated with growth traits at the family level only (family mean r=.29 to .49).

The average number of seedlings growing at the end of the third growing season (ngrwc3) was moderately to strongly correlated with third-year height growth (family mean r=0.60; population mean r=0.83), and weakly to moderately correlated with third-year total height (family mean r=0.35, population mean r=0.66) (Table 16). The actual amount of growth during the cessation period

Table 16: Simple linear correlation coefficients (r) for family means within populations (p<.01) (below diagonal) and population means (p<.05) (above diagonal) for yellow-cedar nurserybed trial seedling growth, phenology and cold-hardiness traits

	Trait[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ht1	-	.67		.53		.66	.36							
2	ht2	.77	-	.68	.53		.55	.59							
3	ht3	.61	.86	-	.84	.75	.53	.71		.66	.61				
4	htgrw2	.39	.89	.79	-	.69	.60	.62		.60	.50				
5	htgrw3	.28	.50	.86	.51	-		.58		.83	.76				
6	rcd2	.61	.67	.60	.53	.38	-								
7	ngrwi3	.41	.57	.66	.56	.51	.42	-	.74						
8	grwi3	.29	.38	.49	.37	.42	.45	.68	-						
9	ngrwc3			.35		.60				-	.80			.56	.60
10	grwc3							.49		.80	-				
11	injacc2											-	.59		
12	injmax2												-	.52	
13	injacc3													-	.40
14	injmax3														-

1. n(pop)=33, n(family)=165; ht1, ht2, ht3, htgrw2, htgrw3, rcd2
2. n(pop)=15, n(family)=44; ngrwi3, grwi3, ngrwc3, grwc3
3. n(pop)=20, n(family)=59; injacc2
4. n(pop)=14, n(family)=40; injmax2
5. n(pop)=33, n(family)=0; injacc3, injmax3

(grwc3) was moderately correlated to third-year total height (population mean r=0.61), and strongly correlated to third-year height growth (population mean r=.76), at the population level only.

Correlations of timing of growth initiation (bud-burst) with early growth traits of Pacific Northwest conifers with determinate growth have usually been weak or nonsignificant, whereas similar correlations with bud-set have been significant and strong (Roche 1969, Rehfeldt 1982, Lines 1987, Campbell *et al.* 1989, Sorenson *et al.* 1990). In this study, the number of seedlings growing during both the growth initiation and cessation period had more of an influence on total height and height growth than the amount of growth during these specific periods.

Cold injury after the second growing season was not statistically ($p>0.05$) correlated with any growth or growth rhythm traits (Table 16). Cold injury during the acclimation period was moderately correlated with cold injury during the period of maximum hardiness (population mean r=0.59).

Cold injury after the third growing season (injacc3, injmax3) which was tested on more populations at the expense of family structure, was moderately correlated with the population average number of seedlings growing during third-year growth cessation (r=0.56 for injacc3, and 0.60 for injmax3) (Table 16).

Development of cold hardiness seems to be more dependant upon the number of seedlings growing into the fall, and less on the actual growth of these seedlings. Cold-hardiness of other Pacific Northwest conifers is related to bud-set in the same way as the number of seedlings growing during growth cessation in this study (Rehfeldt 1982, Kuser and Ching 1980, Lines 1987).

Correlations between cold injury dates within and between the two years of testing were, for the most part insignificant. There was a moderate correlation between injacc2 and injmax2 (population mean r=0.59) and a weak correlation between injmax2 and injacc3 (population mean r=0.40). Poor correlations between injury traits is not surprising given data presented from the first-year study which shows changes in population ranking with respect to cold injury during acclimation.

2.3.3 Genecology

a) seed and germination

No significant correlation of either seed weight or percent filled seed with seed source descriptors was found, although there were significant population differences for both traits. Other studies in Pacific Northwest conifers that have found significant population variation in filled seed weight have reported varying results with respect to macrogeographic correlations including no apparent adaptive pattern for Sitka spruce (Lines 1987), weak or moderate correlations in coastal Douglas-fir (White *et al.* 1981), and strong correlations in coastal Douglas-fir, with increasing seed size on drier sites (Loopstra and Adams 1989).

The number of seed germinating in the second year (grm2) was weakly correlated with latitude (r=-.43) and elevation (.43) (Table 14). Thus, more southern and high elevation populations tended to germinate more during the second year than seed from northern populations or low elevations.

b) growth and phenology

Height 18 weeks after dibbling (ht0.6) and thereafter, was weakly correlated with latitude (r=-.40 to -.45) and moderately correlated with

elevation (r=0.46 to .54) (Table 15). Height growth during cessation (grwc1) was moderately correlated with both latitude (r=-.58) and elevation (r=.57). Number of seedlings growing into September and October (ngrwc1) was not correlated with seed origin, most likely since most of the seedlings were still growing during the course of growth cessation measurements. Final diameter was moderately correlated to latitude (r=-.57).

All growth traits measured during the two-year nurserybed study were significantly correlated with latitude and elevation, with increasing growth by more southern and higher elevation populations (Table 17). Correlation of latitude with second-year diameter was particularly strong (r=-0.82) (Table 17 and Figure 4), while correlations of latitude with total height and height growth were weak to moderate.

Table 17: Simple linear correlation coefficients for six variables in two models relating growth, phenology, and cold-hardiness of yellow-cedar to geographic variables of seed origin (significance level in brackets) from nurserybed trial

Group 1 (N = 33)

	ht3[1]	htgrw2	htgrw3	rcd2	injacc3	injmax3
lat	-.47 (.006)	-.60 (.0002)	-.33 (.06)	-.82 (.0001)	-.43 (.009)	-.53 (.0007)
elev	.57 (.0005)	.59 (.0003)	.32 (.07)	.73 (.0001)	.46 (.004)	.38 (.02)

Group 2 (N = 14)

	ngrwi3	grwi3	ngrwc3	grwc3	injacc3	injmax3
lat	-.11 (.71)	-.09 (.76)	-.62 (.02)	-.53 (.05)	-.57 (.03)	-.55 (.04)
elev	.33 (.25)	.28 (.33)	.51 (.06)	.43 (.13)	.59 (.03)	.32 (.27)

1. See Table 2 for explanation of trait abbreviations

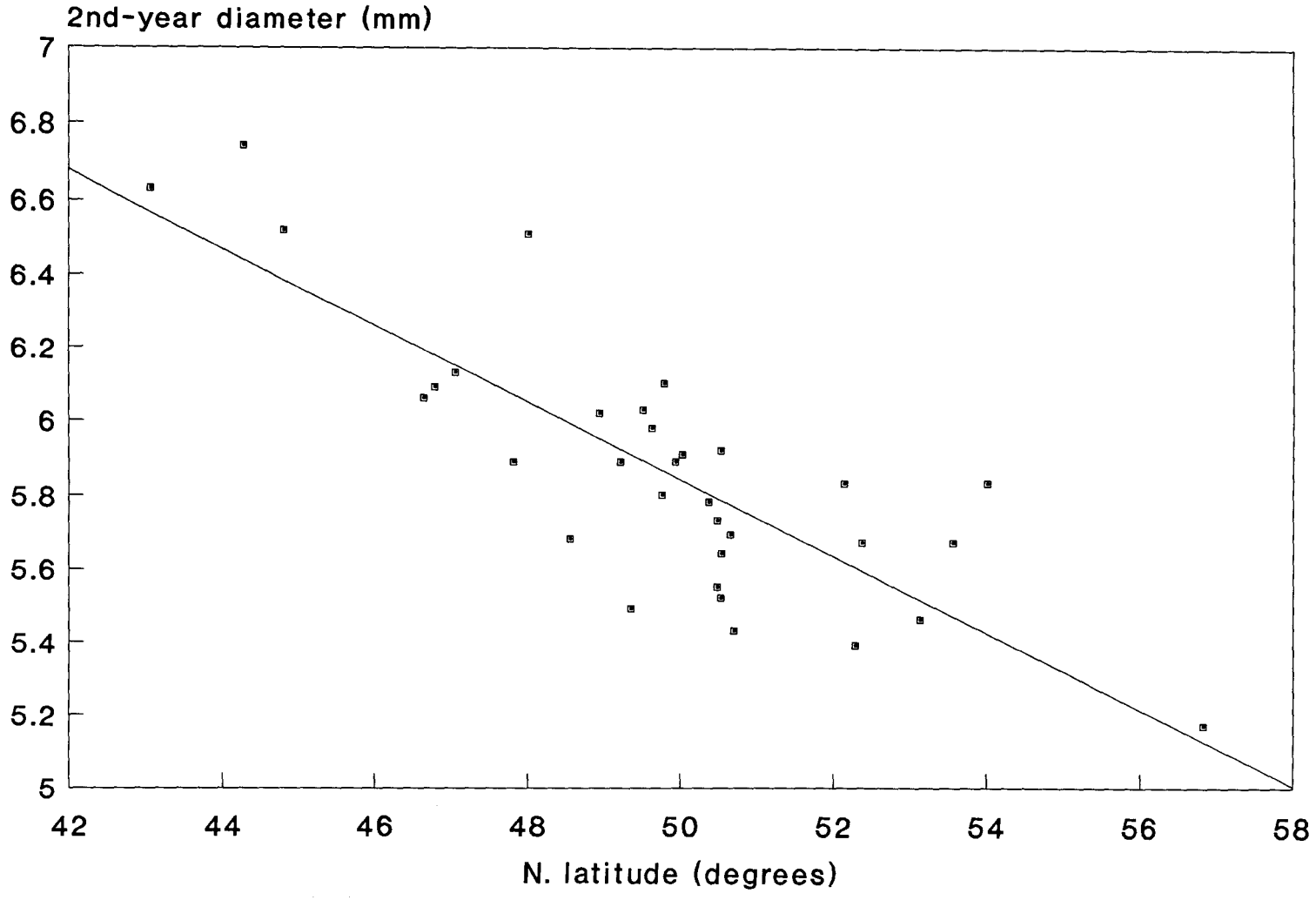


Figure 4: Relationship between second-year root collar diameter and latitude of seed source origin for 33 populations of yellow-cedar grown in a common-garden trial

Growth initiation traits (ngrwi3, grwi3) exhibited no significant trends with geographic descriptors, while growth cessation traits (ngrwc3, grwc3) were significantly correlated with latitude (Table 17).

A plastic response of growth cessation during a late-season warming trend, and differences among populations in this response, can be seen qualitatively among four populations which span the latitudinal distribution of yellow-cedar (Figure 5). Despite significant correlations between number of seedlings growing during cessation and latitude, an increase in temperature before and during days 21 and 28 of measurements for growth cessation (between September 30 and October 7), resulted in an increase in either, or both, the number of seedlings growing, and amount of growth, depending on the population. The number of seedlings growing between day 21 and day 28 decreased for the Oregon population (44% to 35%) while the amount of growth approximately doubled (3.0 mm to 5.9 mm). On the other hand, the Washington population doubled both the number of seedlings growing (33% to 67%) and the amount of growth (2.8 mm to 5.9 mm) during the same period. The British Columbia population doubled the number of seedlings growing (10% to 20%), however, the amount of growth increased by only 57%. Although the Alaska population did not increase in either of the cessation traits, it temporarily halted the declining trend.

Injury to cold after the third-growing season showed trends similar to growth traits with respect to seed source descriptors (Table 17). Correlations of geographic descriptors with cold-injury during both acclimation and assumed maximum hardiness were statistically significant and moderate (r=-0.43 and -0.53 for latitude with injacc3 and injmax3, respectively).

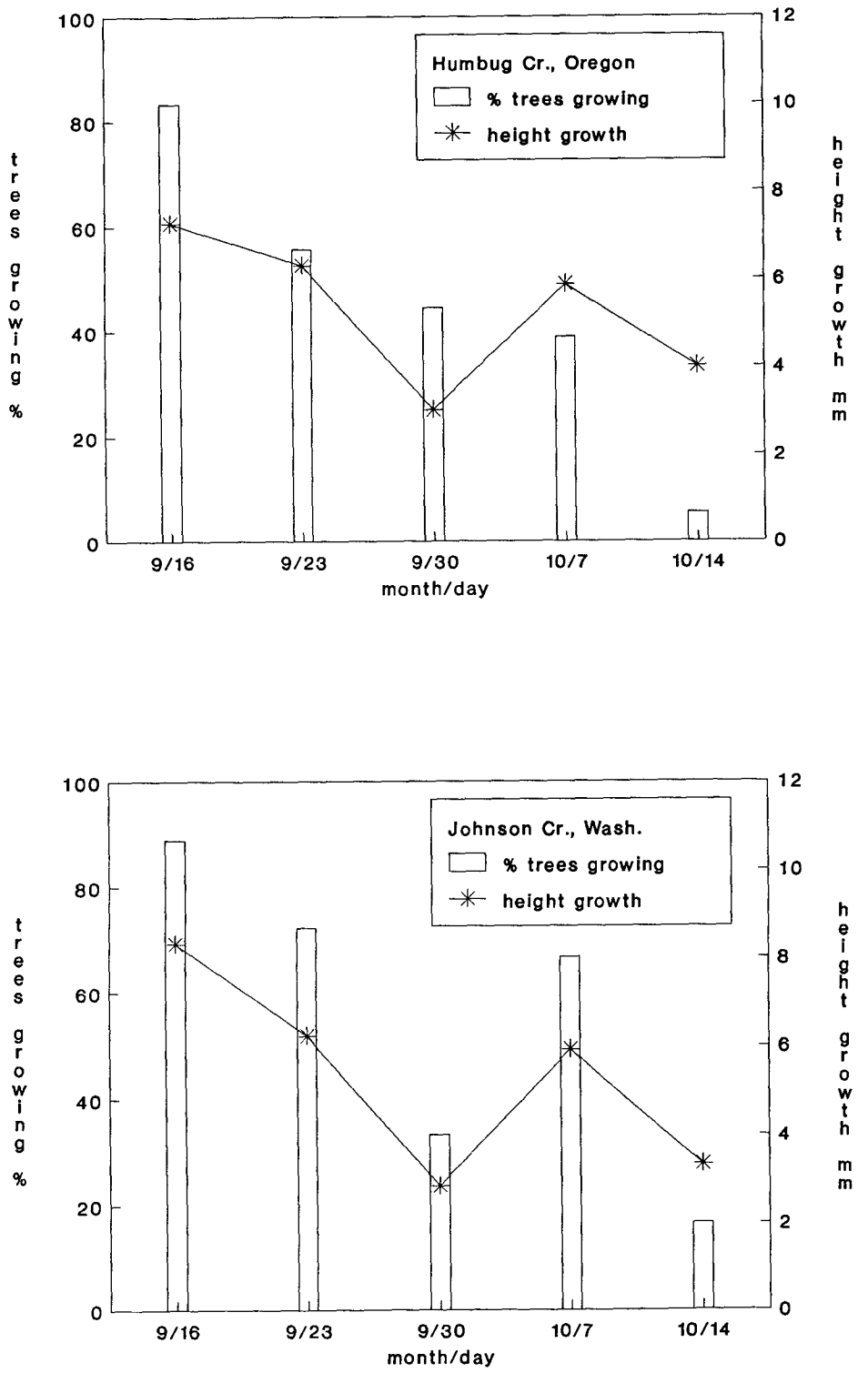


Figure 5: Pattern of height growth cessation for four yellow-cedar populations after the third-growing season in a common-garden trial

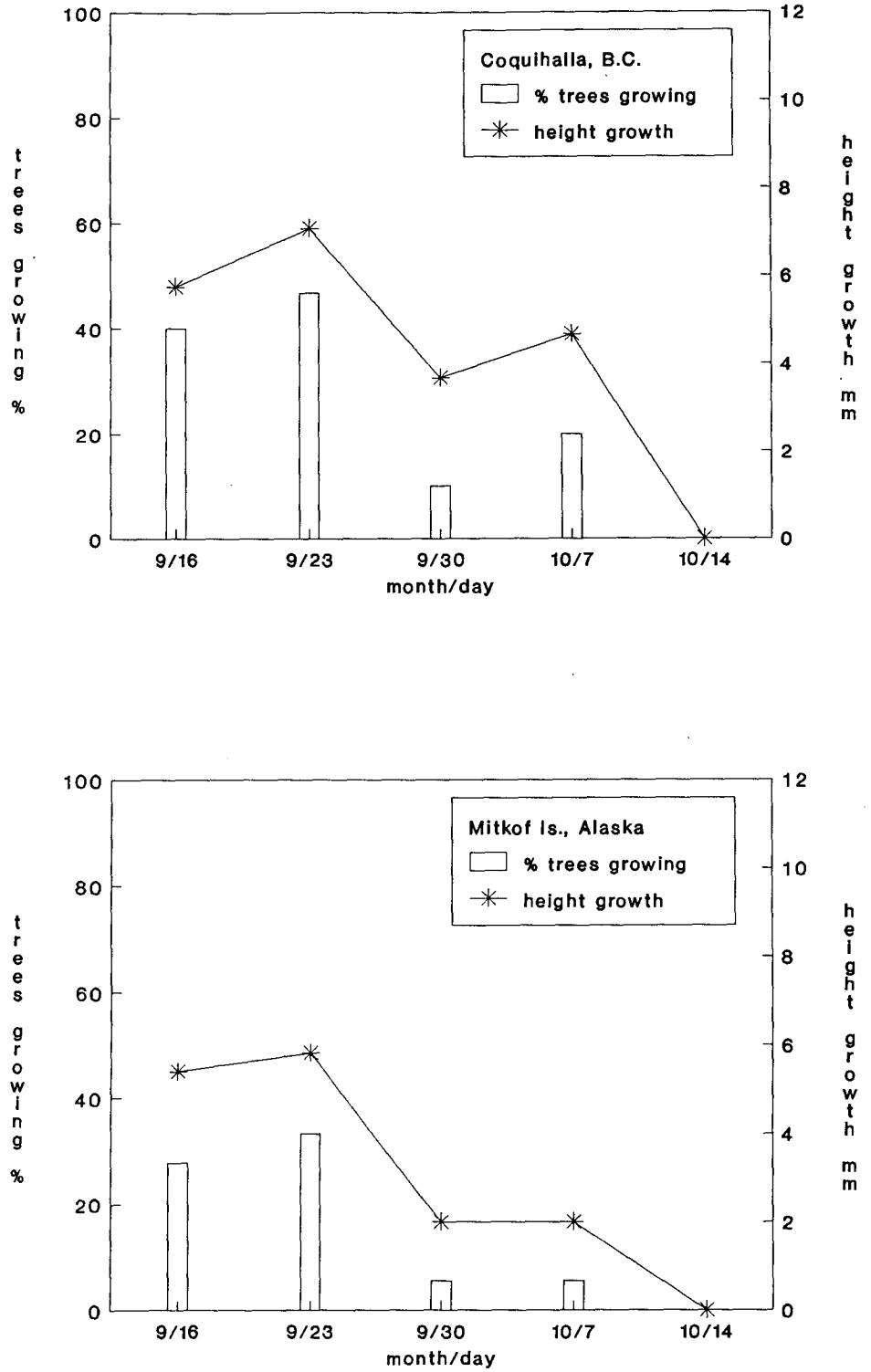


Figure 5: con't

Other Pacific Northwest conifers have shown similar trends for increasing growth and cold susceptibility with decreasing latitude as cited earlier, and correlations of growth cessation with latitude or elevation are common with conifers having extended distributions (e.g. Vaartaja 1959, Rehfeldt 1979b, Kuser and Ching 1980, Lines 1987). However, trends with elevation for yellow-cedar are opposite to other reported studies. Southern populations occur at higher elevations than northern populations (latitude with elevation: r= -.8). Yellow-cedar populations occur above 1200 meters in the southern part of the species distribution, and rarely above 1200 meters in the central part of its range. In northern B.C. and Alaska yellow-cedar occurs mainly below 500 meters. Thus, latitude and elevation can not be separated when discussing seed origin effects on seedling development.

Within central and south British Columbia, yellow-cedar has a more extensive distribution as mentioned earlier. When populations from different elevations from a given latitude are compared with respect to growth or cold-hardiness traits, no adaptive trends are apparent.

c) multivariate analyses

Growth and phenology traits from the first-year greenhouse trial (ht0.12, rcd1, ngrwc1, and grwc1), when subjected to principal component analysis accounted for 58.8% of the total variation with PCA1 and 21.5% for PCA2 (Table 18). PCA1 was loaded by all variables such that populations with larger seedlings and seedlings growing later into the fall contributed more to the factor scores. PCA2 was primarily influenced by ngrwc1 such that populations that had more seedlings growing into the fall contributed more to factor scores. Latitude and elevation accounted for 41.5% of the total

variation in factor scores from PCA1 and 24.3% of total variation in PCA2 (Table 18).

Table 18: Factor loadings and eigenvalues of principal components, percentage of variation accounted for by the principal components, and multiple regression equations for factor scores of the first two principal components for relating seedling germination and first-year greenhouse trial growth and phenology traits of yellow-cedar to geographic variables of seed origin

Trait[1]	Loading	
	pc1	pc2
ngrwc1	.57	.76
grwc1	.90	.03
ht0.12	.87	-.13
rcd1	.68	-.51
Eigenvalue	2.35	0.86
% of variation	58.80	21.50

1. See Table 2 for explanation of trait abbreviations

Factor 1: $\hat{Y}i = 0.447 - .0019\ (LAT) + 5.0 \times 10^{-7}\ (ELEV)^2$

$r^2 = 41.5\%$

Factor 2: $\hat{Y}i = -1.16 + 4.1 \times 10^{-6}\ (LAT * ELEV) - 3.8 \times 10^{-7}\ (ELEV)^2$

$r^2 = 24.3\%$

Table 19 summarizes the results of principal component analysis for the nurserybed trial, for each of the two groups of traits. All growth traits in Group 1 contributed to the loading of the first principal component and accounted for 51.1% of the variability attributed to populations. Populations with larger seedlings contributed to larger factor scores. Index

Table 19: Factor loadings and eigenvalues of principal components and percentage of variation accounted for by the principal component for two models relating yellow-cedar seedling growth, phenology and cold-hardiness from the nurserybed trial, to geographic variables of seed origin

a) Model 1

Trait[1]	Loading	
	PC 1	PC 2
ht3	0.89	-0.34
htgrw2	0.90	-0.26
htgrw3	0.79	-0.28
rcd2	0.74	0.32
injacc3	0.44	0.63
injmax3	0.31	0.78
Eigenvalue	3.07	1.38
% of variation	51.1	22.9

b) Model 2

Trait	Loading
	PC 1
ngrwc3	0.95
grwc3	0.86
injacc3	0.64
injmax3	0.76
Eigenvalue	2.62
% of variation	65.6

1. See Table 2 for explanation of trait abbreviations

of injury during acclimation and maximum hardiness had the greatest influence on factor scores for the second principal component and this component

accounted for an additional 22.9% of the population variability. For PC2, seedlings from populations that were more susceptible to cold contributed more to factor scores.

The quadratic term of latitude and the interaction of latitude with elevation when regressed with factor scores from the first principal component explained 60.4% of the variability (Table 20). Both quadratic terms of latitude and elevation and the interaction of latitude with elevation explained 40.5% of the variability within the second principal component factor scores.

Table 20: Multiple regression equations for two models relating factor scores from the first two principal components for yellow-cedar growth, phenology and cold-hardiness from the nurserybed trial, to geographic variables of seed origin

Model 1	
Factor 1:	$\hat{Y}i = 0.211 - 3.27 \times 10^{-6} (LAT)^2 + 7.32 \times 10^{-4} (LAT*ELEV)$
	$r^2 = 60.4\%$
Factor 2:	$\hat{Y}i = -0.316 + 2.79 \times 10^{-6} (LAT)^2 + 1.24 \times 10^{-6} (ELEV)^2 - 4.17 \times 10^{-6} (LAT*ELEV)$
	$r^2 = 40.5\%$
Model 2	
Factor 1:	$\hat{Y}i = 0.980 - 3.61 \times 10^{-6} (LAT)^2$
	$r^2 = 55.6\%$

Only growth cessation traits and cold-hardiness at acclimation were used for multivariate analysis in Group 2 since growth initiation was not statistically correlated with any of the dependant or independent traits. The first principal component explained 65.0% of the variability attributed

to populations with all traits contributing (Table 19). Seedlings from populations that grew longer into the fall and were more susceptible to cold contributed more to the factor scores. Multiple regression of factor scores from the first principal component explained 55.0% of the variability when regressed on the quadratic term of latitude (Table 20). Thus, southerly and high elevation populations had seedlings which grew longer into the fall and were more frost-susceptible.

