GENETIC VARIATION IN WESTERN RED CEDAR (*Thuja plicata* Donn) SEEDLINGS

by

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ABSTRACT

To determine whether the apparent lack of genetic variation in western red cedar (*Thuja plicata* Donn), as previously inferred by isozyme and terpene studies, would hold true for quantitative seedling traits, a provenance study was initiated to investigate patterns of variation in seedling growth and survival characteristics, cold temperature acclimation, and response to inbreeding.

Seedlings from ten coastal and ten interior provenances, half with family structure (five families / provenance), were grown for three years at one coastal (Vancouver) and one interior (Salmon Arm) location. Twenty-three potted clones were both self-pollinated and polycrossed at Cowichan Lake; resulting progeny were monitored for growth and frost hardiness.

Genetic variation could be detected from the first year, and increased annually. The narrow-sense individual heritability, assuming some inbreeding, of final heights of trees growing in Vancouver was 0.38. Height, root collar diameter, acclimation, and deacclimation exhibited mainly within-population variation, while variation in dry weight measurements, foliar nutrient content, survival at Salmon Arm, and maximum cold hardiness was evident mainly between populations. Coastal / interior differences were noted in first-year heights, branch number, height, survival, and crown dieback at Salmon Arm following a severe winter in which trees suffered major desiccation damage, and in acclimation and deacclimation. In general, adaptive traits appeared to show more between-population differences, while traits under less selective pressure showed mainly within-population variation.

Provenances displaying the greatest variation at the family level were those from Vancouver Island. Between-population variability appeared to be highest in the B.C. interior, and lowest in northern B.C. populations.

Elevation influenced all traits displaying provenance variation. Location effects occurred, and some genotype by environmental interactions were noted. Plasticity was evident in timing of growth initiation and cessation, timing of acclimation and deacclimation, and in depth of maximum hardiness reached per year.

Early traits showed little evidence of inbreeding depression, but there seemed to be a trend towards gradual expression of inbreeding depression over time, at least in traits under selective pressure.

This research showed that western red cedar is much more complex than previously believed, and substantial genetic variation exists in several traits of this species.

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DEDICATION

— Dedicated to Doug —

Many a night I saw the Pleiads, rising thro' the mellow shade,
Glitter like a swarm of fire-flies tangled in a silver braid.
Here about the beach I wander'd, nourishing a youth sublime
With the fairy tales of science, and the long result of Time

- **Alfred Lord Tennyson,** *Locksley Hall,* **1842**

The great tragedy of Science - the slaying of a beautiful hypothesis
by an ugly fact.

- **T.H. Huxley,** *Biogenesis and Abiogenesis,* **1870**

1. INTRODUCTION

1.1. OBJECTIVES

Western red cedar (*Thuja plicata* Donn) is not a major commercial species in British Columbia (at 7.67 million m³ volume harvested, comprising 9.68 % of the Province's total log production in 1993[1]), but is nevertheless considered to be an important value-added timber species on the coast, as the wood is valuable and exhibits some unique properties. Demand for western red cedar wood products has risen in recent years (Minore, 1983), resulting in increased harvesting levels and hence a greater demand for planting stock. Current B.C. nursery sowing request levels for this species call for 10.712 million seedlings provincewide in 1994, up from 2.991 million seedlings which were requested in 1983 (Scott Lohnes, B.C. Ministry of Forests, pers. comm., 1994).

The extent of genetic variability in western red cedar is unknown, although this species has widely been presumed until now to be genetically depauperate, largely based on the results of isoenzyme and leaf extractive studies. However, isozyme variability may not reflect amounts of variation in metric traits (Muona, 1988) and thus inferences about the latter cannot be made based on the former. It is not known whether quantitative traits of interest have the potential for genetic improvement in this species. An understanding of the genetics of western red cedar is

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[1] From unpublished statistics of the B.C. forest industry, as compiled by the Council of Forest Industries, Vancouver, 1994.

therefore desirable to provide knowledge which may later be of use in forest regeneration, forest maintenance, and in the understanding of biodiversity at the species level.

The intent of the present research was to study the extent and pattern of genetic variability in certain growth and adaptive traits of western red cedar seedlings to provide knowledge which will further the understanding of the genetics, growth, and physiology of this species, aid in seed zone delineation, offer information which may be useful in the development of breeding programs for this species, and aid in developing seedlings which may be better suited to their plantation environment.

The objectives of this research were the following:

- to estimate the extent and allocation of genetic variation found in growth, morphological, and survival traits of western red cedar between coastal and interior range populations and between and within provenances and families using quantitative genetic analyses

- to investigate the effect of test location on growth and cold hardiness traits

- to study the effects of self-fertilization on growth and cold hardiness of seedlings

- to aid in the delineation of seed transfer guidelines and to make preliminary recommendations regarding selection and breeding strategies for the western red cedar breeding program

The generalized null hypothesis was that no detectable genetic variation in growth, morphological, and survival traits exists between coastal and interior provenances or between or within populations. The null hypothesis for frost testing was that genetic differences in the responses to cold temperatures do not occur and that the range of variation between and within populations does not increase or diminish from the time when trees begin hardening until total dehardening has occurred. The null hypothesis for self-fertilization studies was that no differences

could be detected between selfed and outcrossed progeny.

The first objective was to partition any variability found in the characteristics studied into genetic and environmental components. Estimates could then be derived for how broad or narrow seed zones should be and whether it is worthwhile to develop intensive breeding programs for this species. Knowledge of seedling growth and physiology gained by this study could be applicable in the regeneration of this species.

The second objective was to determine whether differences could be detected between seedlings growing in a relatively unstressful, favourable coastal site and those grown in a relatively harsh interior environment, and to detect the presence or absence of any genotype by environment interaction. Again, this knowledge would aid in achieving the last objective.

If western red cedar were bereft of genetic heterogeneity, inbreeding might not be detrimental. The intent of the third objective was to investigate such effects in growth and adaptive traits of the progeny of self-pollinated and outcrossed maternal parents.

The fourth objective, to provide knowledge which would assist in the delineation of seed transfer zones and to make preliminary recommendations about selection and breeding of this species, would be based on the results found through the achievement of the first three objectives.

The genetics of western red cedar seedlings were examined by studying populations from the coastal and interior ranges (henceforth referred to as zones) of this species. Provenances (geographic origins of the seed sources) having available

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nonbulked seed were selected from each zone, half with family (open-pollinated maternal half-sib) structure. Seedling samples were grown at one of two sites: a coastal site representative of a mild, favourable environment and an interior site representing a relatively harsh and stressful climate.

To investigate whether variability in seedling growth traits was evident, seedling heights were measured for three years. After the third growing season had concluded, root collar diameters were measured on all trees. A subsample of trees were selected for dry weight measurements, while another subsample was tested for foliar nutrient content. Adaptive traits were represented by seedling survival at two sites and by frost hardiness of trees growing at two locations. By determining the levels of any variation, it may be possible to discover whether or not different processes are acting on different types of characteristics.

To compare the effects of inbreeding with outcrossing, maternal parents were both self-pollinated and polycrossed; the resulting progeny were grown and then measured for height, root collar diameter, dry weight, and cold hardiness. Should evidence of inbreeding depression be found, this might imply that inbreeding may not be the primary mating strategy in natural stands, and some genetic variation must occur in measured traits.

1.2. LITERATURE REVIEW

Western red cedar, a member of the family Cupressaceae, is one of two members of the genus *Thuja* native to North America, and the only *Thuja* species naturally found in the western part of the continent. Western red cedar, actually an arborvitae and not a true cedar, is sometimes referred to as western redcedar.

Western red cedar has a fairly widespread natural range. The coastal portion of the range extends along the Pacific coast from southeastern Alaska, at a latitude of 56°30', to northern California at a latitude of 40°10' (Burns and Honkala, 1990). As well, this species has an inland range occurring along the western slope of the Rocky Mountains from west of Prince George, B.C. (at 54°30' latitude), to northern Montana and Idaho (45°50' latitude) (Minore, 1983; Burns and Honkala, 1990). The coastal and interior populations are separated except for a small contiguous region comprised of scattered patches extending along the B.C. - Washington border.

Interior western red cedar can be found at elevations from 320 m to 2,130 m (Burns and Honkala, 1990). Coastal western red cedar grows from sea level to about 2,300 m in Oregon. In coastal B.C., the upper elevational limit is lower, at about 1,200 m. In Alaska, the upper altitudinal limit is still less, at 910 m (Minore, 1983; Burns and Honkala, 1990), and at its northernmost limit, the presence of western red cedar stops abruptly at 300 m elevation (Pojar and MacKinnon, 1994).

Although western red cedar may form pure stands, it is usually associated with other tree species, and may be present in any stage of forest succession. On the coast, coniferous associates include yellow cedar (*Chamaecyparis nootkatensis* [D. Don] Spach), Port-Orford cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.), coastal

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Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco), *Abies* Mill. species, Sitka spruce (*Picea sitchensis* [Bong.] Carr.), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), mountain hemlock (*Tsuga mertensiana* [Bong.] Carr.), lodgepole pine (*Pinus contorta* Dougl.), western white pine (*Pinus monticola* Dougl.), and Pacific yew (*Taxus brevifolia* Nutt.) (Burns and Honkala, 1990). In the interior, western red cedar grows with interior *Pinus* and *Picea* species, interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco), western hemlock, western larch (*Larix occidentalis* Nutt.), and Pacific yew (Burns and Honkala, 1990).