2.4 SUMMARY

Significant variability was evident at both the population and family within population level in growth and cold-hardiness traits, and at the family within population level for seed and shoot phenology traits. Substantially more genetic variability was found at the family within population level as opposed to the population level in all but two traits, with 2 to 16 times more variation. Narrow-sense heritabilities varied from 0.16 for growth during third-year shoot initiation to 0.64 for first-year height in the nurserybed. Family within population effects for growth traits in the nurserybed are difficult to interpret because of significant interactions of block by family within population.

There were no significant correlations of seed weight with speed of germination or shoot growth. Speed of germination was positively correlated with early shoot growth (ht0.1 to ht0.3) but was negligible with further shoot growth. Time to largest relative growth rate during the first year was a good indication of early growth but not final first-year height. The amount of growth in the late season (grwc1, grwc3) was a good indication of final shoot height (ht0.12, ht3, respectively). Acclimation of cold-hardiness (injacc3, injmax3) was partly dependant upon the number of

seedlings growing in the fall (ngrwc3), and less so on the actual amount of growth of these seedlings.

There was little evidence of adaptive variation for seed and germination traits, however, early growth traits were moderately to strongly correlated with latitude and elevation of seed origin. Growth initiation exhibited no significant trends with geographic descriptors of seed origin, whereas second and third-year shoot height and diameter growth, growth cessation, and injury to cold were correlated to latitude and elevation, such that seedlings from more southerly and high elevation populations were taller, had greater diameter, grew later into the growing season, and were more susceptible to cold injury during acclimation and at maximum hardiness, than more northern populations. Regression of principal component scores on geographic descriptors confirmed the above trends.

There was evidence of phenotypic plasticity of shoot height growth cessation in response to a late warming trend, and substantial population variation in this response.

CHAPTER 3: GREENHOUSE ENVIRONMENT STUDY

3.1 OBJECTIVES

This study involved the testing of different genetic entries in different environments. Yellow-cedar seedlings from a subset of populations and families within populations from the common-garden study were grown under two photoperiods and two moisture regimes during their second growing season. The objectives of the study were to:

1. investigate the effects of different environments on morphological expression and physiological processes;
2. determine the extent of genotype by environment interactions for morphological and physiological traits, and;
3. elucidate the presence of adaptive genetic variation in morphological expression and physiological processes in response to varying environments.

The main study was designed primarily to address objective 2, the extent and presence of genotype by environment interactions. Thus, the design of the trial, and traits measured, were not optimal in detecting adaptive genetic variation. In particular, the southern populations from Oregon were not included, and only one population from the drier coast-transition area was included, because of a lack of seedlings.

However, a trial was performed on a subset of the populations and treatments in the above study, with the purpose of investigating adaptive variation in response to drought. The objective of this study was to investigate the effects of drought on growth and biomass allocation, gas

exchange and water relations of yellow-cedar seedlings from relatively mesic and xeric populations, and from open-pollinated families within these populations.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

One-year-old seedlings from 18 of the 33 populations sown for the common-garden experiment were used for this study. Nine of the populations were represented each by three open-pollinated families, and the other nine were bulked populations consisting of a minimum of six families (Table 21). One-year-old seedlings were lifted from the styro 313 containers (see Chapter 2, Section 2.2.1.6) and repotted into styro 615 containers (volume of cavity=336 ml) with sand. Seedlings were placed in alternative cavities to minimize crown competition for light. The experimental design was a split-plot with the main plot including two blocks each with four greenhouse benches in which one treatment from a 2x2 factorial of two photoperiods and two moisture regimes was randomly assigned to each bench within a block. There were four replications nested within each treatment and block. Each of the 27 open-pollinated families from the nine populations with family structure was randomly assigned to a 3-tree row plot within each replication. Thus, each family was represented by 24 seedlings per treatment (two blocks x four replications x 3-tree row plots) and 96 seedlings in total (four treatments). Six of the bulk populations were represented by one 3-tree row plot per replication (24 seedlings per treatment) and the final three populations by two 3-tree row plots per replication (48 seedlings per population per treatment).

Table 21: Geographic location of yellow-cedar populations used in the greenhouse environment study

Population[1]	N. Latitude	W. Longitude	Elevation (m)
Mitkof Island, AK[2]	56° 49′	132° 57′	300
Porcher Island, B.C.	54° 00′	130° 20′	430
Kwatna Inlet, B.C.[2,3]	52° 08′	127° 23′	488
Holberg, V.I.[2,3]	50° 42′	127° 40′	300
Coal Harbour, V.I.	50° 39′	127° 47′	500
Beaver Cove, V.I.[3]	50° 31′	126° 48′	731
Waukwass Creek, V.I.	50° 29′	127° 18′	750
Bullet Lake, V.I.	49° 56′	126° 21′	579
Squamish, B.C.[3]	49° 47′	122° 55′	900
Mt. Washington, V.I.	49° 45′	125° 20′	1200
Coquihalla, B.C.[3]	49° 37′	121° 02′	1350
Talc Creek, B.C.[3]	49° 30′	121° 40′	800
Yellow Creek, B.C.	49° 12′	124° 41′	1000
Sparton Lake, V.I.	48° 56′	124° 12′	823
Valentine Mt., V.I.[3]	48° 33′	123° 51′	760
Mt. Angeles, WA[2,3]	47° 59′	123° 28′	1750
Huckleberry Ridge, WA[3]	47° 03′	121° 37′	1350
White Pass, WA	46° 38′	121° 25′	1350

1. B.C. = British Columbia, Canada; V.I. = Vancouver Island, B.C.; WA = Washington, U.S.A.; AK = Alaska, U.S.A.
2. Denotes populations used for cold-hardiness testing
3. Denotes populations with family structure (3 families/population)

Seedlings were placed in a fibreglass greenhouse on May 12, 1990. The seedlings experienced ambient photoperiod for the first four days, however, seedlings were not actively growing at this time. Photoperiod treatments began on May 16 with one treatment simulating the daylength of the southern extreme of yellow-cedar's natural range at 41° 30′ latitude (short-day: SD), and the other the northern extreme at 60° latitude (long-day: LD). Photoperiods were adjusted every two weeks to approximate the normal changes occurring at each latitude (Table 22). Blackout cloth was placed on all seedlings at the same time, and incandescent lights were used within the blackout to reach the required daylengths. Photoperiod lights were turned on at 0600 and blackout cloth removed at 0800. Blackout cloth was put on between 1800 and 2000, and lights were turned on according to length of photoperiod at the particular time (Table 22). Thus, any effects of blackout on seedling growth were similar across all treatments. Photoperiod treatments were discontinued on September 15, at which time all seedlings received the natural daylength for Cowichan Lake. Light levels under the blackout varied from 10 to 20 $\mu mol/m^2/s$, which is below the compensation point for yellow-cedar (Grossnickle and Russell 1991). Thus, the light levels under the blackout were designed to elicite a phytochrome response as opposed to active photosynthesis.

All seedlings were regularly watered as needed and fertilized with a balanced (20:20:20) soluble fertilizer at 0.5 g/l until the moisture regime treatments began on July 16. During the six weeks following July 16, the seedlings in the wet treatment (W) were watered as needed but not fertilized. Seedlings in the dry treatment (D) were not watered until a subsample of seedlings reached predawn shoot water potentials of -1.5 MPa or less, on

Table 22: Photoperiod schedule (hours of daylight) for yellow-cedar greenhouse environment study

Treatment	Hours of Daylight								
	05/16	06/1	06/15	07/1	07/15[1]	08/1	08/15	09/1[2]	09/15[3]
SD (41° 30′)	14.0	14.5	15.0	15.0	14.5	14.0	13.5	13	12
LD (60°)	17.0	18.0	19.0	19.0	18.0	17.0	15.0	13.5	12

1. Start of drydown treatment
2. End of drydown treatment
3. Photoperiod treatment ended, seedlings received natural photoperiod for Cowichan Lake

average, as measured by a pressure chamber (Soil Moisture Corp. Model 3005) according to Ritchie and Hinkley (1975). At this time, the seedlings were watered enough to completely moisten the dry soil and to raise the next day's predawn shoot water potential to between -0.8 and -1.0 MPa. This cycle was repeated a number of times (Figure 6) during the six-week period, after which the droughted seedlings were watered to saturation, and all seedlings were fertilized on a regular schedule for the rest of the growing season.

3.2.2 Traits Measured

a) morphological traits

Heights of the main stem were taken on every seedling every 3 weeks from May 14 to September 17 (ht1.1 to ht1.7). Six weeks after the last periodic measurement, final height (ht2) and root collar diameter (rcd2) were measured. As well, seedlings from replicates 1, 3, and 4 in block 1 were destructively sampled after the growing season, and the following additional traits measured:

1) number of secondary branches greater than 1 mm (brno);
2) length of largest branch mid-way up seedling (brlgth);
3) acute angle of branch measured in 2) above, from main stem (brang);
4) root dry weight (rtwt);
5) main-stem dry weight (stwt), and;
6) secondary branch dry weight (brwt).

At the time of sampling, root volume was less than soil volume for all seedlings.

b) cold-hardiness measurements

On November 5, cold-hardiness was measured using the same techniques as described in Chapter 2, Section 2.2.3.1 c). Five populations from each

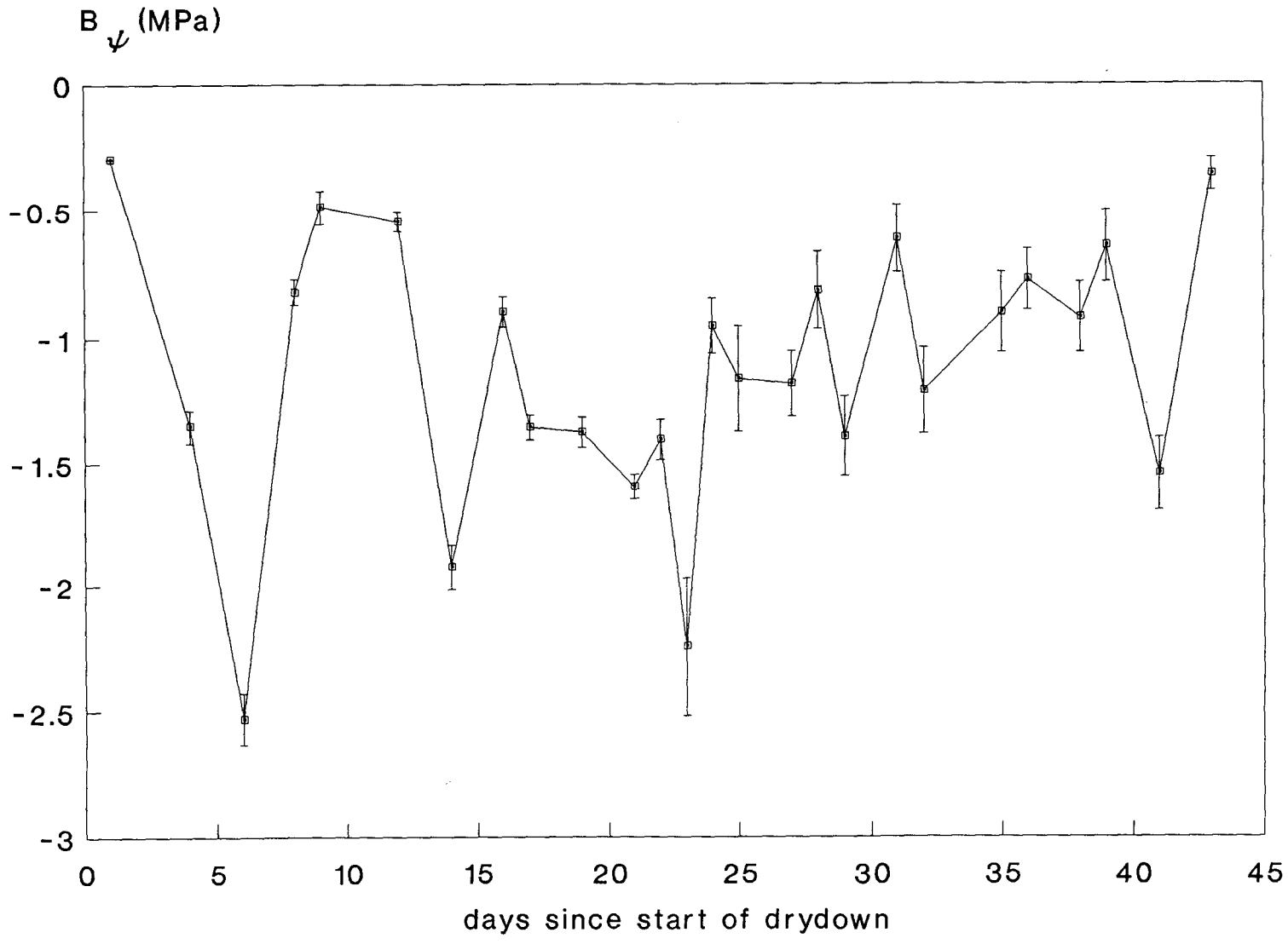


Figure 6: Average predawn shoot water potential for 2-year-old yellow-cedar seedlings during a 6-week drydown (+/- s.e.)

treatment were tested with bulk tissue taken from either two seedlings from each of three open-pollinated families or six seedlings from each bulk population (Table 21). Populations were chosen to maximize the latitudinal differences among the sources included in the greenhouse environment study. Three freezing temperatures were used, along with a control, with five vials per population per treatment per temperature. Samples were taken from replicates 3 and 4, block 2. Index of injury (injacc) at each test temperature was calculated as previously described in Chapter 2, Section 2.2.3.1 c).

c) gas exchange and water potential measurements

At 13 times during the cyclic soil drying, seedlings in the long-day, dry treatment (LDD) were measured for gas exchange. One hour after blackout was removed (0800), stomatal conductance (g_s), CO_2 assimilation rate (A), and transpiration rate (Tr) were measured using a closed system, gas exchange analyzer (LI-6200, LI-COR Inc., Lincoln, NE). Measurements were taken on six seedlings from two families from each of three populations: Kwatna Inlet, mid-coast, windward slope, low elevation; Coquihalla, coast-interior transition, high elevation, and; Mt. Angeles, high elevation, coastal leeward slope,and six seedlings from one bulk population: Mitkof Island, Alaska, northern coast, windward slope, low elevation (Table 23). A lateral branch (4-6 cm) in the upper crown of each seedling was enclosed in a 0.25 l cuvette. Three seedlings per family or bulk population from replicate 2, block 1 were measured during the first 45 minutes, and the next three seedlings (replicate 3, block 1) from each family or bulk population were measured during the next 45 minutes. Measurements were only taken on clear days.

Table 23: Populations used for yellow-cedar gas exchange and water relations drought study and mean annual precipitation data for the closest weather station

Population	N. Lat	W. Long	Elev (m)	Closest Climatic Data						
				Station	Lat	Long	Elev	Annual ppt (mm)	Summer[1] ppt (mm)	No. of Days[2]
Mitkof Island, AK	56° 49′	132° 57′	300	Wrangell, AK	56° 28′	132° 23′	37	2012	484	49
Kwatna Inlet, BC[3]	52° 08′	127° 23′	488	Bella Bella, BC	52° 10′	128° 9′	12	2672	591	56
Coquihalla, BC[3]	49° 37′	121° 02′	1350	Hell's Gate, BC	49° 47′	121° 27′	122	1199	114	27
Mt. Angeles, WA	47° 59′	123° 28′	1750	Hurricane Ridge, WA	47° 59′	123° 28′	1700	1270	-	-

1. Rainfall during May, June, July and August
2. Number of days with 0.2 mm or more of rain (0.1 mm for Wrangell, AK)
3. Populations used for water relations study

The above design, measurements taken mid-morning on clear days, and split into two replications, minimized environmental differences within and between measurement dates. PAR averaged 430 μmol m^{-2} s^{-1} (s.e.=7.1) and 512 μmol m^{-2} s^{-1} (s.e.=6.9), and VPD 1.8 kPa (s.e.=0.22) and 1.95 kPa (s.e. =0.29) during the first and second replications respectively, over all measurement dates. These values of PAR and VPD represent ranges which have a minimal effect on A and g_s of yellow-cedar seedlings (Grossnickle and Russell 1991).

Prior to each gas exchange date, predawn shoot water potentials ($\beta\psi$) were measured on the same seedlings using a pressure chamber, for the first five measurement dates only.

Foliage surface areas were estimated after the drydown period using a LI-3100 area meter (Li-COR Inc., Lincoln, NE). Foliage areas were multiplied by 2.45 to geometrically convert the foliage surface. As well, surface areas were adjusted to allow for differential growth during the drydown period. Gas exchange measurements were adjusted to reflect actual surface areas.

d) water relation measurements

Pressure-volume analysis was performed on six seedlings from each of two families from each of two populations, Kwatna Inlet, B.C. and Coquihalla, B.C. (Table 23), before, and after, the 6-week cyclic drydown period. Due to a lack of seedlings, only one family (16-5) was in common with the gas exchange study. Pressure-volume data were collected for two seedlings from each family (n=8) on each of 3 days for both pre- and post-drydown. Seedlings were removed from the greenhouse, transplanted in 11-litre pots, rehydrated, sealed in plastic, and placed in the dark for 12 hours prior to data collection. A lateral shoot was removed from a seedling at 0800 hours

and its saturated weight measured. Pressure-volume curves were then determined on the lateral shoot for each seedling by collecting measurements of water potential and shoot mass at periodic intervals while, between measurements, shoots transpired outside the pressure chamber on the laboratory bench (Hinckley *et al*. 1980). Approximately 12-18 paired pressure chamber and weight measurements were taken over a 6-8 hour period. Dry weights of shoots were measured after oven-drying at 70° C for 24 hours.

Table 24 summarizes all traits that were either measured or derived in the greenhouse environment study, along with the codes or symbols used in the text, and in tables and figures.

3.2.3 Statistical Analyses

3.2.3.1 Morphological traits

a) anova models

Data were analyzed by ANOVA using PROC GLM TYPE III sums of squares (SAS Inst. 1985). Four different linear models were used depending on the objective of the analyses and the trait (Tables 25 and 26). Height, root collar diameter, and growth (periodic and relative) were analyzed using the complete data set (two blocks and four replications per block) (Table 25a and 25b), whereas the traits from destructive sampling (dry weights) and additional seedling crown descriptors were measured and analyzed on a subset of the data (three replications within block one) (Table 26a and 26b). Using the appropriate ANOVA model described above, each trait was analyzed according to two additional models: population structure only (all 18 populations included) (Model 1, Tables 25a and 26a), and; population and

Table 24: Description of measured and derived traits used in the greenhouse environment study for yellow-cedar (symbols used to describe trait in text in parenthesis)

1. Morphological traits	
Total height (ht1,ht2)	main stem length measured from soil to tip of leader in cm for initial and final dates, respectively
Height growth (htgrw2)	total shoot elongation in cm during the study period
Relative growth rate (rgr2)	relative growth in mm/mm/year during the study period
Periodic heights (ht1.1-ht1.7)	growth in mm over seven 3-week periods during the study
Periodic relative growth rate (rgr1.1-rgr1.7)	relative growth rates in mm/mm/week over seven 3-week periods during the study
Root collar diameter (rcd2)	final diameter measured at the root collar diameter in mm
Root, mainstem, and branch dry weights (rtwt, stwt, brwt), and total dry weight (totdw)	dry weights in gm of 2-year-old seedlings after the study period
Branch number (brno)	number of branches greater than 1mm on 2-year-old seedlings after the study period
Branch length (brlgth)	length of longest branch in midcrown in mm of 2-year-old seedlings after the study period
Branch angle (brang)	acute angle of longest branch in midcrown in degrees of 2-year-old seedlings after the study period
2. Cold-hardiness traits	
Injury to cold (injacc)	index of injury (%) to cold after the study period during acclimation
Lethal temperature (LT_{50})	temperature (°C) at which index of injury equals 50%
3. Gas exchange traits	
Predawn shoot water potential ($\beta\psi$)	MPa
CO_2 assimilation rate (A)	$\mu mol/m^2/s$
Stomatal conductance (g_s)	cm/s

Table 24: (con't)

3. Gas exchange traits (con't)	
Transpiration (tr)	$mmol/m^2/s$
Water use efficiency (WUE)	$\mu mol\ CO_2/mmol\ H_2O$
4. Water relation traits	
Osmotic potential at saturation ($\psi_{\pi(sat)}$) and at turgor loss point ($\psi_{\pi(tlp)}$)	MPa
Maximum bulk modulus of elasticity (ϵmax)	MPa
Relative water content at turgor loss point (RWC_{tlp})	%
Dry weight fraction (DWF)	gm dry weight/gm saturated weight
Symplastic fraction at full turgor (V_o)	
Shoot turgor pressure (ψ_P)	MPa•g/g

Table 25: ANOVA model for yellow-cedar greenhouse environment study: height, height growth and diameter. a. Model 1: population structure only; b. Model 2: population and family (population)

a.

Source[1]	df	E(MS)[4,5]
L	1	$\sigma^2_e + nprbw\emptyset_l$
W	1	$\sigma^2_e + nprbl\emptyset_w$
B	1	$\sigma^2_e + nprlw\sigma^2_b$
R(LWB)	18	$\sigma^2_e + n\sigma^2_{pr(blw)} + np\sigma^2_{r(blw)}$
P	17	$\sigma^2_e + nrblw\sigma^2_p$
P*R(LWB)	408	$\sigma^2_e + n\sigma^2_{pr(blw)}$
error	*[2]	σ^2_e

b.

Source[1]	df	E(MS)[4,5]
L	1	$\sigma^2_e + nfprbw\emptyset_l$
W	1	$\sigma^2_e + nfprbl\emptyset_w$
B	1	$\sigma^2_e + nfprlw\sigma^2_b$
R(LWB)	18	$\sigma^2_e + n\sigma^2_{f(p)r(blw)} + nf\sigma^2_{pr(blw)} + nfp\sigma^2_{r(blw)}$
P	8	$\sigma^2_e + nbrlw\sigma^2_{f(p)} + nfbrlw\sigma^2_p$
P*R(LWB)	192	$\sigma^2_e + n\sigma^2_{f(p)r(blw)} + nf\sigma^2_{pr(blw)}$
F(P)	18	$\sigma^2_e + n\sigma^2_{f(p)r(blw)} + nbrlw\sigma^2_{f(p)}$
F(P)*R(LWB)	432	$\sigma^2_e + n\sigma^2_{f(p)r(blw)}$
error	*[3]	σ^2_e

1. L = light, W = water, B = block, R = replication, P = population, F = family, (L,W = fixed; B, R(LWB), P, F(P) = random)
2. Error df vary from 3227 to 3235
3. Error df vary from 1862 to 1869
4. n, f, p, r, b, w, l = number of trees per family per population per replication per block per watering regime per photoperiod treatment, number of families per population, number of populations, number of replications per block, number of blocks, number of watering regimes, and number of photoperiod treatments, respectively
5. $\emptyset_l$, $\emptyset_w$, σ^2_b, $\sigma^2_{r(blw)}$, σ^2_p, $\sigma^2_{pr(lwb)}$, $\sigma^2_{f(p)}$, $\sigma^2_{f(p)r(lwb)}$, σ^2_e = variance components due to effect of photoperiod, watering regime, block, replication within the interaction of photoperiod, watering regime and block, population, interaction of population and replication, family within population, interaction of family within population and replication, and random error, respectively

Table 26: ANOVA model for yellow-cedar greenhouse environment study: dry weights and morphological traits. a. Model 1: population structure only; b. Model 2: population and family (population)

a.

Source[1]	df	E(MS)[4,5]
L	1	$\sigma^2_e + k\emptyset_l$
W	1	$\sigma^2_e + k\emptyset_w$
R(LW)	8	$\sigma^2_e + n\sigma^2_{pr(lw)} + np\sigma^2_{r(lw)}$
P	17	$\sigma^2_e + nrlw\sigma^2_p$
P*R(LW)	136	$\sigma^2_e + n\sigma^2_{pr(lw)}$
error	*[2]	σ^2_e

b.

Source[1]	df	E(MS)[4,5]
L	1	$\sigma^2_e + k\emptyset_l$
W	1	$\sigma^2_e + k\emptyset_w$
R(LW)	8	$\sigma^2_e + n\sigma^2_{f(p)r(lw)} + nf\sigma^2_{pr(lw)} + nfp\sigma^2_{r(lw)}$
P	8	$\sigma^2_e + nrlw\sigma^2_{f(p)} + nfrlw\sigma^2_p$
P*R(LW)	64	$\sigma^2_e + n\sigma^2_{f(p)r(lw)} + nf\sigma^2_{pr(lw)}$
F(P)	18	$\sigma^2_e + nrlw\sigma^2_{f(p)}$
F(P)*R(LW)	144	$\sigma^2_e + n\sigma^2_{f(p)r(lw)}$
error	*[3]	σ^2_e

1. L = light, W = water, R = replication, P = population, F = family, (L,W = fixed; R(LW), P, F(P) = random)
2. Error df vary from 800 to 1061
3. Error df vary from 446 to 630
4. n, f, p, r, w, l = number of trees per family per population per replication per watering regime per photoperiod treatment, number of families per population, number of populations, number of replications, number of watering regimes, and number of photoperiods, respectively
5. $\emptyset_l$, $\emptyset_w$, $\sigma^2_{r(lw)}$, σ^2_p, $\sigma^2_{pr(lw)}$, $\sigma^2_{f(p)}$, $\sigma^2_{f(p)r(lw)}$, σ^2_e = variance components due to the effect of photoperiod, watering regime, replication within the interaction of photoperiod and watering regime, population, interaction of population and replication, family within population, interaction of family within population and replication, and random error, respectively

family within population (only the nine populations with family structure included) (Model 2, Tables 25b and 26b).

In all of the above models, the photoperiod and watering regime treatments were considered fixed. In the growth models, populations, families within populations, blocks, and replications within blocks were considered random, and in the dry weights and crown descriptor models, populations, families within populations and replications were considered random. Appropriate F-tests were constructed using Satterthwaite's approximation (Milliken and Johnson 1984).

For each of the four above models, and all traits, a full model including all interactions was run. In no case were any genetic (population or family within population) interactions with photoperiod, watering regime, and photoperiod by watering regime interactions, large or significant. Thus, Tables 25 and 26 represent reduced models, with non-significant interactions pooled into the error term. Tables 25 and 26 outline the expected mean squares for a completely balanced model.

b) analyses of covariance for dry matter partitioning

In order to investigate differential partitioning of dry matter to roots, stems, and branches in response to varying environment and genetic treatments, dry weight components were adjusted for correlative effects with total dry weight. In general, seedling growth conforms to the theory of allometric growth, such that:

$$O_i = a(O_j)^b \qquad (3.1)$$

where, O_i and O_j are the dry weights of two different organs, and a and b are allometric constants (Ledig and Perry 1965, Bongarten and Teskey 1987). If O_i represents either root, stem, or branch dry weight, and O_j total plant dry weight, then the above equation suggests that O_i changes in a linear fashion with an increase in plant size, such that,

$$\log(O_i) = a + b*\log(O_j). \qquad (3.2)$$

By testing for parallel or divergent regression lines, differences in dry matter allocations by treatments can be investigated.

In order to investigate differences in allocation of dry matter in response to the four environments used in this study, long-day, dry (LD), long-day, wet (LW), short-day, dry (SD) and short-day, wet (SW), dry weight data (roots (rtwt), stems (stwt), and branches (brwt)) were submitted to an analysis of covariance using Proc GLM (SAS Inst 1985) individually for each of the nine populations that had family structure (n=27 per population per environment), and all populations considered together according to the following model:

$$Y_{ijk} = ß_o + ß_{oi} + ß_1 x_{ijk} + ß_{1i} x_{ijk} + \alpha_{j(i)} \qquad (3.3)$$

where, Y_{ijk} is the log(root, stem, or branch dry weight) of the kth seedling, k=1,...,27, in the jth replication j=1,...,3, of the ith environment, i=1,...,4, $ß_o$ and $ß_1$ are average regression coefficients, $ß_{oi}$ and $ß_{1i}$ are environment effect coefficients, x_{ijk}, the covariate, is the log(total dry weight), and $\alpha_{j(i)}$ is the replication(environment) effect. Significant differences among environments is indicative of differences in environment means (intercept) and significant differences in environment by log(total dry weight) effects are indicative of differences in regression slopes between environments (Bongarten and Teskey 1987).

Differences among populations were evaluated within each environment using the same model as above except substituting environment effects with population effects. Again, significant population effects indicate differences in population means (intercept) and population by log (total dry weight) effects are indicative of different regression slopes between environments.

Pairwise comparisons of slopes among populations within environments were conducted for those models with significant effects using the standardized t-statistic (Neter and Wasserman 1974). Seedlots having similar slopes were then tested for least-square differences among adjusted means.

c) nonparametric stability measurements

An alternative approach to investigating genotype by environment interactions, rank stability measures (Nassar and Huhn 1987), was used. The two nonparametric measures of stability used were:

$$S_i^{(1)} = 2 \sum\sum \mid r_{ij} - r_{ij'} \mid / [N(N-1)] \qquad (3.4)$$

$$S_i^{(2)} = \sum (r_{ij} - r_{i.})^2 / (N-1), \quad r_{i.} = \sum r_{ij}/N \qquad (3.5)$$

where,

r_{ij} = rank of the ith genotype in the jth environment;

N = number of environments.

The first expression ($S_i^{(1)}$) is a measurement of the mean of the absolute rank differences of a genotype over all environments it was tested in. A value of 0 is indicative of maximum stability. The second measure ($S_i^{(2)}$) is a measure of the variance among the ranks over all environments and again, a measure of 0 means maximum stability (Nassar and Huhn 1987). Phenotypic means of the

ith genotype in the jth environment were corrected for genotypic effects as follows:

$$x_{ij}^{*} = x_{ij} - (x_{i.} - x_{..}) \qquad (3.6)$$

where,

x_{ij}^{*} = corrected mean of the ith genotype in the jth environment;

$x_{i.}$ = mean of ith genotype over N environments;

$x_{..}$ = overall mean of K genotypes and N environments.

Since each genotype is ranked separately by environment, and genotypic effects are removed, the rank stability measures are attributed to genotype by environment interactions only. A Chi-square test statistic was used to test the null hypothesis that all genotypes are equally stable for each trait measured (see Nassar and Huhn 1987).

d) correlations of morphology with geographic descriptors

Simple linear correlations were performed using SAS Proc CORR (SAS Inst. 1985) for all growth and morphology traits within each environment, with geographic descriptors.

3.2.3.2 Cold-hardiness measurements

Index of injury was calculated for each population at each test temperature for each vial as described in Section 2.2.3.1 c). Index of injury data were subjected to ANOVA using SAS PROC GLM Type III sums of squares (SAS Inst. 1985). All main effects were considered fixed. Temperature at which 50% index of injury occurs (LT_{50}) was determined after regressing index of injury values for each combination of population and environment on test temperature using SAS Proc REG (SAS Inst. 1985).

3.2.3.3 Gas exchange and shoot water potential measurements

Response models for paired values of predawn shoot water potential to A, g_s, and water use efficiency (WUE=A/Tr) and A to g_s, were developed using curve-fitting regression analysis separately by population and by family within population. Model selection was determined using SAS REG (SAS Inst. 1985) with stepwise selection of dependant variables, with variables being entered/dropped if $p>0.05$ for partial F-test. Dependant variables included x, x^2, x^3, $1/x$, $1/x^2$, and ln(x).

3.2.3.4 Water relation measurements

Pressure-volume curves were developed using a software program developed by Schulte (1988), using non-linear, least-squares analysis (Schulte and Hinckley 1985). Pressure-volume curves define the osmotic potential at saturation ($\psi_{\pi(sat)}$) and turgor loss point ($\psi_{\pi(tlp)}$), maximum bulk modulus of elasticity (ϵ_{max}), relative water content at turgor loss point ($RWC_{(tlp)}$), and symplastic fraction at full turgor (V_o). Dry weight fraction (DWF) was calculated as grams dry weight/grams saturated weight for each shoot. Least-square means for each trait by population and by family within population were calculated for both pre- and post-drydown pressure-volume curves, and multiple pair-wise tests performed (SAS Inst. 1985).

Response models for shoot turgor pressure (ψ_p) to relative water content were fitted using the same methods as described above for gas exchange data, by population and family within population for both pre- and post-drydown.

3.3 RESULTS AND DISCUSSION

3.3.1 Morphological Traits

a) shoot growth

Overall, seedlings from the long-day, wet (LW) environment were taller and had both the greatest height growth and relative height growth (Table 27). Seedlings grown under the short-day, dry (SD) regime were the shortest, and had the least height growth. Seedlings grown under both the short-day, wet (SW) and long-day, dry (LD) environments were similar, and intermediate, between the LW and SD regimes, for shoot height growth response (Table 27).

Seedlings grown under the SW had the greatest root collar diameter, and seedlings grown under both the LD and SD treatments the least. The root collar diameter of seedlings grown under the LW were intermediate (Table 27).

The above results are similar to the results presented for other *Cupressaceae* species when grown under similar environmental stresses, including yellow-cedar (Arnott *et al.* 1992) and western redcedar (Major *et al.* 1993).

Significant differences ($p<0.01$) were evident for total height, height growth, and relative height growth for both the photoperiod and soil moisture treatments, and for the soil moisture treatment only, for root collar diameter (Table 28). There were no significant interactions of photoperiod with soil moisture for any of the growth traits, as mentioned earlier.

Periodic shoot elongation presented as relative growth rate (mm/mm/wk) was sensitive to environments (Tables 27 and 28, Figure 7). Significant differences were apparent between the short and long photoperiod treatments during weeks four to six (rgr1.2) and between weeks seven and nine (rgr1.3). Photoperiod treatments started at the beginning of the study.

Table 27: Least square means (standard errors) for shoot growth and morphology traits of yellow-cedar measured in the greenhouse study during and after the second growing season, by environments

Trait[1]	Environments[2]			
	LD	LW	SD	SW
a. shoot growth				
rcd2	5.12 (.02)b[3]	5.51 (.02)ab	5.11 (.02)b	5.66 (.03)a
ht2	324.7 (1.6)b	355.8 (1.7)a	300.1 (1.5)c	327.6 (1.6)b
htgrw2	129.4 (1.2)b	158.1 (1.4)a	106.7 (1.0)c	133.5 (1.1)b
rgr2	0.513 (.005)b	0.592 (.005)a	0.445 (.004)c	0.530 (.005)b
rgr1.1	0.073 (.0012)a	0.077 (.0011)a	0.070 (.0009)a	0.074 (.0009)a
rgr1.2	0.084 (.0010)a	0.083 (.0010)a	0.068 (.0009)b	0.070 (.0009)b
rgr1.3	0.054 (.0008)a	0.054 (.0008)a	0.044 (.0007)b	0.048 (.0007)b
rgr1.4	0.010 (.0003)b	0.035 (.0006)a	0.008 (.0003)b	0.030 (.0005)a
rgr1.5	0.005 (.0002)b	0.021 (.0004)a	0.005 (.0002)b	0.019 (.0004)a
rgr1.6	0.020 (.0004)a	0.018 (.0004)a	0.016 (.0004)a	0.016 (.0003)a
rgr1.7	0.016 (.0003)a	0.007 (.0002)b	0.013 (.0003)a	0.008 (.0002)b
b. morphology				
rtwt	4.84 (.068)b	5.31 (.092)ab	5.10 (.076)ab	5.90 (.085)a
stwt	2.24 (.032)b	2.56 (.038)a	2.09 (0.30)b	2.50 (.038)a
brwt	5.65 (.078)c	6.59 (.092)a	5.23 (.076)d	6.20 (.088)b
totwt	12.5 (.163)b	14.2 (.203)a	12.0 (.169)c	14.3 (.190)a
brlgth	86.9 (.929)b	99.0 (1.112)a	79.8 (.968)c	86.8 (1.043)b
brno	26.8 (.222)a	27.6 (.239)a	25.7 (.231)b	27.4 (.231)a
brang	62.4 (.738)a	58.7 (.713)a	61.6 (.799)a	54.2 (.771)a

1. See Table 24 for explanation of trait abbreviations
2. LD = long day, dry; LW = long day, wet; SD = short day, dry; SW = short day, wet
3. Different letters in the same row denotes significance at p = 0.05 using multiple, pair-wise t-tests

Table 28: Mean squares for yellow-cedar seedling growth traits from the greenhouse environment study: a. Model 1: population structure only; b. Model 2: population and family (population)

a.	Trait[1]							
Source[2]	ht2[3]	htgrw2[3]	rgr2	rgr1.1[4]	rgr1.2	rgr1.3	rgr1.4	rgr1.5
L	288.82 ***[5]	257.81 ***	2.17 ***	0.63	148.32 ***	33.58 **	5.58	1.30
W	571.62 ***	519.61 ***	4.42 ***	16.12 *	0.18	3.86	361.82 ***	154.01 ***
R(LWB)	16.52 ***	11.25 ***	0.12 ***	2.45 **	5.37 ***	4.10 ***	1.58 ***	0.66 ***
P	21.98 ***	16.73 ***	0.46 ***	17.94 ***	12.31 ***	4.73 ***	0.95 ***	0.28 ***
P*R(LWB)	2.89 ***	1.59 ***	.02 ***	1.26 ***	0.88 ***	0.49 *	0.20 ***	0.09 *
error	2.05	0.016	0.016	0.84	0.70	0.43	0.15	0.08
b.								
L	216.52 **	241.51 ***	2.28 ***	2.39	151.35 ***	37.19 **	5.04	0.57
W	529.48 ***	409.24 ***	3.05 ***	8.57	0.34	3.01	312.45 ***	133.45 ***
R(LWB)	24.13 ***	12.41 ***	0.12 ***	2.80 ***	6.20 ***	3.41 ***	1.67 ***	0.47 ***
P	26.81	9.06	0.27	4.75	11.10	5.27	1.65 *	0.48
P*R(LWB)	2.58	1.49	0.02	1.19	0.78	0.47	0.18	0.09
F(P)	47.81 ***	17.25 ***	0.16 ***	8.51 ***	5.83 ***	2.68 ***	0.64 ***	0.21 ***
F(P)*R(LWB)	2.50 ***	1.46 ***	0.02 ***	1.16 ***	0.85	0.47 *	0.20 ***	0.08
error	1.49		0.013	0.65	0.63	0.40	0.13	0.08

1. See Table 24 for explanation of trait abbreviations
2. L = light, W = water, B = block, R = replication, P = population, F = family (population)
3. ms (ht2)x10^3, ms (htgrw2) x 10^3
4. ms (rgr1.1 to rgr1.7) x 10^{-3}
5. Probability levels: * p<.05; ** p<.01; *** p<.001

Table 28: (con't)

a.	Trait[1]		
Source[2]	rgrl.6	rgrl.7	rcd2
L	3.63 *	0.78 *	6.87
W	0.70	13.23 ***	161.20 ***
R(LWB)	0.60 ***	0.11	3.96 ***
P	1.15 ***	0.31 ***	3.34 ***
P*R(LWB)	0.18 ***	0.08 ***	0.58 **
error	0.13	0.06	0.70
b.			
L	3.19 *	0.73 *	10.14
W	4.32 *	13.20 ***	48.41 ***
R(LWB)	0.62 ***	0.17 **	3.64 ***
P	1.39 *	0.43 *	2.44
P*R(LWB)	0.19	0.09	0.54
F(P)	0.44 ***	0.15 **	2.87 ***
F(P)*R(LWB)	0.16 ***	0.08 ***	0.63 ***
error	0.12	0.06	0.43

1. See Table 24 for explanation of trait abbreviations
2. L = light, W = water, B = block, R = replication, P = population, F = family (population)
3. ms (ht2)x10^3, ms (htgrw2) x 10^3
4. ms (rgrl.1 to rgrl.7) x 10^{-3}
5. Probability levels: * p<.05; ** p<.01; *** p<.001

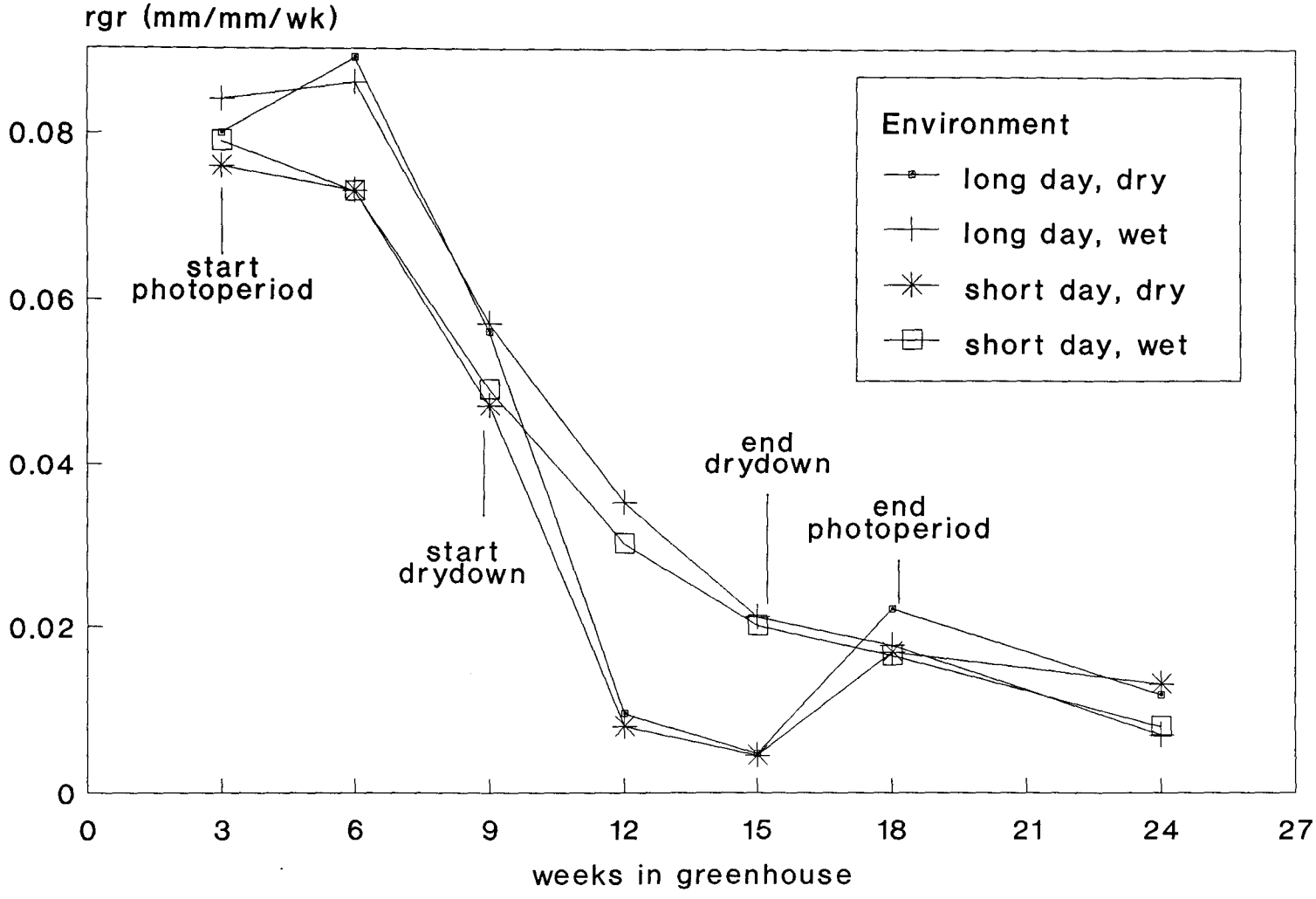


Figure 7: Average periodic relative growth rate over a 24-week period for yellow-cedar during their second year grown in four environments

Differences among the soil moisture treatments became pronounced during the 10- to 12-week period (rgr1.4) which immediately followed the start of the cyclic drydown, and continued for the duration of the drydown (week 13 to 15; rgr1.5). Photoperiodic effects were not significant during the drought period, although seedlings from the long-day, wet environment had a greater relative growth rate than seedlings from the short-day, wet environment.

During the three-week period following the drought, depending on the model, there were no, or small, significant effects between the wet and dry environments, and photoperiodic effects were again significant (Table 28). During the last 6 weeks (rgr1.7), the relative growth rate of the seedlings that had undergone a drought was significantly greater than seedlings growing under the normal watering regime ($p<0.001$). Similarly, growth of the seedlings that had experienced the short photoperiod, the photoperiods being equal since September 15, was greater than the seedlings that had experienced the longer photoperiod ($p<0.05$).

Again, these results are similar to those reported in the literature for *Cupressaceae* species. In studies with seedlings of incense-cedar (Harry 1987), western redcedar (Krasowski and Owens 1991), and yellow-cedar (Arnott *et al.* 1992), growth was either slowed or arrested, depending on the treatment, during the treatment period, and growth rapidly increased, and in most cases, surpassed, the growth of the controls upon release of the stress. The results reported here, and in the literature for indeterminate species, are different than those for determinate species. It has been shown that for determinate species, short photoperiods and moisture stress applied during the active growing season, usually results in decreased growth, formation of a vegetative bud, and subsequent dormancy (e.g. Wareing 1956, Irgens-Moller

1957, Vaartaja 1959, Nienstadt and Olson 1961, Lavender *et al*. 1968, Heide 1974, Nelson and Lavender 1976, Bongarten and Teskey 1987, Joly *et al*. 1989, Grossnickle *et al* 1991).

In Model 1, in which all 18 populations were included without family structure, differences among populations were evident for all shoot growth traits measured (Table 28a). When family structure was included in the ANOVA with the nine populations and three families per population (Model 2), populations accounted for little of the variability and were significant ($p<.05$) for only three of the traits measured (rgr1.4, rgr1.6, and rgr1.7) (Table 28b). All traits had significant family within population effects.

More variation was attributed to populations under Model 1 as compared to Model 2. This difference can be explained by a combination of more southern and northern populations included in Model 1, and the effect of families within population on estimation of population mean squares. Levels of variance attributed to families within populations (Model 2) and populations (Model 1) are similar to those presented in Chapter 2, with two to six times more variation attributed to families within populations for second-year growth traits.

No population or family within population interactions with environments were evident in either model for all shoot growth traits. In most cases, the mean squares for these interactions were very small, with F ratios resulting in $p>0.5$.

There were significant replication by population interactions in Model 1 and replication by family within population interactions in Model 2 for shoot growth traits (Table 28). Thus, although population effects were significant in Model 1 and family within population effects in Model 2,

these are not strictly interpretable. However, the amount of variation attributed to these interactions are minimal relative to genetic effects. The major cause of these interactions is most likely small plot size.

In the only other comprehensive study reported in the literature with a *Cupressaceae* species involving genetic structure and environment stress, Harry (1987) showed that shoot relative growth rate response to moisture stress was very sensitive, showed very little genetic variability, and no significant genotype by environment interactions. Genetic differences among families within stands were apparent at the beginning of the study prior to the start of moisture stress, however, during the study very little variation was attributed to genetics and most to differences among environments (Harry 1987). In the present study, genetic variability among families within populations, although significant, did decrease substantially during the course of the study and most of the variability was attributed to differences between either or both the photoperiod and soil moisture treatments.

b) dry weight production

Seedlings grown under both the LW and SW environment produced the greatest total dry weight, seedlings grown under SD the least, and LD intermediate (Table 27). Distribution of dry matter was influenced by the environments such that seedlings grown under LW and SW had more stem weight and branch weight than those in the LD and SD environments, and seedlings grown under the SW regime had more root weight than those grown under LD.

Total dry weight differed only between the two soil moisture treatments according to ANOVA (Table 29). Stem weight was strongly influenced by drought, but only marginally so by photoperiod, whereas branch weight was

Table 29: Mean squares for yellow-cedar dry-weight and branching traits from the greenhouse environment study: a. Model 1: population structure only; b. Model 2: population and family (population)

a.

Source[2]	Trait[1]						
	rtwt	stwt	brwt	totdw	brang	brlgth[3]	brno
L	30.89[4]	3.13	39.32**	5.10	1700	222.40**	100.57
W	70.18	33.29***	217.38***	700.51***	7438	218.54**	356.6**
R(LW)	17.14***	1.25***	2.29	17.32*	1520***	11.90***	28.34
P	3.85**	2.56***	12.07***	28.77***	565***	11.05***	91.59***
P*R(LW)	1.61	0.41*	2.53*	8.54	186*	3.42	17.44
error	1.40	0.33	2.01	7.83	153	3.01	15.67

b.

L	36.02	6.67*	33.10*	4.58	813	255.45**	244.56**
W	76.80	35.69***	186.46***	676.56***	7470	220.50**	569.25**
R(LW)	23.73***	1.14	4.29	41.53***	1646***	14.24***	17.22
P	1.56	2.81	10.66	23.78	613	10.77	136.0
P*R(LW)	1.59	0.43***	2.89	9.15	179***	3.60	14.29
F(P)	3.83**	3.44***	14.53***	44.00***	666***	24.32***	155.13***
F(P)*R(LW)	1.84***	0.39***	2.45***	9.29***	192***	3.28**	13.97
error	1.13	0.23	1.57	5.88	123	2.37	12.30

1. See Table 24 for explanation of trait abbreviations
2. L = light, W = water, R = replication, P = population, F = family (population)
3. ms (brlgth) x 10^{-2}
4. Probability levels: * p<.05; ** p<.01; *** p<.001

influenced by both treatments. Root weight did not differ for any of the treatments. There were no interactions between photoperiod and soil moisture for any of the dry weight traits.

Significant differences among populations in Model 1 (Table 29a) and for family within populations for Model 2 (Table 29b) were present for total dry weight and its components. There were no genotype by environment interactions in any of the dry weight traits. There were significant replication by genetic interactions for a number of the traits, mostly in Model 2 (Table 29b). Similar to shoot growth traits, the amount of variation attributed to these interactions were minimal.

Caution should be used in interpreting the statistical results of the components of total dry weight since the distribution of dry matter is influenced by total dry weight. However, the above analyses were presented in order to facilitate comparisons to other reported literature involving indeterminate species and to compare to the ANCOVA results presented later.

In the study with yellow-cedar (Arnott *et al.* 1992), shoot dry weight was significantly greater in the seedlings grown under normal watering regimes compared to those growing under drought, and no significant difference was found between watering regimes for root dry weight. As well, no significant differences were found for any dry weight component between long (16 hours) and short (8 hours) photoperiods. However, unlike the study reported here, Arnott *et al.* (1992) found significant moisture by photoperiod interactions for shoot dry weight and total dry weight.

Major *et al.* (1993) showed similar results for western redcedar such that 1-year-old seedlings grown under regular watering regimes and long- or short-days produced more shoot dry weight than seedlings grown under drought

stress in either long- or short-days. As well, no differences were evident among treatments for root dry weight.

c) dry weight partitioning

Within each population, and considering all populations together, there were no significant differences in either environment means (intercept) or environment by log(total dry weight) effects (results not shown). The covariate, log(total dry weight), was significant ($p<0.001$) in every case. Thus environments did not significantly change yellow-cedar growth allometric parameters. Allocation of dry matter to roots, stems and branches within each environment did not differ among populations and was only significant for slope for roots and stems in the long-day, dry environment (Table 30).

The above results are in contrast to those presented in the last section for dry weight traits not adjusted for total weight. With total dry weight taken into consideration, moisture and photoperiodic effects had no significant impact on dry matter allocation and populations had minimal impact. Bongarten and Teskey (1987), reported significant differences in dry weight allocation in loblolly pine populations in response to moisture. In general, seedlings grown under the dry regime had an increase in the allocation of root weight at the expense of stem weight. This response varied significantly by population. There was no significant change in dry matter allocation to branches in response to moisture, however, there was a significant difference among populations (Bongarten and Teskey 1987).

The allometric relationship has been used in past studies to investigate the changes in root:shoot dry weight ratios with environment treatments or genetics (e.g. Ledig and Perry 1965, Ledig *et al.* 1970, Cannell

Table 30: Mean squares from analysis of covariance for yellow-cedar dry weight traits from the greenhouse environment study, by environment

Source[2]	Environments[1]								
	LD			LW			SD		
	root[3]	stem	branch	root	stem	branch	root	stem	branch
P	0.037[4]	0.068	0.012	0.005	0.023	0.003	0.017	0.025	0.017
R	0.077	0.021	0.038*	0.154***	0.116*	0.042**	0.041	0.044	0.025
P*R	0.031*	0.032	0.015*	0.024	0.026	0.006	0.025*	0.048*	0.018*
Cov	4.973***	4.978***	7.418***	6.917***	8.02***	8.58***	4.978***	5.976***	10.06***
P*Cov	0.035*	0.067***	0.011	0.004	0.019	0.003	0.015	0.022	0.015
R*Cov	0.063*	0.017	0.030*	0.083*	0.063	0.027*	0.043*	0.040	0.026
error	0.015	0.020	0.008	0.020	0.027	0.006	0.013	0.025	0.009

1. LD = long day, dry; LW = long day, wet; SD = short day, dry; SW = short day, wet
2. P = population, R = replication, Cov = $\log_e$ (total dry weight)
3. Root = $\log_e$ (root dry weight), stem = $\log_e$ (stem dry weight), branch = $\log_e$ (branch dry weight)
4. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

Table 30: (con't)

Source[2]	Environments[1]		
	SW		
	root	stem	branch
P	0.011	0.023	0.008
R	0.047	0.016	0.035*
P*R	0.021	0.035	0.013
Cov	6.560***	7.733***	9.814***
P*Cov	0.011	0.023	0.007
R*Cov	0.049*	0.006	0.038*
error	0.014	0.030	0.009

1. LD = long day, dry; LW = long day, wet; SD = short day, dry; SW = short day, wet
2. P = population, R = replication, Cov = $\log_e$ (total dry weight)
3. Root = $\log_e$ (root dry weight), stem = $\log_e$ (stem dry weight), branch = $\log_e$ (branch dry weight)
4. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

and Willett 1976, Cannell *et al.* 1978). In general, for determinate conifers, it has been shown that log(root:shoot dry weight ratio) decreases linearly with decreasing log(total plant dry weight). As well, log(shoot dry weight) has been shown to be linearly correlated with log(root dry weight). In this study in which an indeterminate species was used, the log(root, stem, and branch dry weights) were found to be significantly correlated with log(total dry weight). However, there was no observed relationship between log(shoot dry weight) and log(root dry weight), or log(root:shoot dry weight ratio) with log(total dry weight).

As Ledig *et al.* (1970) pointed out, many observed changes in allocation of dry weight with environmental or genetic treatments were not significant when data were adjusted for total dry weight. Such was the case with this study. Changes in allocation of biomass to roots may not happen in indeterminate species in response to stress since shoot growth is not arrested permanently for the season and buds are not formed (Krasowski and Owens 1991). Upon release of the stress, growth resumes, and at times, at a greater rate than for shoots that have not undergone the stress.

d) seedling crown traits

The number of branches greater than 1 mm and length of longest branch at mid-crown were both sensitive to environments such that seedlings grown under LW environment produced the most branches and longest mid-crown branch on average, as compared to seedlings grown under the SD regime (Table 27). Both traits were influenced by photoperiod and moisture treatments (Table 29) and showed significant genetic variability at the population level under Model 1 and at the family within population level in Model 2. Again there

were no significant interactions of photoperiod and watering regime or environments and genotypes.

The acute angle of the mid-crown branch was not influenced by environment (Tables 27 and 29), however, there were genetic differences at both the population (Model 1) and family within population (Model 2) level. It is well documented in the literature that branch angle is a highly heritable trait, and as such, environment has a minimal impact on its phenotypic expression.

e) stability

According to Huhn's rank stability measures (Table 31), there were no significant differences in stability among the 18 populations or the 27 families for all traits measured. Only three of the test statistics were significant for the variance of rank deviations ($p<0.05$). Thus, results from both the ANOVA and rank stability measures are consistent and indicate the lack of genotype by environment interactions. Consistent results between mean squares for genotype by environment and rank stability measures have been reported elsewhere (Skroppa 1984, St. Clair and Kleinschmit 1986).

f) trait correlations with population origin

Correlations of growth and morphology traits with geographic descriptors were minimal. This is not surprising since the populations used in this study did not represent the entire range of yellow-cedar's geographic

Table 31: Chi-square tests for rank stability statistics of 18 populations and 27 families of yellow-cedar grown over four environments for shoot growth and morphological traits

Trait[1]	Population[2]		Family[3]	
	S1	S2	S1	S2
ht2	23.62[4]	25.14	26.33	37.15
htgrw2	25.20	37.77 **	22.76	33.35
rgr2	27.32	35.47 **	25.48	29.92
rcd2	20.41	23.15	29.19	36.17
rtwt	20.26	20.98	23.51	28.27
stwt	19.66	20.25	17.76	25.33
brwt	18.78	21.97	29.14	42.58 *
totdw	12.48	14.73	26.35	29.09
brno	19.89	27.24	31.42	35.56
brlgth	19.76	19.41	23.87	35.33

1. See Table 24 for explanation of trait abbreviations
2. $\chi^2_{0.05;17} = 27.6$
3. $\chi^2_{0.05;26} = 38.9$
4. Probability levels: * $p<.05$; ** $p<.01$

distribution. The more southerly populations from Oregon were not included in this study (lack of seedlings) and, as discussed in Chapter 2, significant adaptive trends are only apparent when seedlings from these populations are included.

Correlations of growth and morphology traits with latitude and elevation by environment are presented in Table 32 for those combinations which were significant ($p<0.05$). The most striking result is the stronger and more abundant correlations using plants grown under the intermediate

Table 32: Simple linear correlation coefficients[1] for growth and morphology traits based on population mean and geographic descriptors of seed origin for 2-year-old yellow-cedar grown under four environments (n=17)

Environment[3]	Trait[2]				
	ht2	rcd2	rtwt	stwt	brwt
LD					
latitude	-.47	-.58	-.52	-.48	
elevation					-.57
LW					
latitude		-.46			
elevation	.48				
SD					
latitude		-.57			.43
elevation		.46			
SW					
latitude		-.50	-.40		
elevation		.57			-.43

1. Only those correlation coefficients in which $p<.05$ are presented
2. See Table 24 for explanation of trait abbreviations
3. LD = long day, dry; LW = long day, wet; SD = short day, dry; SW = short day, wet

stressful environments (LD and SW). Seedlings grown under only one stress, for example drought, had significant correlations between latitude of seed origin and total height, root collar diameter, root weight and shoot weight. When grown under no moisture or light stress, only root collar diameter was significantly correlated with latitude.

There was no evidence from this study of photoperiodic ecotypes. If photoperiodic ecotypes are present, then seedlings originating from populations with lower probabilities of early fall frosts (i.e. more

southerly populations) should grow relatively more into the fall than seedlings from populations with a greater chance of early fall frost (i.e. more northern populations) when grown under shortened photoperiods (Vaartaja 1959, 1962). This was not the case for the results presented from this study for all morphological traits analyzed. Apparent discrepancies with the results from Chapter 2, in which more southerly populations inherently had greater shoot elongation and diameter growth, and grew longer into the fall in a common-garden at approximately 49° latitude could be explained by the small differences in duration of daylength between the two photoperiods and by the lack of Oregon populations in this study. Demonstration of photoperiodic ecotypes for indeterminate species (Vaartaja 1959, 1962) required the comparisons of very long photoperiods (18-20 hours) versus very short periods (<8 hours).

On the other hand, the evidence of strong plastic control of shoot growth and other morphological traits, along with the apparent lack of population by environment interactions, is indicative of less population differentiation in photoperiodic response for yellow-cedar.

3.3.2 Cold-hardiness

All main effects except photoperiod treatments were large and significant for index of injury (Table 33). There were significant interactions for watering regime and population, temperature and population, and watering regime, temperature and population. Seedlings from the short-day, dry environment were the most cold-susceptible with an LT_{50} of -12.6° C, and seedlings from the long-day, wet environment were the least susceptible

Table 33: ANOVA model and mean squares for cold-hardiness testing (index of injury) of two-year-old yellow-cedar seedlings from the greenhouse environment study

Source[1]	df	Mean Squares	
L	1	85.7[2]	
W	1	428.7	***
T	2	16527.8	***
P	3	699.6	***
LW	1	21.3	
LT	2	65.7	
LP	3	53.2	
WT	2	43.8	
WP	3	449.1	***
TP	6	146.0	**
LWT	2	82.1	
LWP	3	87.2	
LTP	6	28.7	
WTP	6	105.1	*
LWTP	6	0.7	
error	140	39.6	

1. L = light, W = water, T = temperature, P = population
2. Probability levels: * $p<.05$; ** $p<.01$; *** $p<.001$

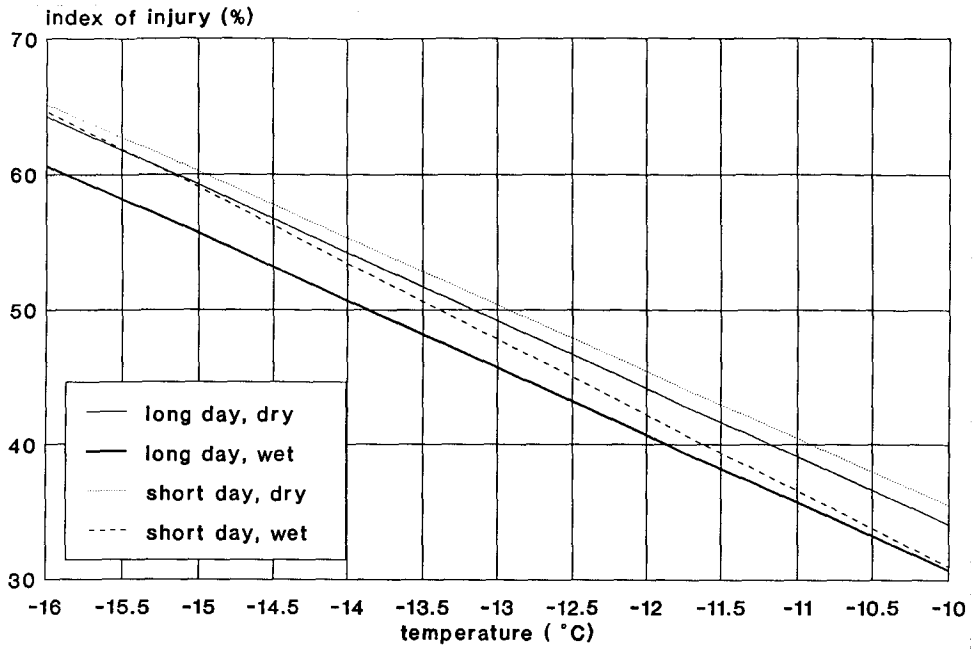
(LT_{50} = -13.9° C) (Table 34). The most southern population (Mt. Angeles) was the most cold-susceptible with an LT_{50} of -12.2° C, and Kwatna Inlet, a mid-coast population was the least susceptible (LT_{50} = -14.2° C) (Table 34).

Table 34: Regression equations for index of injury and test temperature, and LT_{50} values for yellow-cedar populations from the greenhouse environment study, by environment

Population[1]	Treatment[2]	Regression Equation[3]	r^2	LT_{50}[4] (°C)
11	LD	$\hat{Y}i = 5.07 + 13.14\ x$	.94	-14.26
	LW	$\hat{Y}i = 0.36 + 14.44\ x$	.88	-14.32
	SD	$\hat{Y}i = 1.51 + 15.40\ x$	.99	-13.46
	SW	$\hat{Y}i = -13.82 + 18.05\ x$	.94	-14.60
19	LD	$\hat{Y}i = 10.34 + 13.15\ x$	.89	-13.06
	LW	$\hat{Y}i = 15.27 + 11.93\ x$	.99	-12.72
	SD	$\hat{Y}i = 1.57 + 15.53\ x$	.99	-13.35
	SW	$\hat{Y}i = 8.29 + 14.69\ x$	.96	-12.51
31	LD	$\hat{Y}i = 17.70 + 12.98\ x$	.98	-11.46
	LW	$\hat{Y}i = -11.19 + 18.57\ x$	.91	-14.32
	SD	$\hat{Y}i = 28.80 + 11.02\ x$	.91	-9.78
	SW	$\hat{Y}i = -3.63 + 17.41\ x$	.97	-13.25
42	LD	$\hat{Y}i = -20.66 + 21.75\ x$	.85	-13.75
	LW	$\hat{Y}i = -10.84 + 17.53\ x$	.97	-14.40
	SD	$\hat{Y}i = -9.04 + 18.19\ x$	.90	-13.74
	SW	$\hat{Y}i = -7.25 + 18.42\ x$	.94	-13.32

1. 11 = Kwatna Inlet, B.C.; 19 = Holberg, Vancouver Island; 31 = Mt. Angeles, WA; 42 = Mitkof Island, AK
2. LD = long day, dry; LW = long day, wet; SD = short day, dry; SW = short day, wet
3. $\hat{Y}i$ = predicted index of injury (%); x = test temperature (°C)
4. LT_{50} = temperature that will result in a predicted index of injury of 50%

Figure 8 graphically illustrates the cold-hardiness regression equations by environment, and Figures 9 to 12 separately for each population by environment. As well, the same figures show the relative growth rate during the last 6 weeks of measurement (rgrl.7) by environment, and separately for each population by environment. As illustrated in Table 27 and Figure 7, upon release of both the drought treatment and the short photoperiod, the relative growth rates surpassed those of the seedlings that



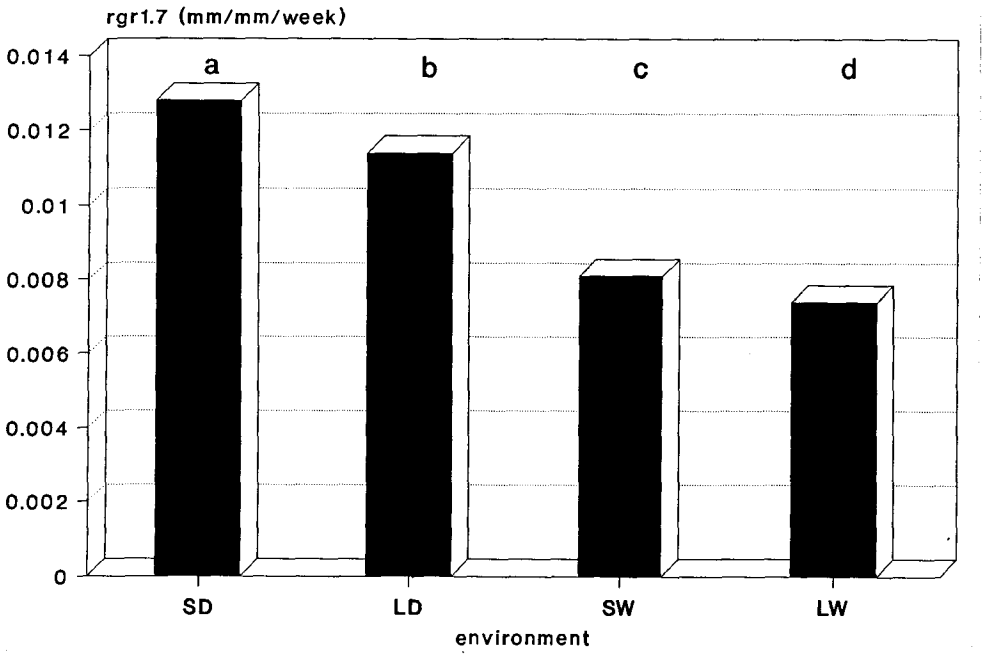
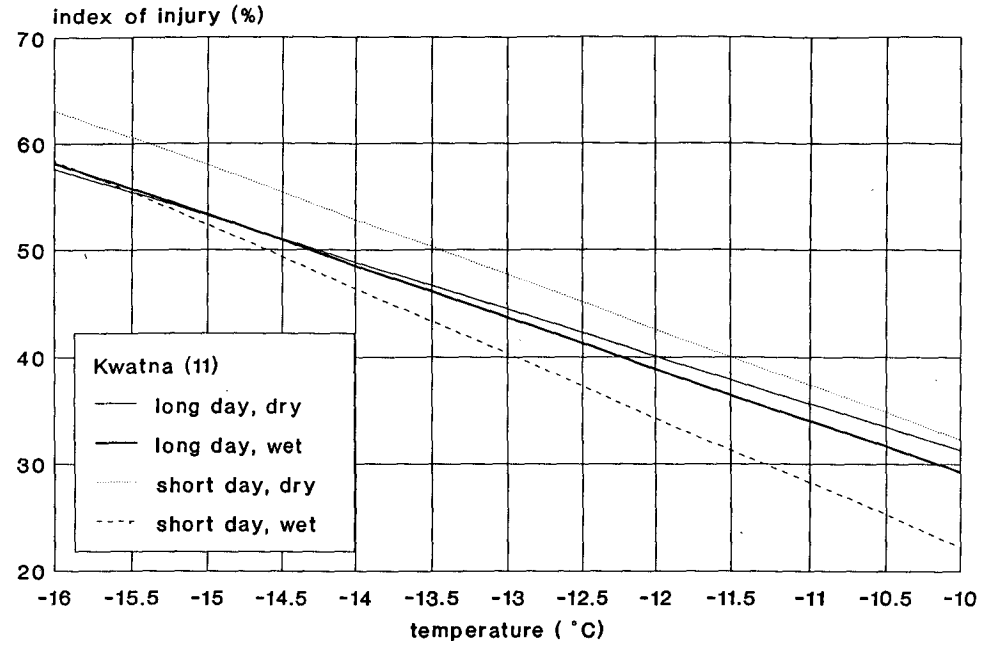


Figure 8: Index of injury predicted response to temperature from regression analysis and relative growth rate for a six-week period prior to cold-hardiness testing for 2-year-old yellow-cedar seedlings grown under four environments. Different letters between environments for the growth data denotes significance at $p<.05$ according to Duncan's New Multiple Range Test



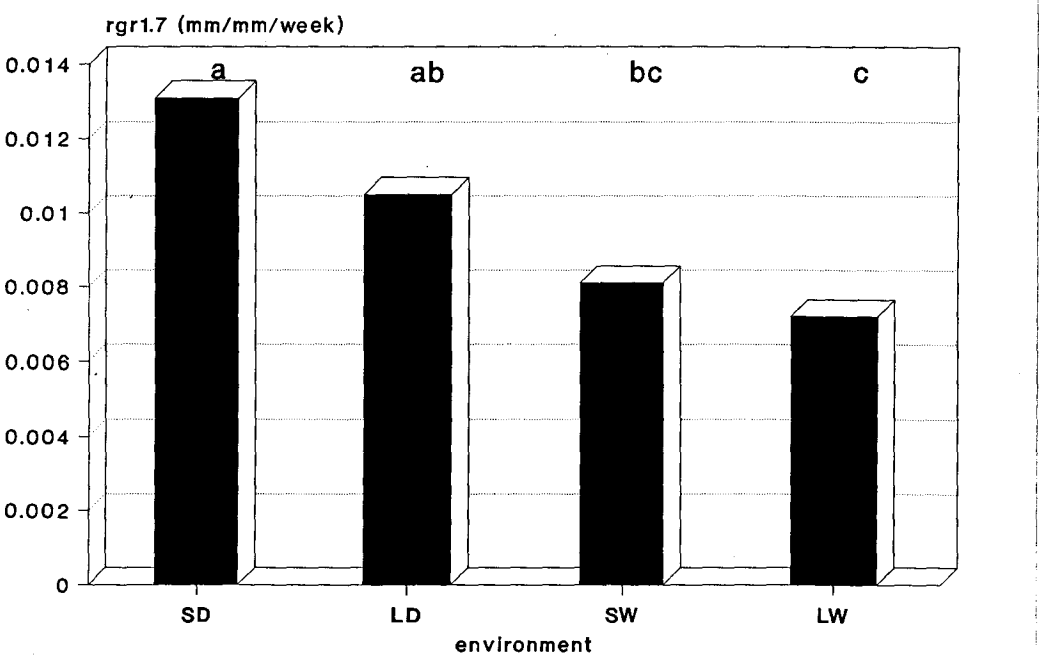
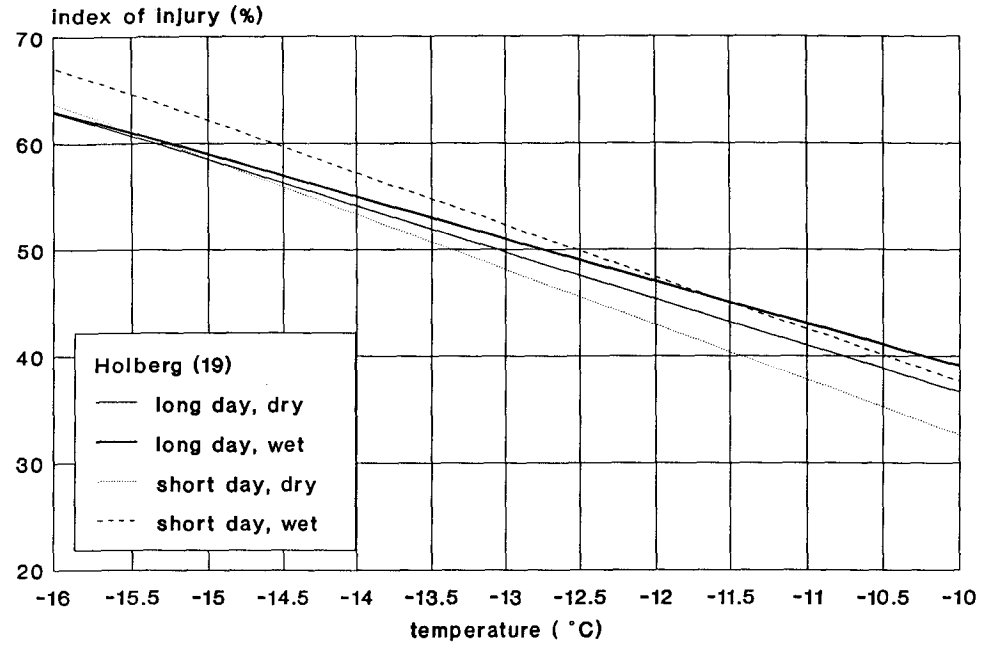


Figure 9: Index of injury predicted response to temperature from regression analysis and relative growth rate for a six-week period prior to cold-hardiness testing for 2-year-old yellow-cedar seedlings from Kwatna Inlet grown under four environments. Different letters between environments for the growth data denotes significance at $p<.05$ according to Duncan's New Multiple Range Test



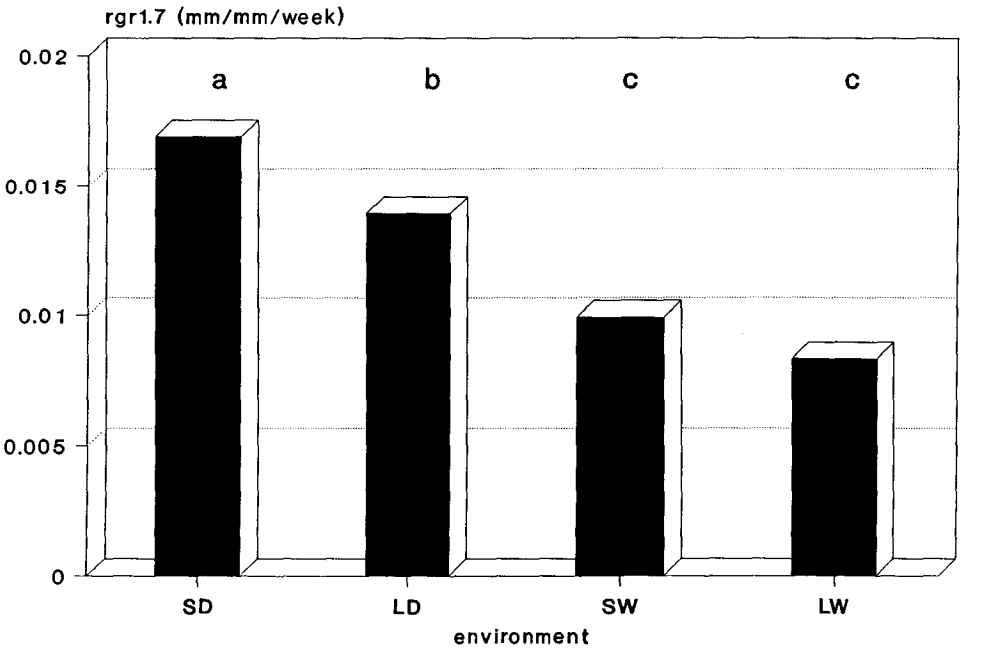
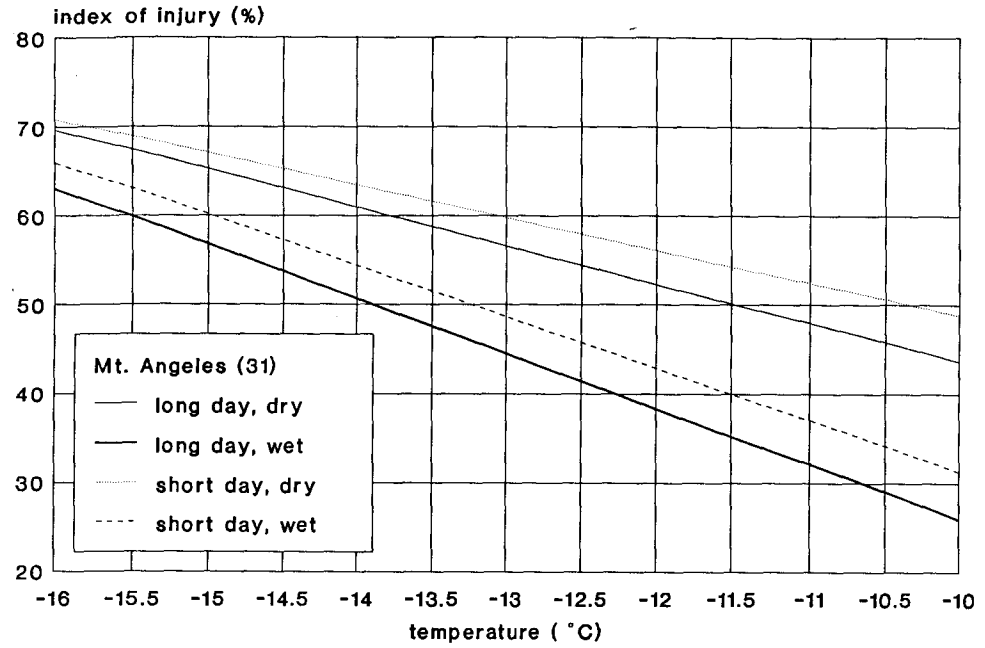


Figure 10: Index of injury predicted response to temperature from regression analysis and relative growth rate for a six-week period prior to cold-hardiness testing for 2-year-old yellow-cedar seedlings from Holberg grown under four environments. Different letters between environments for the growth data denotes significance at $p<.05$ according to Duncan's New Multiple Range Test



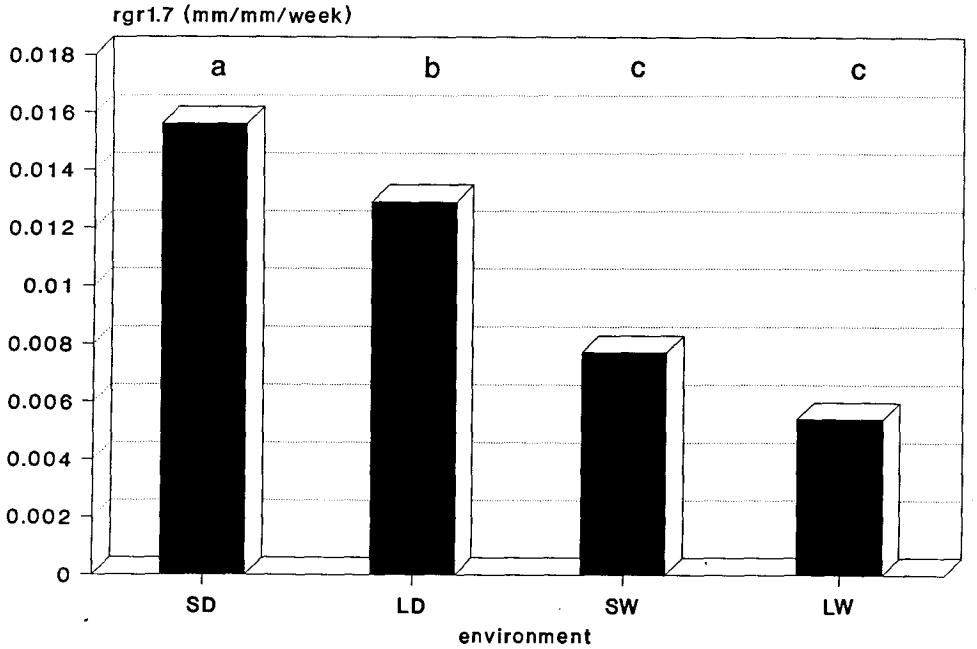
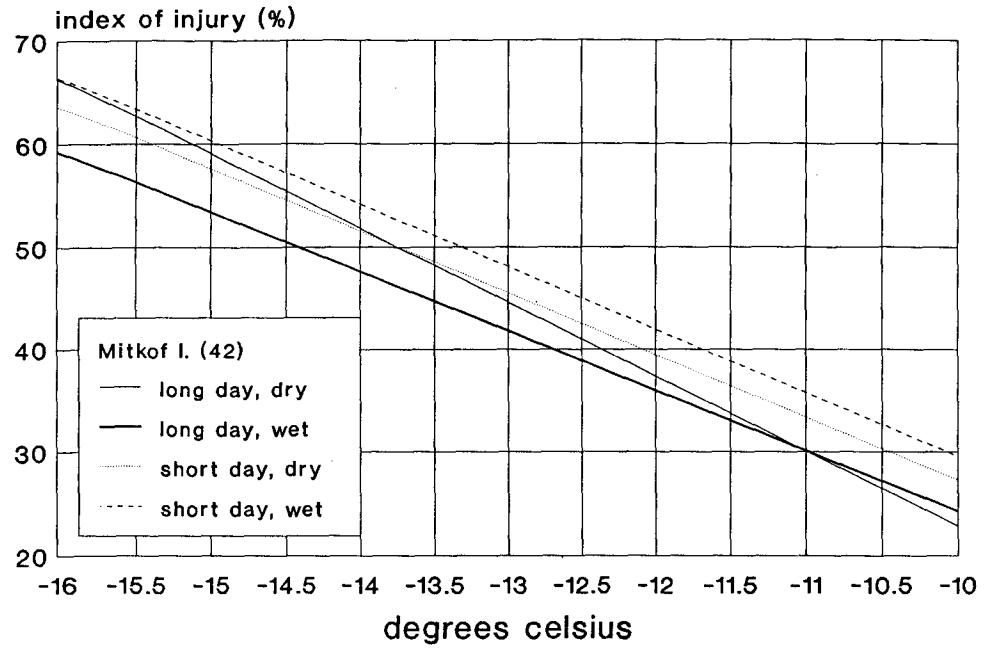


Figure 11: Index of injury predicted response to temperature from regression analysis and relative growth rate for a six-week period prior to cold-hardiness testing for 2-year-old yellow-cedar seedlings from Mt. Angeles grown under four environments. Different letters between environments for the growth data denotes significance at $p<.05$ according to Duncan's New Multiple Range Test



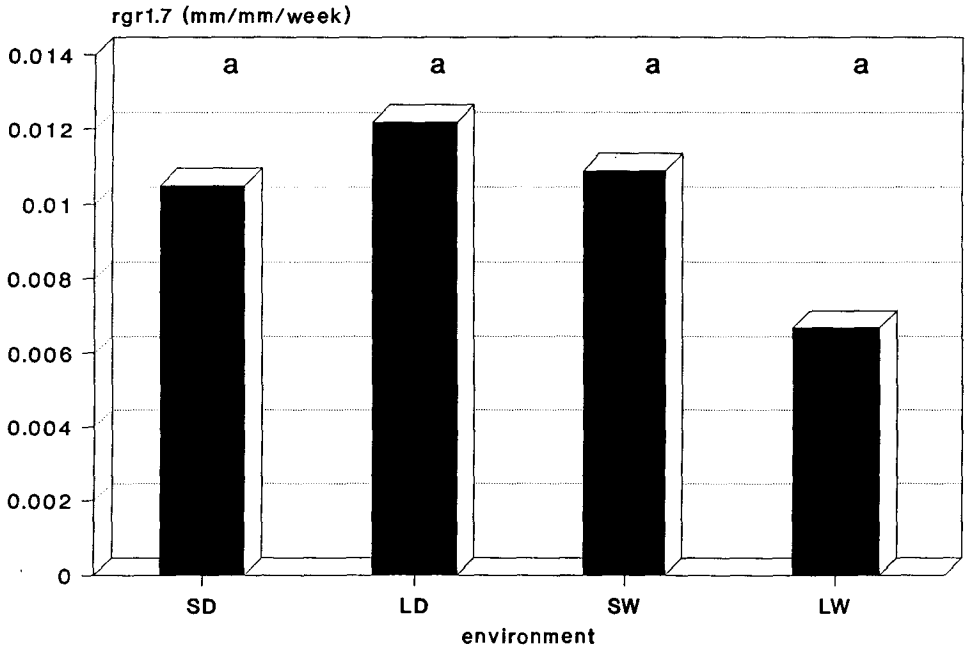


Figure 12: Index of injury predicted reponse to temperature from regression analysis and relative growth rate for a six-week period prior to cold-hardiness testing for 2-year-old yellow-cedar seedlings from Mitkof Island grown under four environments. Different letters between environments for the growth data denotes significance at $p<.05$ according to Duncan's New Multiple Range Test

were growing in the wet and long photoperiod environment, respectively. Thus, as can been seen in Figure 8, seedlings from the short-day, dry environment continued to grow the most into late September and October, and were the most susceptible to cold. Seedlings from the long-day, wet environment had the slowest relative growth rate and were the most cold-resistant.

This trend generally can be seen at the population level, however, deviations from this pattern are evident, and account for the significant genotype by environment interactions shown in Table 33. Seedlings originating from Mt. Angeles population illustrate the above relationship the best (Figure 11), with a three-fold difference in relative growth rate and a 4.5° C difference in LT_{50} between seedlings from the short-day, dry environment and those from the long-day, wet environment.

The above results for the effects of environment on cold-hardiness are different than most of the literature. As stated earlier, short photoperiod or mild drought stress during the growing season, can result in greater cold-hardiness during the acclimation period for determinate conifer species. This is an indirect response related to decreased shoot growth and earlier budset for seedlings grown under stress (Glerum 1985).

Increased cold-hardiness for indeterminate species grown under short photoperiods has been reported for yellow-cedar (Arnott *et al.* 1992), eastern white cedar (Colombo and Raitenan 1991) and for western redcedar (Major *et al.* 1993). However, in the yellow-cedar study (Arnott *et al.* 1992), cold-hardiness was measured immediately following the end of the photoperiod treatment, in the study on eastern white cedar (Colombo and Raitenan 1991), the short photoperiod was never released during cold-hardiness testing, and

in the western redcedar study (Major *et al.* 1993), the seedlings were immediately planted in the field upon release of the photoperiod treatment. Thus, shoot growth was not able to resume in any of the above studies prior to cold-hardiness testing. Silim (1991) reported minimal differences in cold-hardiness of both yellow-cedar and redcedar seedlings in response to decreasing photoperiod or moisture stress.

The results of this study are consistent with others (Arnott *et al.* 1992, Krasowski and Owens 1991) with respect to increased shoot growth of stressed seedlings following release of the stress. In this study, and the above reported studies, measurements of shoot growth continued after release of the environment treatments while the seedlings remained in containers in a nursery environment. Thus, because cold-hardiness testing in this study was done 6 weeks after the release of the stress treatments, the results were influenced by the plastic response of yellow-cedar shoot growth. In subsequent cold-hardiness testing in the study by Arnott *et al* (1992), it was found that decreased photoperiods and moisture stress had a transitory effect on increasing cold-hardiness of yellow-cedar seedlings (P. Puttonen, B.C. Ministry of Forests, pers. comm.).

Increased cold susceptibility of more southern populations is consistent with results presented earlier (Chapter 2). Significant interactions of watering regime with population and temperature with population, are for the most part, scale effects.

3.3.3 Gas Exchange and Shoot Water Potential

The initial drydown cycle resulted in considerable variation in predawn shoot water potential (ßψ) at day 6, with seedlings from Coquihalla (16)

having the highest (-2.2 MPa), and Mitkof Island (42) the lowest (-3.7 MPa) average ßψ (Figure 13). Figure 14 illustrates the variation between two families within the Coquihalla population.

Both A and g_s responded to decreasing predawn shoot water potential in a pattern typical for yellow-cedar (Grossnickle and Russell 1991, Arnott *et al.* 1992). Net photosynthesis declined in a steady concave fashion from approximately -0.4 MPa and reached the compensation point between -2.0 and -2.7 MPa (Figure 15 and Table 35), similar to results presented for yellow-cedar 1-year-old seedlings by Grossnickle and Russell (1991) during a winter drydown and by Arnott *et al.* (1992) for 1-year-old yellow-cedar rooted cuttings during a summer drydown. Stomatal conductance declined rapidly at high ßψ values from -0.25 to -0.75 MPa, and tapered off until g_s reached near zero at ßψ less than -2.0 to -3.0 MPa (Figure 16 and Table 35). Grossnickle and Russell (1991) reported similar results, however, g_s declined more rapidly in response to soil moisture deficits and reached near zero at higher ßψ.

In the study by Grossnickle and Russell (1991), g_s declined more rapidly than A with decreasing shoot water potential, and declined gradually well above the winter turgor loss point (-2.4 MPa), owing to stomatal limitations. This study showed similar patterns, however, stomata closed at or near the turgor loss point after the drydown (see Table 38).

As the severity of drought increased, A decreased gradually with a decrease in g_s until approximately 0.4 cm s^{-1} at which time A decreased rapidly with a decrease in g_s (Figure 17). Arnott *et al.* (1992) reported a similar response for 1-year-old yellow-cedar rooted cuttings during a summer drydown.

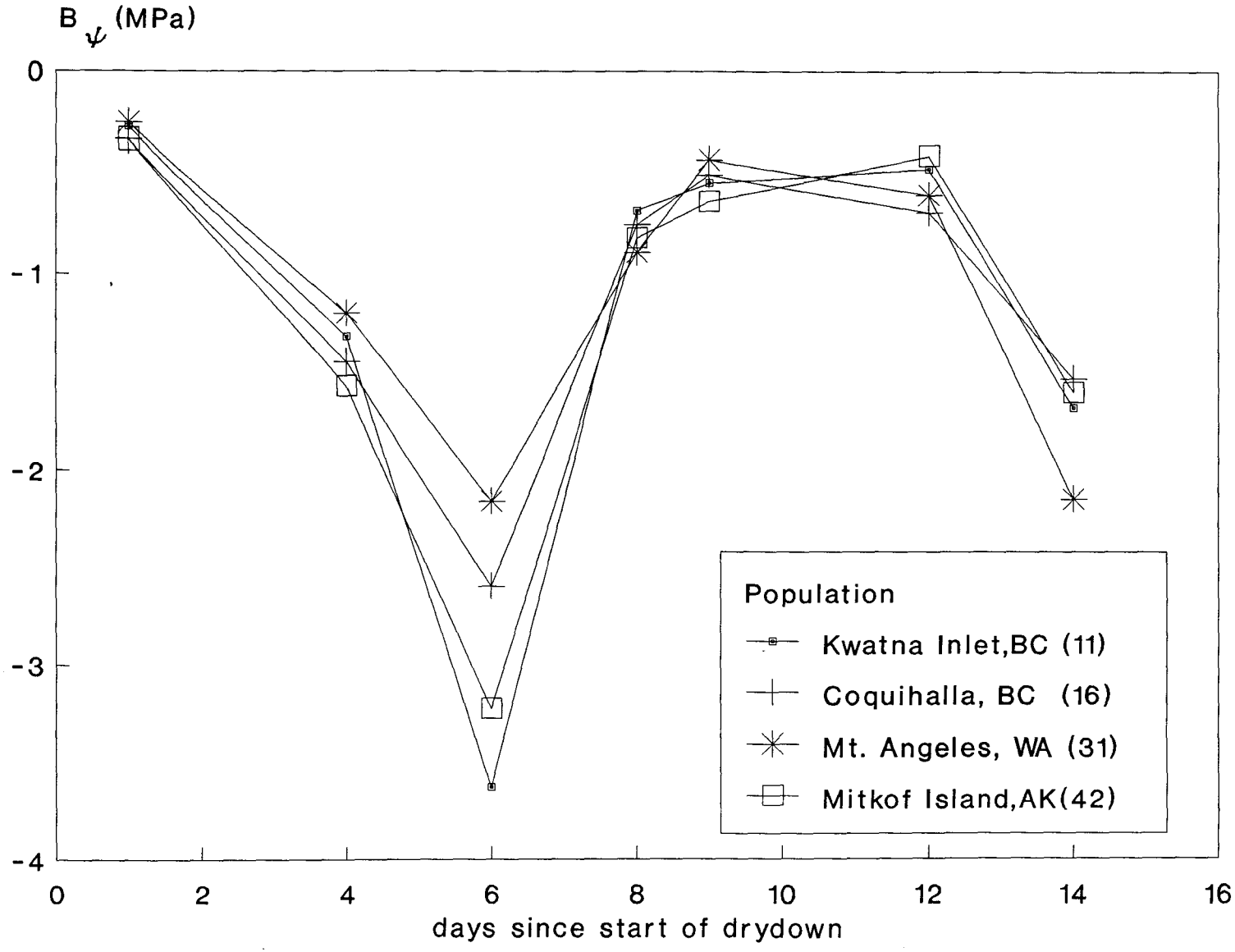


Figure 13: Average predawn shoot water potential for 2-year-old yellow-cedar seedlings from four populations for the first two drydown cycles of a 6-week drydown

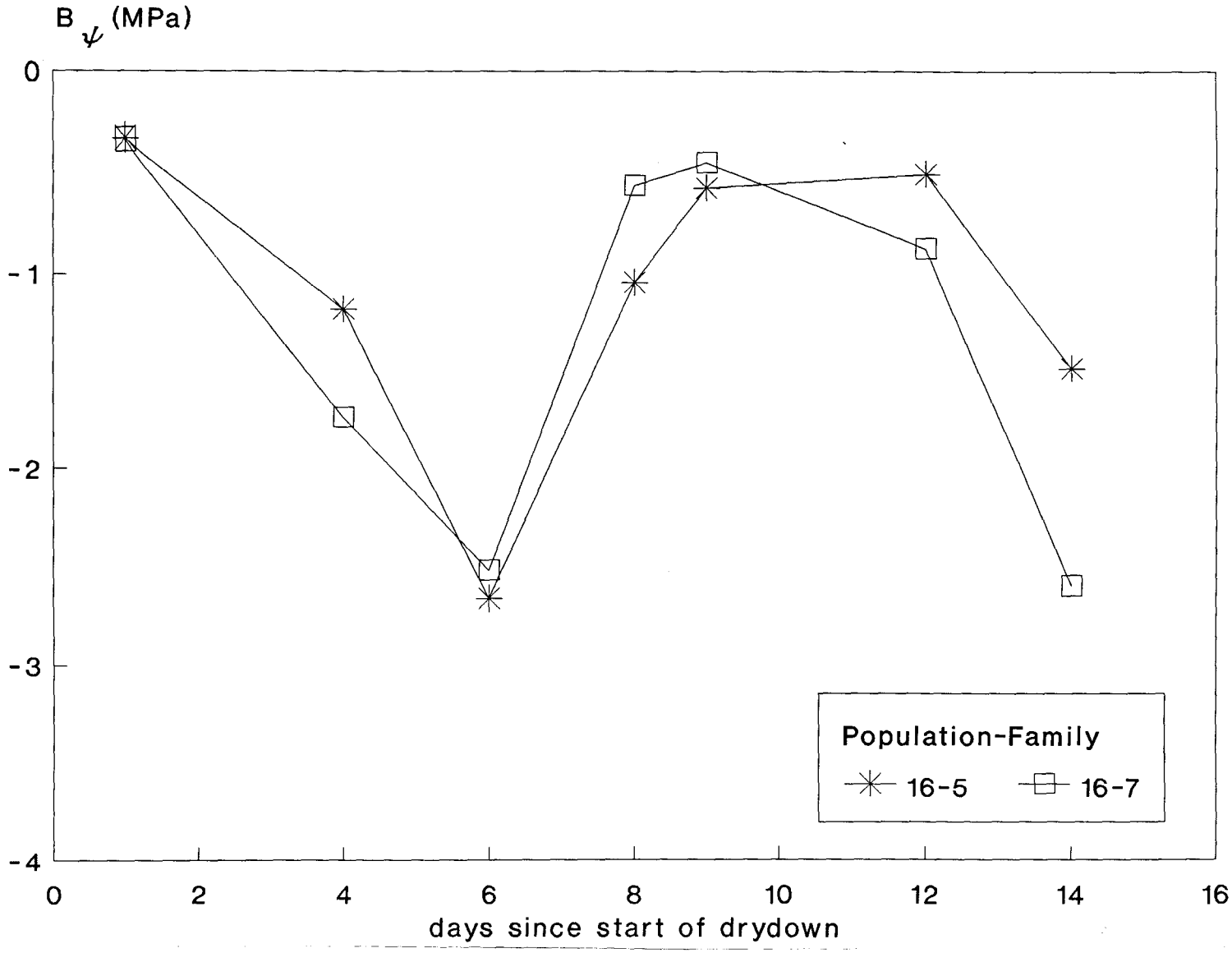


Figure 14: Average predawn shoot water potential for 2-year-old yellow-cedar seedlings from two open-pollinated families from Coquihalla population (16) for the first two drydown cycles of a 6-week drydown

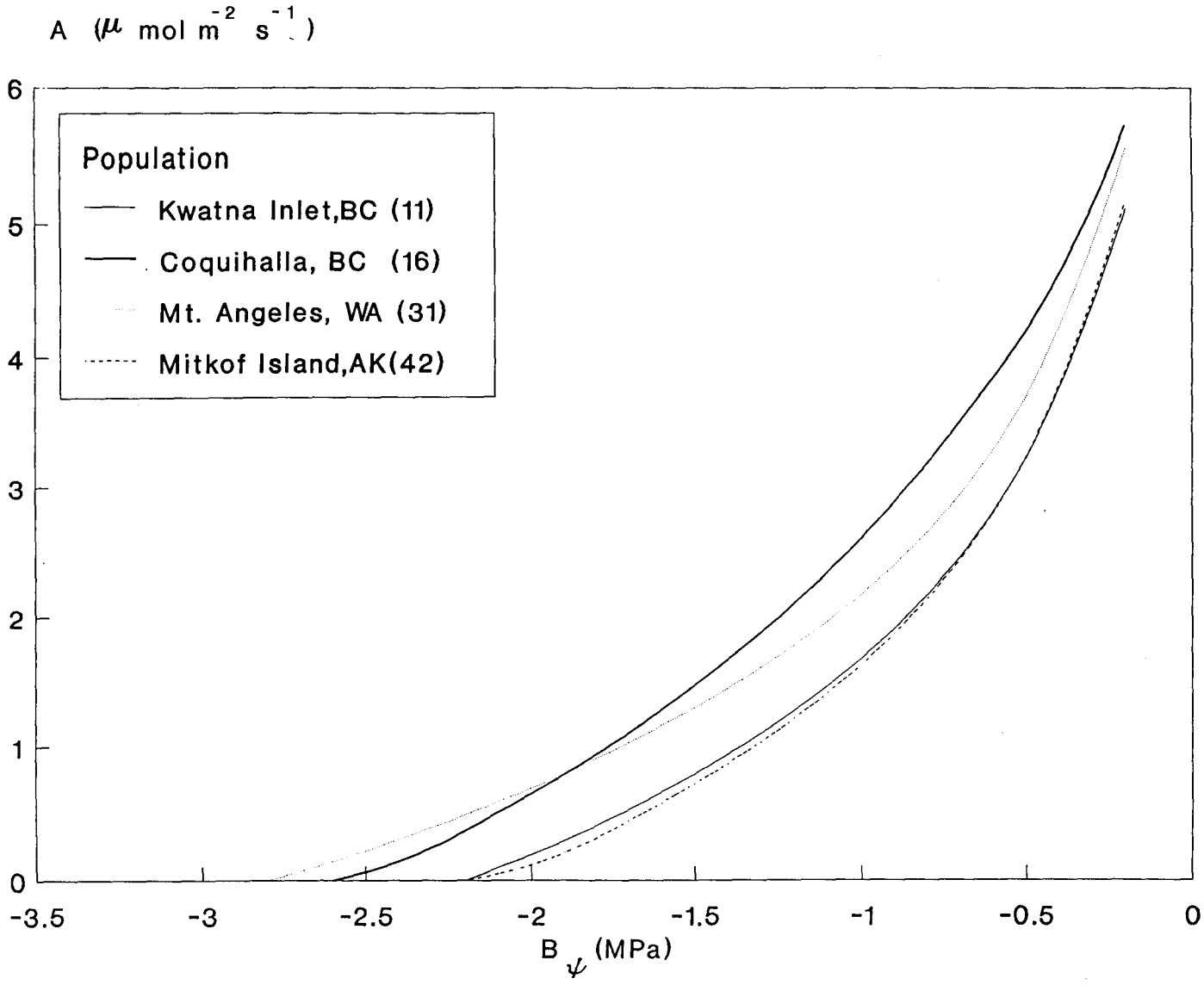


Figure 15: Net photosynthesis (A) predicted response to predawn shoot water potential (ßψ) from regression analysis for 2-year-old yellow-cedar seedlings from four populations (see Table 35 for regression equations)

Table 35: Regression equations for gas exchange and shoot water potential data for yellow-cedar populations

a. $y = A$; $x = ß\psi$[1]

Population[2]	Regression Equation	r^2	n
11	$\hat{Y}_i = 1.66 - 2.14 (\ln x)$	0.83	51
16	$\hat{Y}_i = 2.73 - 0.152 (1/x^2) - 2.97 (\ln x)$	0.58	49
31	$\hat{Y}_i = 2.15 - 2.12 (\ln x)$	0.75	48
42	$\hat{Y}_i = 1.61 - 2.21 (\ln x)$	0.91	25

b. $y = g_s$; $x = ß\psi$

Population	Regression Equation	r^2	n
11	$\hat{Y}_i = -0.009 + 0.029 (1/x)$	0.84	51
16	$\hat{Y}_i = -0.018 + 0.054 (1/x) - 0.005 (1/x^2)$	0.72	49
31	$\hat{Y}_i = -0.005 + 0.028 (1/x)$	0.82	48
42	$\hat{Y}_i = -0.014 + 0.033 (1/x)$	0.77	25

c. $y = A$; $x = g_s$

Population	Regression Equation	r^2	n
11	$\hat{Y}_i = 8.21 + 0.002 (1/x) + 1.58 (\ln x)$	0.83	128
16	$\hat{Y}_i = 10.74 + 0.006 (1/x) + 2.26 (\ln x)$	0.73	127
31	$\hat{Y}_i = 10.99 - 347 (x^3) + 0.009 (1/x) + 2.36 (\ln x)$	0.83	118
42	$\hat{Y}_i = 7.54 + 5.6 \times 10^{-6} (1/x^2) + 1.40 (\ln x)$	0.89	64

1. A = net photosynthesis ($\mu mol/m^2/s$)
   $ß\psi$ = predawn shoot water potential (MPa)
   g_s = stomatal conductance (cm/s)
2. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.; 31 = Mt. Angeles, WA; 42 = Mitkof Island, AK

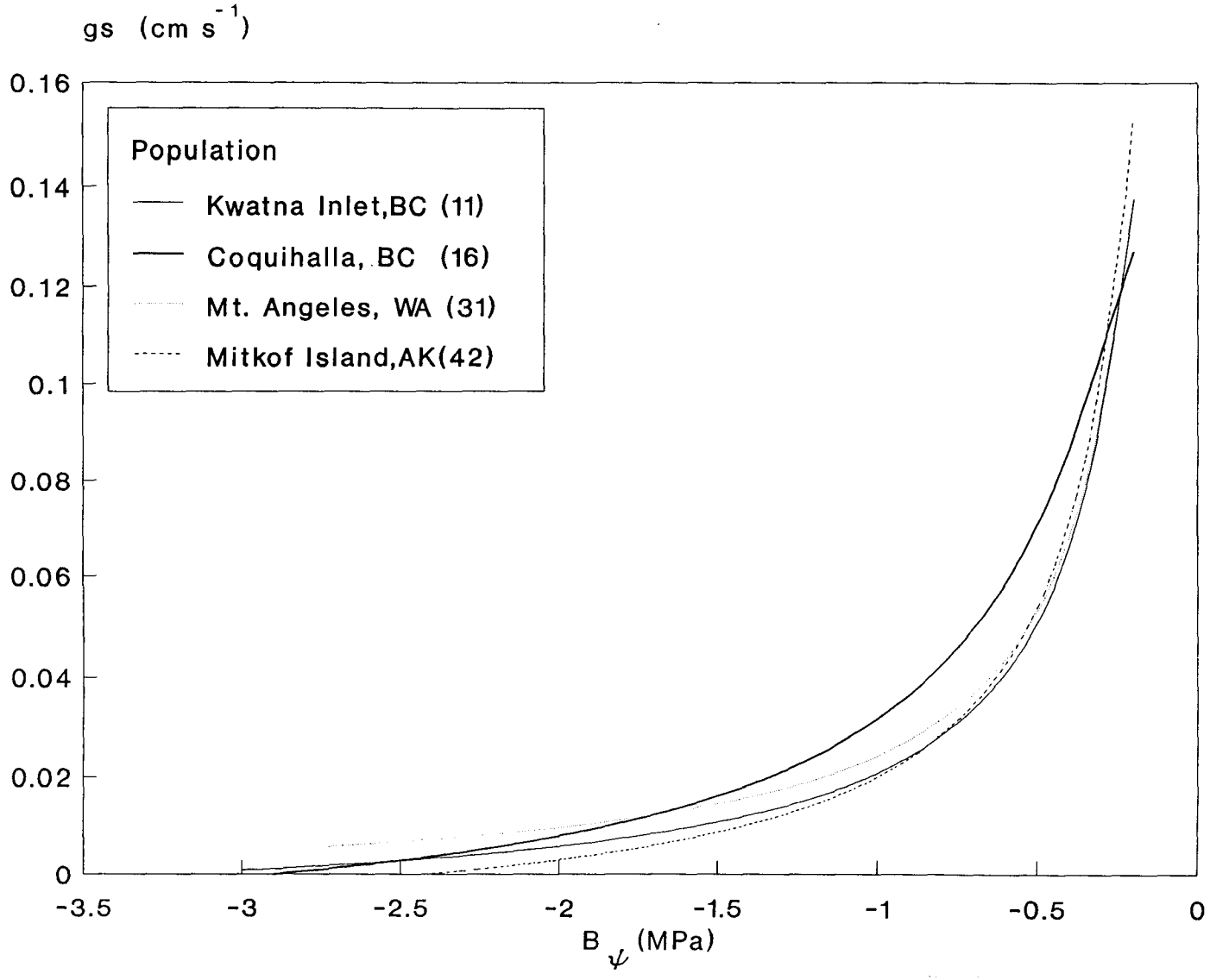


Figure 16: Stomatal conductance (g_s) predicted response to predawn shoot water potential (ßψ) from regression analysis for 2-year-old yellow-cedar seedlings from four populations (see Table 35 for regression equations)

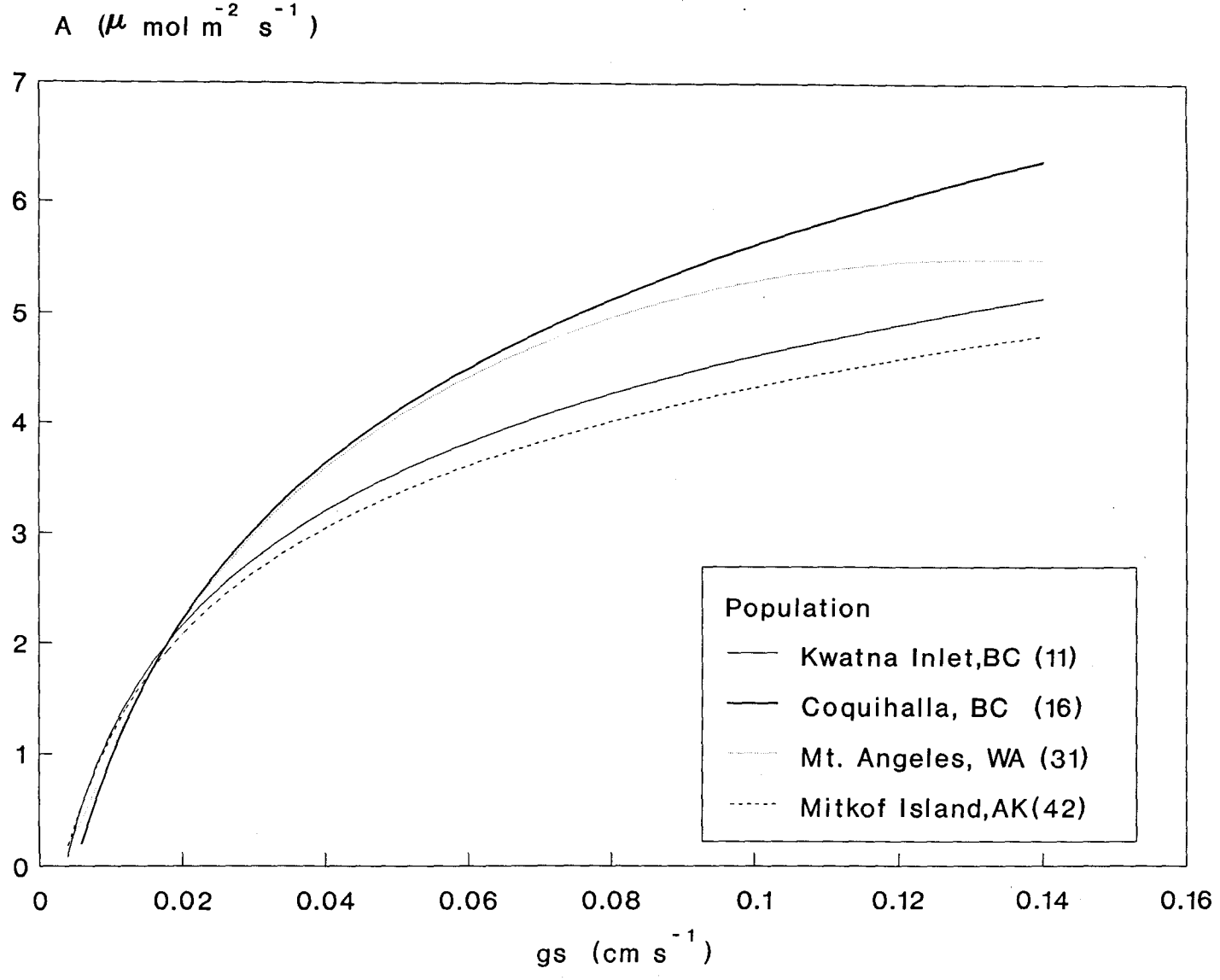


Figure 17: Net photosynthesis (A) predicted response to stomatal conductance (g_s) from regression analysis for 2-year-old yellow-cedar seedlings from four populations (see Table 35 for regression equations)

Kwatna Inlet and Mitkof Island, both windward, northern coastal, low elevation populations with relatively wet and mild summers (see Table 23), showed a more rapid decline in A and g_s with a decrease in $\beta\psi$ than Coquihalla, a high elevation, coastal:interior transition population with relatively drier and warmer summers (Figures 15 and 16). Mt. Angeles, a coastal, leeward, southern exposure, high elevation population was intermediate. As well, seedlings from both Kwatna Inlet and Mitkof Island populations showed a more gradual increase in A with an increase in g_s, while Coquihalla seedlings had the greatest increase, and Mt. Angeles seedlings, intermediate (Figure 17). At a given g_s, both Coquihalla and Mt. Angeles seedlings maintained higher levels of A than Kwatna Inlet or Mitkof Island, except at values greater than 0.25 cm s^{-1}.

Seedlings from Coquihalla had higher A and g_s under well-watered conditions (>-0.5 MPa) and were able to maintain higher levels of A and g_s under greater water stress. Seedlings from Mt. Angeles maintained intermediate levels of A with increasing water stress. Seedlings from Kwatna Inlet and Mitkof Island essentially reached the compensation point for CO_2 uptake at -2.0 MPa, whereas seedlings from Coquihalla and Mt. Angeles continued photosynthesis until -2.6 and -2.75 MPa, respectively.

These results compare favourably to those of Kelliher and Tauer (1980) with eastern cottonwood and Abrams *et al.* (1990) with green ash, in which plants from xeric habitats exhibited less stomatal sensitivity to drought, and greater photosynthesis (Levitt's (1980b) drought avoiders, water spenders). Seedlings from both Coquihalla and Mt. Angeles populations, both areas that receive substantially less summer rainfall than Kwatna Inlet and Mitkof Island populations, had less stomatal sensitivity and higher photosynthetic capacity under drought.

Considerable variation is evident among families within populations (Table 36). Using Coquihalla seedlings as an example, family 16-5 had a more gradual decrease in both A and g_s with decreasing water stress, and greater A with a given level of g_s (Figures 18, 19, 20). Those families that had the highest gas exchange at high base water potentials also had the highest under greater stress. Family 16-5, however, had greater A when g_s was high as compared to 16-7, but had similar rates under conditions of limiting soil moisture.

Different trends among populations with respect to water use efficiency were evident (Figure 21 and Table 37). At low ßψ (<-0.5 MPa) all populations had similar WUE values. However, both Coquihalla and Mt. Angeles seedlings maintained higher WUE values with increasing water stress than either Kwatna Inlet or Mitkof Island (no regression equation was fitted to the data). Kwatna Inlet seedlings had decreased WUE values after a ßψ of -0.7 MPa , and Mitkof Island seedlings had low WUE with $\beta\psi$ values less than -0.5 MPa.

At lower ßψ, both Coquihalla and Mt. Angeles seedlings maintained a higher WUE over Kwatna Inlet and Mitkof Island seedlings because of continued higher A with decreasing g_s. Seedlings from Mt. Angeles had a lower WUE than Coquihalla at low ßψ because of a combination of lower A and higher g_s. Seedlings from areas that experience warmer and drier weather during the summer growing season are able to assimilate CO_2 at a higher level during water stress while minimizing water loss due to transpiration as compared to seedlings from the cooler, wetter areas.

3.3.4 Water Relations

Overall, both $\psi_{\pi tlp}$ and $\psi_{\pi sat}$ decreased indicating active osmotic adjustment, and cells became more inelastic (increased ϵ_{max}) in response to

Table 36: Regression equations for gas exchange and shoot water potential data for yellow-cedar families

a. $y = A$; $x = ß\psi$[1]

Family[2]	Regression Equation	r^2	n
11-1	$\hat{Y}_i = 1.62 + 0.079\ (x^3) - 2.98\ (\ln x)$	0.91	26
11-5	$\hat{Y}_i = 1.42 - 1.88\ (\ln x\ )$	0.90	24
16-5	$\hat{Y}_i = 3.04 - 2.87\ (\ln x)$	0.78	23
16-7	$\hat{Y}_i = 1.23 + 0.03\ (x^3) - 1.89\ (\ln x)$	0.80	26
31-2	$\hat{Y}_i = 1.60 + 0.066\ (x^3) - 2.76\ (\ln x)$	0.94	21
31-4	$\hat{Y}_i = 8.47 - 6.88\ (x) - 0.798\ (x^3) - 0.131\ (1/x^2)$	0.70	26

b. $y = g_s$; $x = ß\psi$

Family	Regression Equation	r^2	n
11-1	$\hat{Y}_i = -0.013 + 0.036\ (1/x)$	0.87	26
11-5	$\hat{Y}_i = -0.023 + 0.037\ (1/x) + .022\ (\ln x)$	0.91	24
16-5	$\hat{Y}_i = -0.01 + 0.048\ (1/x)$	0.79	23
16-7	$\hat{Y}_i = -0.015 + 0.001\ (x^2) + 0.03\ (1/x)$	0.95	26
31-2	$\hat{Y}_i = -0.005 + 0.029\ (1/x)$	0.92	21
31-4	$\hat{Y}_i = -0.005 + 0.028\ (1/x)$	0.73	26

c. $y = A$; $x = g_s$

Family	Regression Equation	r^2	n
11-1	$\hat{Y}_i = 8.95 + 0.003\ (1/x) + 1.74\ (\ln x)$	0.86	64
11-5	$\hat{Y}_i = 6.76 - 0.012\ (1/x) + 3.3\times10^{-5}\ (1/x^2) + 1.06\ (\ln x)$	0.84	63
16-5	$\hat{Y}_i = 11.8 - 520\ (x^3) + 0.012\ (1/x) + 3.13\ (\ln x)$	0.66	62
16-7	$\hat{Y}_i = 7.41 + 0.003\ (1/x) + 1.45\ (\ln x)$	0.80	64
31-2	$\hat{Y}_i = 9.94 + 0.008\ (1/x) + 2.13\ (\ln x)$	0.88	53
31-4	$\hat{Y}_i = 9.10 + 1.69\ (\ln x)$	0.76	64

1. A = net photosynthesis (μmol/m^2/s)
   $ß\psi$ = predawn shoot water potential (MPa)
   g_s = stomatal conductance (cm/s)
2. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.; 31 = Mt. Angeles, WA

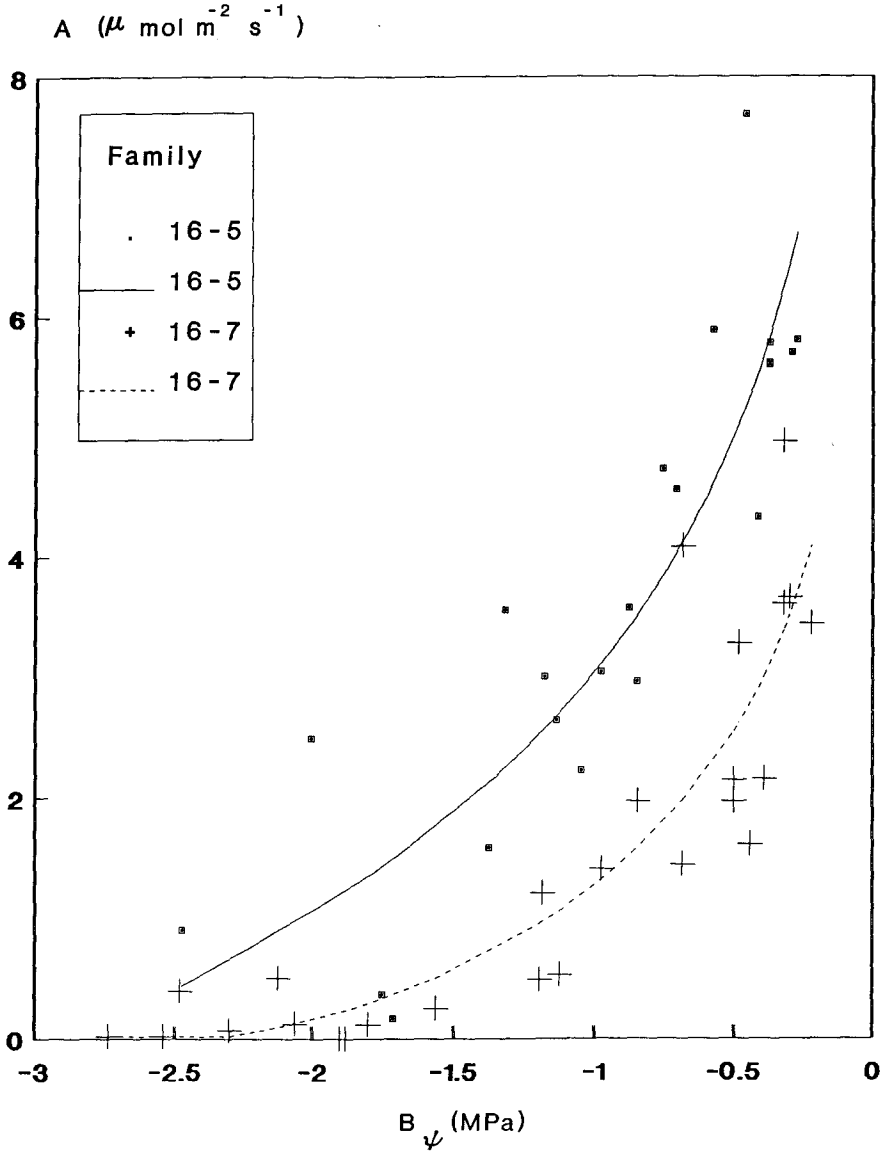


Figure 18: Net photosynthesis (A) predicted response to predawn shoot water potential (ßψ) from regression analysis for 2-year-old yellow-cedar seedlings from two open-pollinated families from Coquihalla population (see Table 36 for regression equations)

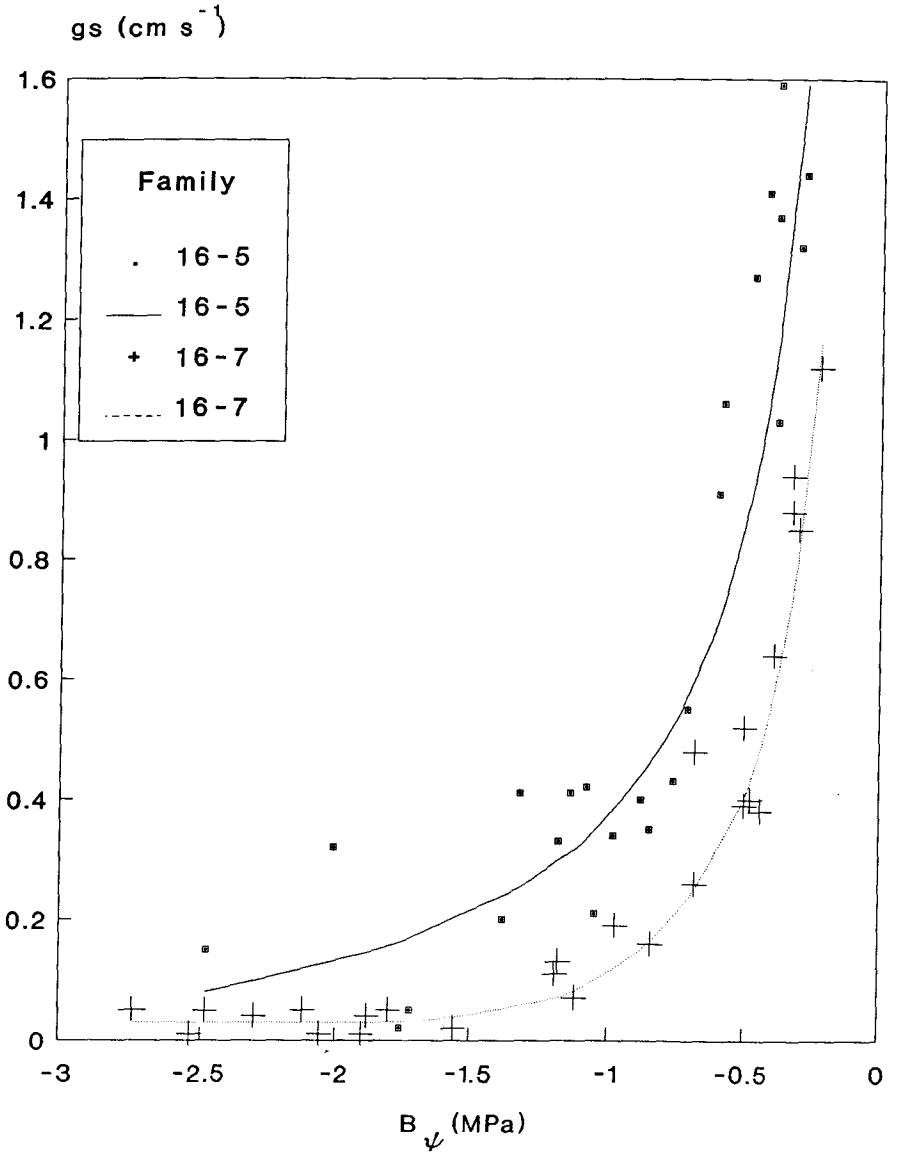


Figure 19: Stomatal conductance (g_s) predicted response to predawn shoot water potential (ßψ) from regression analysis for 2-year-old yellow-cedar seedlings from two open-pollinated families from Coquihalla population (see Table 36 for regression equations)

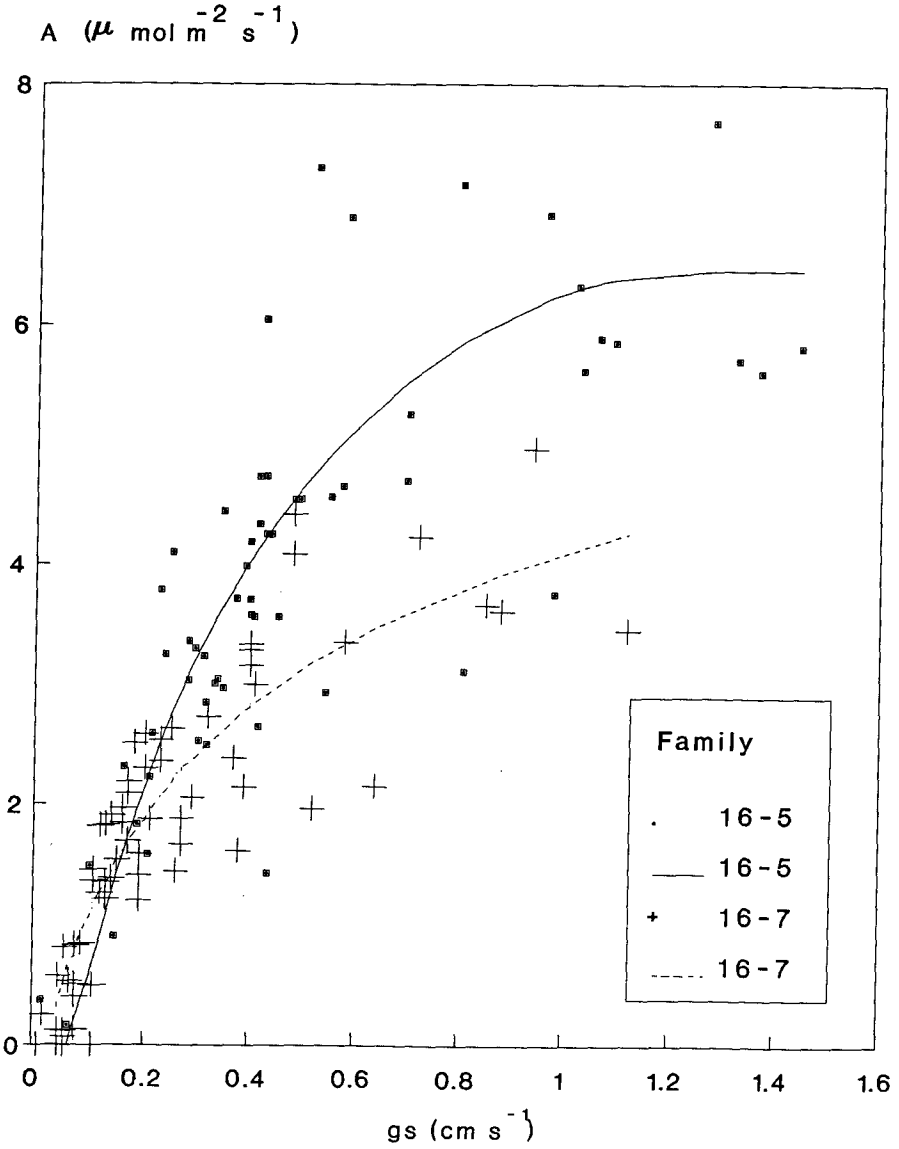
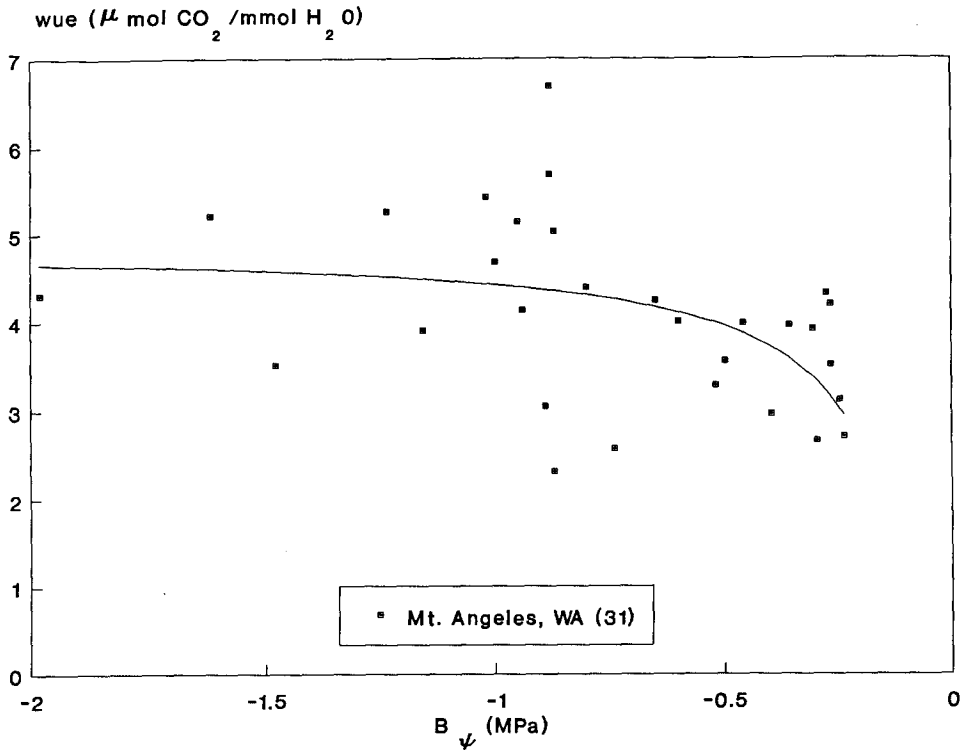


Figure 20: Net photosynthesis (A) predicted response to stomatal conductance (g_s) from regression analysis for 2-year-old yellow-cedar seedlings from two open-pollinated families from Coquihalla population (see Table 36 for regression equations)



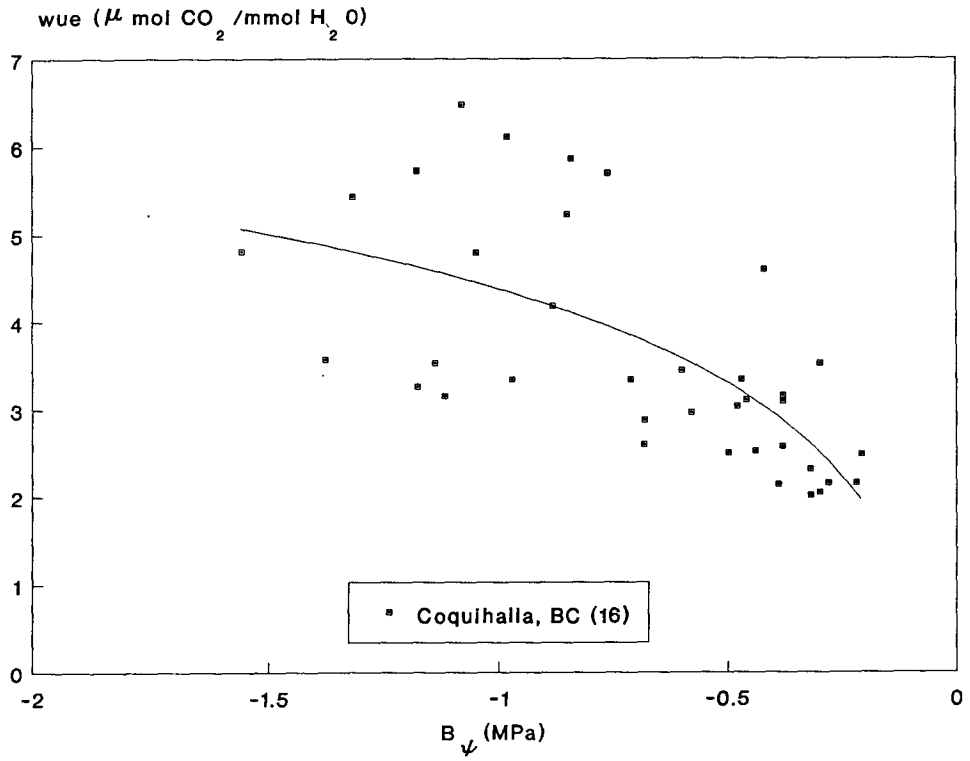
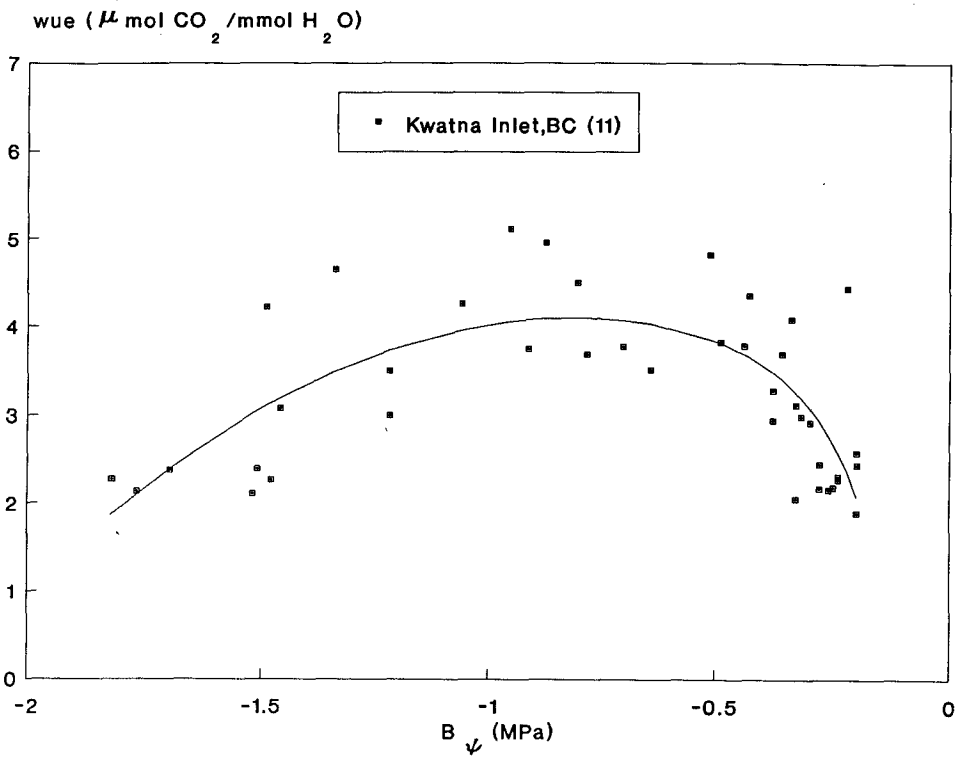


Figure 21: Water use efficiency (WUE) predicted response to predawn shoot water potential (ßψ) from regression analysis for 2-year-old yellow-cedar seedlings from four populations (see Table 37 for regression equations)



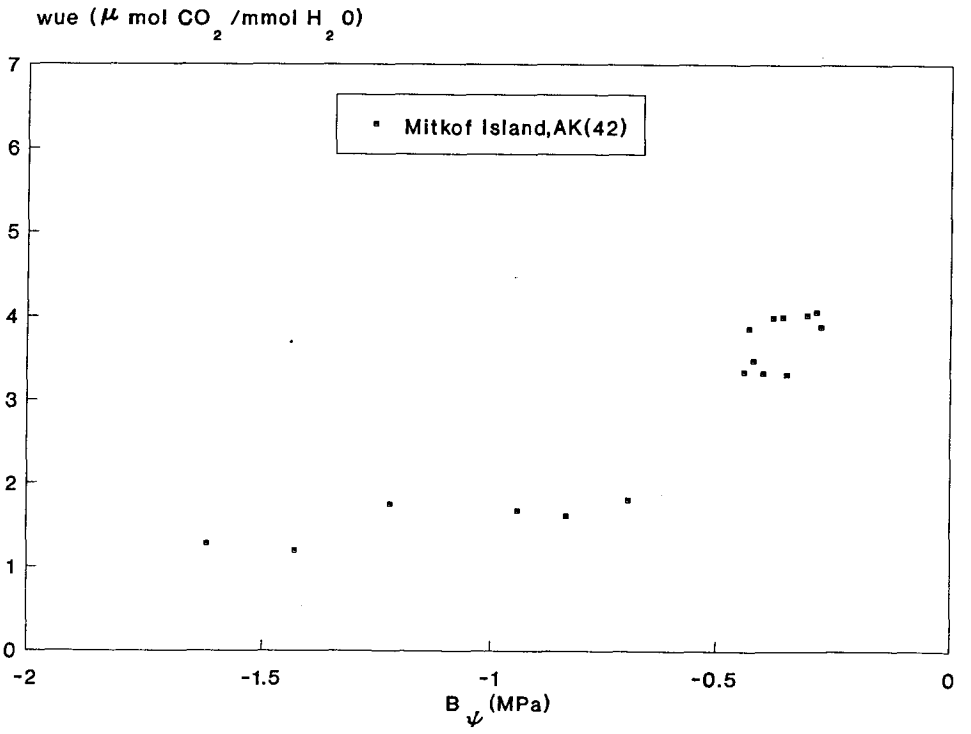


Figure 21: (con't)

Table 37: Regression equations for water use efficiency and shoot water potential data for yellow-cedar populations

y = WUE; x = ßψ[1]

Population[2]	Regression Equation	r^2	n
11	5.09 - 0.48 (x^3) - 0.598 (1/x)	0.49	41
16	4.38 + 1.55 (ln x)	0.45	41
31	4.89 - 0.468 (1/x)	0.33	36

1. WUE = water use efficiency (μmol CO_2/mmol H_2O)
   ßψ = predawn shoot water potential (MPa)
2. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.; 31 = Mt. Angeles, WA

the summer drought (Table 38). This is in contrast to the results of Arnott *et al.* (1992) in which 1-year-old yellow-cedar rooted cuttings did not osmotically adjust or change cell wall elasticity properties after a drydown. Osmotic adjustment in response to a drought has been reported in some woody species (Seiler and Johnson 1985, Abrams *et al.* 1990, Parker and Pallardy 1985) while not in others (Bahari *et al.* 1985, Joly and Zaerr 1987, Seiler and Cazell 1990). Cell wall elasticity as measured by ϵ_{max} has been reported to increase (Ellsworth and Reich 1992, Grossnickle 1992), decrease (Bahari *et al.* 1985, Joly and Zaerr 1987), not change (Arnott *et al.* 1992, Grossnickle 1992), or vary according to ecotype (Parker and Pallardy 1985, Abrams *et al.* 1990) following a drought during the active growing season.

Coquihalla seedlings had significantly lower $\psi_{\pi sat}$ and higher symplastic water at full turgor (V_o) as compared to Kwatna Inlet seedlings prior to the drydown (Table 38). Both $\psi_{\pi tlp}$ and ϵ_{max} were also lower, but not significantly. After the 6-week drydown, none of the shoot water parameters were significantly different between the two populations, except for V_o.

Table 38: Least-square means for shoot water relation parameters of yellow-cedar for pre- and post-drydown by a) population and b) family(population) (standard error in parenthesis)

a. Population[2]	Parameter[1]					
	$\psi_{\pi(tlp)}$	$\psi_{\pi(sat)}$	ϵ_{max}	$RWC_{(tlp)}$	Vo	DWF
Pre-drydown						
11	-1.70 (.04)a[3]	-1.21 (.03)a	6.80 (.45)a	74.5 (1.7)a	0.892 (.02)b	.253 (.004)a
16	-1.82 (.05)a	-1.35 (.03)b	7.74 (.45)a	74.3 (1.2)a	0.975 (.01)a	.258 (.006)a
Post-drydown						
11	-2.08 (.06)b	-1.58 (.05)c	10.18 (.39)b	77.5 (1.0)a	0.923 (.02)b	.294 (.005)b
16	-2.04 (.07)b	-1.55 (.06)c	10.20 (.50)b	76.6 (.71)a	0.968 (.01)a	.302 (.003)b
b. Family	$\psi_{\pi(tlp)}$	$\psi_{\pi(sat)}$	ϵ_{max}	$RWC_{(tlp)}$	SYM	DWF
Pre-drydown						
11-4	-1.71 (.06)a[3]	-1.22 (.04)a	7.32 (.43)c	74.5 (2.7)ab	.906 (.02)bc	.257 (.006)c
11-8	-1.69 (.06)a	-1.20 (.04)a	6.91 (.62)c	74.4 (2.2)ab	.879 (.03)c	.248 (.006)c
16-5	-1.87 (.07)ab	-1.42 (.05)bc	8.81 (.78)b	76.7 (1.6)ab	.974 (.01)a	.275 (.004)b
16-9	-1.79 (.06)a	-1.31 (.03)ab	7.02 (.35)c	72.6 (1.4)b	.976 (.02)a	.246 (.007)c
Post-drydown						
11-4	-2.16 (.06)c	-1.62 (.05)d	9.51 (.45)ab	75.7 (1.5)ab	.939 (.03)ab	.297 (.008)a
11-8	-2.02 (.09)bc	-1.55 (.07)cd	10.73 (.55)a	78.9 (1.1)a	.910 (.03)bc	.292 (.006)a
16-5	-2.01 (.05)bc	-1.55 (.03)cd	10.57 (.60)ab	77.3 (1.4)ab	.986 (.01)a	.305 (.005)a
16-9	-2.06 (.13)bc	-1.55 (.10)cd	9.91 (.79)ab	76.1 (0.8)ab	.942 (.02)ab	.300 (.004)a

1. See Table 24 for explanation of trait abbreviations
2. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.
3. Different letters in the same column within Table 38a and within Table 38b denotes significance at P=0.05 according to multiple, pair-wise t-tests

Both families from Coquihalla (16-5 and 16-9) had lower $\psi_{\pi tlp}$ and $\psi_{\pi sat}$ prior to drydown than the families from Kwatna (11-4 and 11-8), with family 16-5 being significantly lower for $\psi_{\pi sat}$ (Table 38). Family 16-5 also had the highest ϵ_{max} value and DWF prior to drydown. Both families from Coquihalla had significantly higher symplastic water than seedlings from the Kwatna families.

Seedlings from both populations osmotically adjusted (i.e. significant decreases in both $\psi_{\pi tlp}$ and $\psi_{\pi sat}$ after the drydown), with seedlings from Kwatna adjusting more than Coquihalla (Table 38). Family 11-4 had the greatest reduction and family 16-5, the least. After the 6-week drydown, none of the shoot water parameters were significantly different among the four families.

Cell walls became more inelastic in both populations after experiencing the 6-week drydown (Table 38). Although both increases in ϵ_{max} were significant, seedlings from Kwatna had a greater change (3.4 MPa). At the family level, Kwatna family 11-8 had the greatest increase (3.8 MPa), and Coquihalla family 16-5 the least (nonsignificant change of 1.8 MPa).

There was a slight, though insignificant increase in relative water content at turgor loss point for both populations, and little change in symplastic water (Table 38). All four families showed no significant changes in both RWC and symplastic water. Dry weight fraction increased significantly for both populations (approximately 4.1% for both populations), and all four families significantly increased their dry weight fraction, following the drydown.

Thus, following a 6-week drought, both populations reacted similar in adaptation to drought, with osmotic adjustment and increased cell wall inelasticity, resulting in more solutes per cell which would allow for greater maintenance of turgor during drought. Inelastic cell walls allow

tissue water potential to decrease rapidly with a change in water content. This may help to maintain a favourable gradient for moisture uptake from drying soils without resulting in large tissue water deficits (Abrams *et al.* 1990).

Overall, yellow-cedar seedlings from both populations, had a decrease in shoot turgor potential (ψ_p) at a given RWC following the drydown (Table 39, Figure 22). However, the post-drydown curves had a steeper slope resulting in the turgor loss point occurring at a greater RWC than pre-drydown seedlings. Seedlings from Kwatna had a lower ψ_p with decreasing RWC than Coquihalla seedlings both before and after the drydown (Figure 22). The slope of the curves between the two populations either at pre- or post-drydown were similar.

Coquihalla family 16-5 had a greater decrease in ψ_p with a drop in RWC before drydown, reaching turgor loss point at a RWC of 76%, whereas the other three families reached turgor loss point at below 70% (Figure 23 and Table 39). Although Kwatna families 11-4 and 11-8 had similar ψ_p at high RWC, family 11-8 had a greater decrease in ψ_p after the drydown, reaching turgor loss point at approximately 78% RWC, and 11-4 the least drop in ψ_p reaching turgor loss point at a RWC of 74% (Figure 23). Both families 16-5 and 16-9 had similar post-drydown curves starting at higher ψ_p than both Kwatna families 11-4 and 11-8, and having similar RWC at turgor loss point, which was intermediate between the two Kwatna families.

3.3.5 Morphological and Physiological Adaptations to Drought

Table 40 summarizes shoot growth traits and dry weight allocation by population and moisture regime averaged for only those families and populations in which gas exchange data was taken. As stated earlier, yellow

Table 39: Regression model equations for yellow-cedar elasticity curves for pre- and post-drydown by population and family within population

$y = \psi_P$; x = RWC[1]

Population[2]	Regression Equation	r^2	n
Pre-drydown			
11	$-7.75 + 5.21 (x^2) + 3.68 (1/x)$	0.89	145
16	$-7.32 + 5.35 (x^2) + 3.29 (1/x)$	0.96	129
Post-drydown			
11	$-12.1 + 7.78 (x^2) + 5.79 (1/x)$	0.94	122
16	$-11.2 + 7.62 (x^2) + 5.18 (1/x)$	0.94	123

Family	Regression Equation	r^2	n
Pre-drydown			
11-4	$4.93 - 14.5 (x) + 10.74 (x^2)$	0.90	83
11-8	$-4.58 + 5.71 (x^2) - 5.04 (\ln x)$	0.90	67
16-5	$-8.87 + 6.16 (x^2) + 4.06 (1/x)$	0.97	48
16-9	$-5.99 + 4.67 (x^2) + 2.60 (1/x)$	0.96	80
Post-drydown			
11-4	$-8.35 + 6.13 (x^2) + 3.7 (1/x)$	0.94	60
11-8	$-15.1 + 9.09 (x^2) + 7.5 (1/x)$	0.95	61
16-5	$-12.3 + 8.22 (x^2) + 5.78 (1/x)$	0.93	66
16-9	$-10.1 + 7.04 (x^2) + 4.61 (1/x)$	0.95	56

1. ψ_p = turgor pressure (MPa)
   RWC = relative water content at turgor loss point
2. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.

cedar seedlings were strongly influenced by moisture stress, in that drought resulted in reduced shoot and lateral branch extension, root collar diameter, and total dry weight.

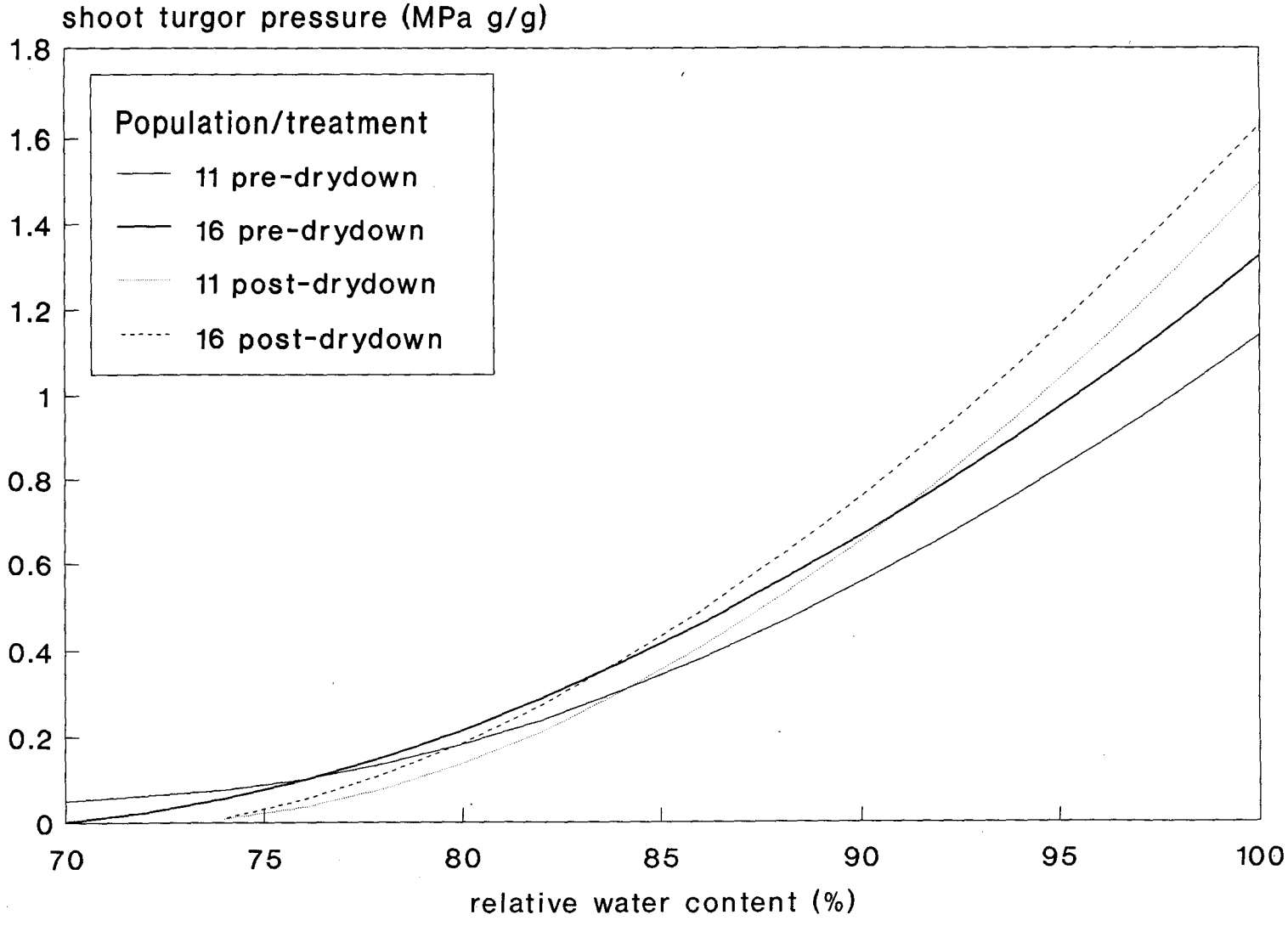
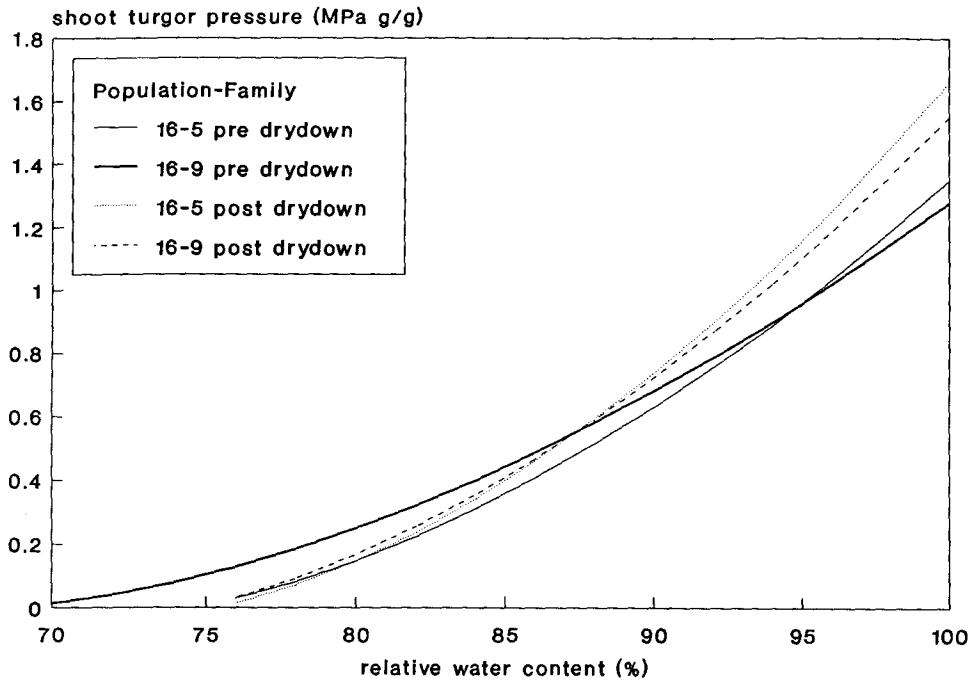


Figure 22: Turgor pressure predicted response to relative water content from regression analysis for 2-year-old yellow-cedar seedlings from two populations before and after a 6-week drydown (see Table 39 for regression equations)



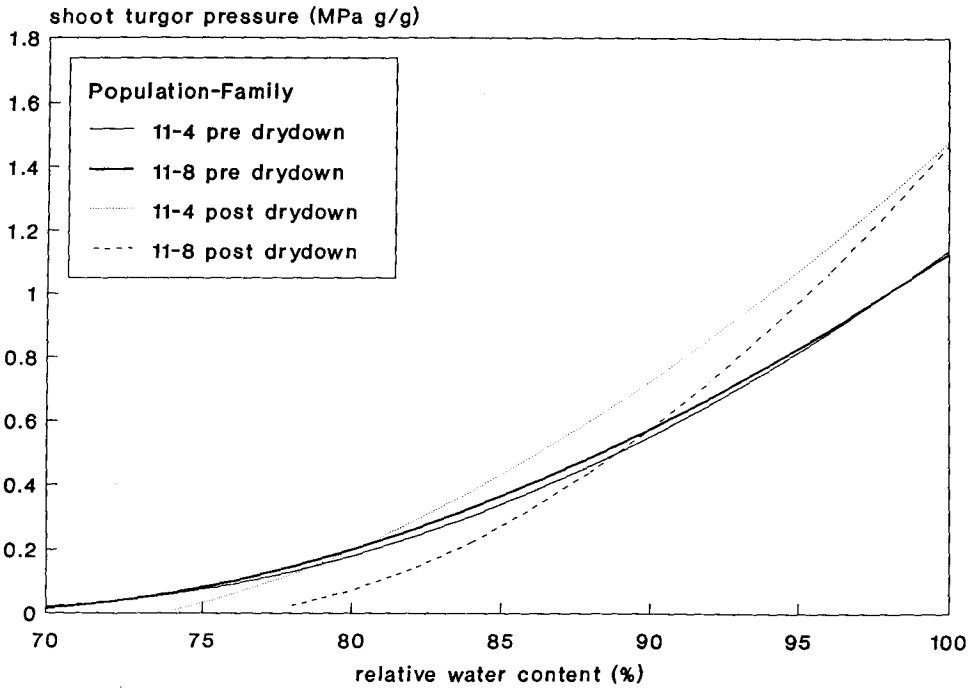


Figure 23: Turgor pressure predicted response to relative water content from regression analysis for 2-year-old yellow-cedar seedlings from two open-pollinated families within each of two populations (see Table 39 for regression equations). Figure 23a: Kwatna Inlet, Figure 23b: Coquihalla

Table 40: Least-square means of morphological traits for yellow-cedar gas exchange study, by moisture regime

	Trait[1]							
	ht2	rgr2	rcd2	brlgth	totdw	root[2]	stem[2]	branch[2]
dry								
11[3]	323.7ab[4]	0.589a	4.91b	82.1a	11.5b	1.50b	0.737a	1.68a
16	307.6b	0.465b	5.02b	73.3b	11.4b	1.63a	0.719a	1.55b
31	338.9a	0.500b	5.38a	86.7a	13.8a	1.54ab	0.797a	1.61a
42	324.7ab	0.528ab	5.19ab	83.8a	12.7ab	1.51b	0.710a	1.67a
wet								
11	355.9c	0.641a	5.44b	97.5a	15.0a	1.62b	0.930b	1.87a
16	344.5d	0.572b	5.75ab	79.8b	13.3a	1.74a	0.885b	1.77b
31	392.0a	0.623a	5.91a	104.6a	15.9a	1.67ab	1.05a	1.77b
42	367.6b	0.680a	5.82ab	87.7ab	15.4a	1.63b	0.818b	1.90a

1. See Table 24 for explanation of trait abbreviations
2. $\log_e$ (dry weight) adjusted for $\log_e$ (total dry weight)
3. 11 = Kwatna Inlet, B.C.; 16 = Coquihalla, B.C.; 31 = Mt. Angeles, WA; 42 = Mitkof Island, AK
4. Different letters in the same column within each environment denotes significance at the $p<0.05$ level using multiple, pair-wise t-tests

Rankings of populations were similar between the two moisture regimes for all traits, especially for those populations in which traits were significantly different (i.e. no genotype by environment interaction).

Seedlings from the Coquihalla population, an interior:coastal transition site which receives 20% of the average summer rainfall at Kwatna Inlet (see Table 23), had less shoot and lateral branch growth, and less allocation of carbohydrates to branches, than seedlings from the three coastal populations. However, Coquihalla seedlings allocated more dry matter production to root dry weight than Kwatna Inlet or Mitkof Island seedlings under both moisture regimes. Seedlings from the more xeric site (Coquihalla) developed a more conservative phenotype, smaller height and diameter growth, decreased lateral branch growth, and more allocation of carbohydrates to roots and less to stem and branches. This seedling morphology should allow for increased soil water absorption through the exploration of a larger volume of soil and increased absorption efficiency per unit root area, and decreased transpirational surface (Levitt 1980b).

Seedlings from Mt. Angeles population, a south-facing coastal leeward site which also receives substantially less rainfall than Kwatna Inlet or Mitkof Island (see Table 23), also allocated more carbohydrates to roots under both moisture regimes.

In this study, seedlings from Coquihalla had the greatest ability to assimilate CO_2 at all levels of stress studied and to maintain greater CO_2 uptake at a given level of stomatal conductance greater than 0.2 cm sec^{-1}. As well, these seedlings had the greatest water use efficiency when under stress. Seedlings from both populations osmotically adjusted, however, seedlings from Coquihalla, a xeric habitat, had lower $\psi_{\pi(sat)}$ prior to the

drydown. A similar result was found for green ash (Abrams *et al.* 1990). Lower osmotic potentials under saturation would allow for the maintenance of positive turgor if osmotic adjustment was not possible such as a rapid increase in soil water deficit.

Both populations had similar tissue capacitance before and after the drought, with similar changes in shoot turgor pressure with decreasing RWC. Coquihalla seedlings, however, consistently maintained higher ψ_p at a given RWC, thus possibly being able to maintain stomatal opening at lower RWC.

Thus, besides their conservative morphology, seedlings from Coquihalla had increased photosynthetic efficiency per unit area foliage and greater stomatal conductance under both well-watered and drought conditions, lower osmotic potential prior to drought, and higher turgor pressure at a given relative water content. These physiological adaptations to drought, as well as the ability to osmotically adjust and increase cell wall inelasticity following a drought, will allow for the maintenance of positive turgor and cell growth (Hsiao 1973), and minimize physical damage to cellular processes due to drought (Levitt 1980a). The above morphological and physiological adaptations to drought have been reported for other woody tree species from xeric habitats (Kelliher and Tauer 1980, Parker and Pallardy 1985, Bongarten and Teskey 1986, Joly *et al.* 1989, Abrams *et al.* 1990, Kubiske and Abrams 1992).

The environments used in this study were not completely indicative of environments where yellow-cedar occurs. In particular soil temperature, which has shown to have an influence on gas exchange in yellow-cedar (Grossnickle and Russell 1991), may influence a population's adaptive response to moisture stress with respect to morphological development.

3.4 SUMMARY

Environments had a large effect on growth and morphology of yellow-cedar. Shoot elongation exhibited phenotypic plasticity, responding to both decreased photoperiod and water-stress through decreased shoot growth, and relative growth rates greater than the non-stressed seedlings upon release of the treatments. There were no significant treatment interactions of photoperiod and moisture regime.

Significant differences were evident among the 18 populations analyzed without family structure for all growth and morphology traits. In the reduced dataset in which only nine populations were included, differences among families within populations were large and significant for all traits, and accounted for most of the genetic variation, with little or none attributed to populations. Significant interactions of replication by genetic entry for some of the traits, makes the interpretation of genetic main effects difficult.

In all growth and morphology traits analyzed, there was minimal evidence for significant genotype by environment interactions at both the population and family within population level, with both photoperiod treatments and water regimes.

Environments had minimal impact on dry weight partitioning. Population differences were also small, except when seedlings were grown under long-days and moisture stress. As well, there were no interactions of populations with environments.

Genetic variation in gas exchange and water relation parameters in response to a drought was evident with 2-year-old yellow-cedar seedlings among and within populations. With respect to gas exchange, seedlings from

the most xeric site (Coquihalla) had less stomatal sensitivity to drought, resulting in greater carbon assimilation and water use efficiency at lower predawn shoot water potentials as compared to seedlings from more mesic sites (Kwatna Inlet and Mitkof Island). Seedlings from Mt. Angeles, an intermediate site with respect to moisture availability, had an intermediate response. Within population variation was evident in gas exchange response to drought in all populations with family structure.

Both populations studied with respect to water relation parameters (Kwatna Inlet and Coquihalla) exhibited similar responses to drought, osmotically adjusting and increasing cell wall inelasticity, and reaching similar levels of osmotic potential and cell wall elasticity after the drought. Seedlings from Coquihalla had lower osmotic potentials at saturation before the drydown as compared to seedlings from Kwatna Inlet. There were significant differences among families for osmotic potential at saturation, cell wall elasticity, symplastic water, and dry weight prior to the drydown, and for symplastic water after the drydown.

Seedlings from Coquihalla, a xeric habitat, had less shoot and lateral branch extension and less biomass allocated to branches and more to roots as compared to mesic sources under both well-watered and drought conditions.

CHAPTER 4: CONCLUSIONS

Although yellow-cedar appears to occupy a unique and limited niche within the Pacific Northwest, its range is extensive latitudinally and it exhibits a wide ecological amplitude in response to soil moisture and nutrient availability. In the absence of competition from other associated conifers, the range of yellow-cedar would most likely be (or has been in the past) more extensive. This view is supported by past climatic and geological events (Critchfield 1984) and the existence of isolated, inland populations in central Oregon and southern British Columbia. The combination of an extensive latitudinal range and geographic isolation, coupled with a wide ecological amplitude and indeterminate growth habit, would seem to suggest aspects of both a specialist and generalist adaptive mode.

In this study, the following generalities for morphological and physiological seedling traits that measured the range of the annual developmental sequence of yellow-cedar, can be stated:

1. significant population and family within population genetic variation exists for many of the traits measured;
2. genetic variation among families, for most traits, is 2 to 16 times greater than population variability;
3. seedling traits are under moderate to strong additive genetic control;
4. genetic variation at the population level is moderately correlated with seed origin for most traits, and;
5. seedlings exhibit phenotypic plasticity in response to environmental changes.

The above generalities on population effects and associations with geography are more relevant when all populations measured in this study are considered. If southern populations are removed, (i.e. Oregon populations), then correlations of traits with seed origin are, for the most part, nonsignificant. This can be seen by the minimal trait correlations with seed origin and the lack of evidence for photoperiodic ecotypes in the environment study presented in Chapter 3, in which no Oregon populations were included.

A result similar to the above can be seen for population differences in adaptive responses to drought. In this study, morphological and physiological responses to drought were only apparent among populations from areas that greatly differed in moisture availability (i.e. coastal, windward versus coast:interior transition).

Thus, it seems possible that yellow-cedar populations in the extreme environmental ranges of the species, (i.e. southern and continental populations), have responded to environmental selection pressures, most likely aided by reduced gene flow due to spatial isolation and poor sexual reproduction (Russell *et al.* 1990), by changes in gene frequency. At the same time, however, the species has maintained a substantial amount of both genetic variation and phenotypic plasticity within populations. Yellow-cedar seems to have evolved an intermediate adaptation mode with less genetic differentiation associated with geography than coastal Douglas-fir, Sitka spruce, and western hemlock, and more geographic differentiation than western white pine and western redcedar.

This study has shown that yellow-cedar responds readily to changes in environment. However, there was minimal evidence that phenotypic plasticity was under genetic control as illustrated by the lack of interaction of

populations and environments and families and environments. Possible explanations for this are:

1. Responding to photoperiod for initiating shoot growth cessation is not a primary adaptation signal for indeterminate species. In studies with *Thuja plicata* and *T. occidentalis*, Vaartaja (1959, 1962) showed that very short days (less than 8 hours) were required to generate a significant morphological response between seedlings from southern and northern populations. Thus, in this study, the minimal difference between the two photoperiod treatments was enough to produce an overall species response, but not any differential response among populations or families within populations;

2. The moisture stress applied in this study was quite severe, and most seedlings completely stopped growing. This may have completely masked any interactions of genotype and environment. Perhaps a milder stress would have been more appropriate for detecting genetic variability in plasticity.

3. Temperature, possibly in combination with photoperiod, may be a more important environmental signal for yellow-cedar growth rhythms. This was illustrated qualitatively in Chapter 2 by the differential response of populations in growth cessation in the fall to an ambient warming trend.

Thus, the lack of genetic control of phenotypic plasticity in yellow-cedar may be more an artifact of the actual treatments and their levels chosen for this study, as opposed to any real biological absence.

This thesis represents a comprehensive study on the effects of genetics, environments, and their interactions, for seedlings of a non-bud forming species. Interpreting and integrating morphological and physiological characteristics which infer fitness attributes to seedlings of

yellow-cedar, allowed for an indepth look at adaptive strategies to heterogeneous environments for an indeterminate species. In particular, the following approaches were new for indeterminate species:

1. describing shoot growth initiation and cessation components for populations and environments and correlating them to cold-hardiness, growth, and geographic parameters of seed origin, and;
2. describing drought resistant populations, integrating morphological and physiological characteristics, including gas exchange and water relations.

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