In B.C., western red cedar is predominantly found in the Coastal Douglas-fir, Coastal Western Hemlock, and Interior Cedar Hemlock biogeoclimatic zones (Krajina *et al.*, 1982; Meidinger and Pojar, 1991). The ecological amplitude of western red cedar appears broad with regard to soil moisture and richness. This tree grows in poor to rich soil nutrient regimes and in dry to very wet available soil moisture regimes, and is very resistant to prolonged spring flooding (Krajina *et al.*, 1982; Rushforth, 1987).

This species has a high shade tolerance, although less than that of western hemlock, Pacific yew, and Pacific silver fir (*Abies amabilis* [Dougl.] Forbes). On dry sites, western red cedar is shade-requiring (Krajina *et al.*, 1982). The frost tolerance of this species is not as high as that of some of its associated coniferous species, and where sufficient precipitation is present, its range seems to be limited by low temperature extremes (Burns and Honkala, 1990). Winter desiccation damage can also be severe (Miller, 1978). Adams and Mahoney (1991) observed that transpirational stress was more detrimental to this species than light competition, although growth reductions were influenced by below-ground competition.

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Western red cedar, as with all members of the Cupressaceae, has indeterminate shoot growth, without winter bud formation or preformed shoots. Thus meristems do not benefit from the protection of bud scales in winter. The foliage has little cutin and wax and hence is poorly protected from excessive transpiration (Burns and Honkala, 1990). However, shoot growth is opportunistic and elongation occurs as long as climatic conditions are favourable. Shoots have a longer period of growth than that of any associated conifers (Burns and Honkala, 1990). This species has been known to undergo shoot elongation into December in coastal B.C., but shoot growth in the spring takes longer to commence than does that of its determinate associates.

Western red cedar contains extractives, notably the thujaplicins, in its heartwood, which act as natural fungitoxins and make western red cedar one of the most resistant species to pathogen attacks, contributing to the longevity of this species. However, over the centuries, biodegradation of these extractives by fungi may gradually occur (Burns and Honkala, 1990). Nevertheless, this species can live for about 1,000 years.

Naturally occurring asexual propagation is thought to be common, at least in Idaho stands (Minore, 1983). Polyploidy has been noted in members of the Cupressaceae (Minore, 1983). Haploid and triploid members of *Thuja plicata* have been found, as have 4n *Juniperus*. Wright (1976) speculated that ploidy may confer an adaptational strategy for the invasion of new habitats, as polyploids often exhibit faster than normal growth and so may have an advantage in occupying a site.

The recent history of western red cedar has been difficult to reconstruct, in part because pollen among members of the family Cupressaceae is indistinguishable

and also difficult to separate from *Taxus* pollen (Hebda and Mathewes, 1984) and in part because of incomplete and sporadic pollen records. Critchfield (1984), in examining the impact of the Pleistocene on the genetic structure of conifers in North America, could find no explanation in fossil records for the presumed reduced variability of western red cedar as reported in earlier studies.

Due to severe climatic conditions during the last ice age, even in nonglaciated areas, it is unlikely that any populations of western red cedar were able to survive on nunataks which have been hypothesized for parts of the Queen Charlotte Islands, Alaska, and Vancouver Island. It is not known whether this species took refuge in one or in several disjunct refugia south of the ice sheets' borders.

The last glaciation was believed to have reached as far south as 47° to 48° latitude in western North America (Booth, 1987). Near the coast, the Puget lobe of the Cordilleran ice sheet reached to just south of the Puget lowland. In Idaho, the ice sheet reached as far south as about the southern shore of Lake Pend Oreille. A record of pollen dating back to 13,800 years before present (BP) at Davis Lake, situated just south of the Puget lobe and hence beyond the limit of the last glaciation, shows the presence of Cupressaceae pollen back to 13,800 years BP (Baker, 1983). Sporadic pollen records of the Cupressaceae are evident from Davis Lake to northern California, but go no further south than that, at about 39° latitude (Baker, 1983).

The ice sheets receded in an inland direction from the west coast of North America beginning about 14,000 years BP (Hughes, 1987; Heusser, 1989), and began receding in Idaho sometime between 14,000 and 11,000 years ago (Waitt and Thorson, 1983). Thus species reinvasion could have occurred at that time according to rates of migration had conditions been favourable. However, it appears that

climatic and edaphic conditions were not favourable for western red cedar habitation (Heusser, 1983). It is believed that warming began about 12,500 years BP (Barnosky *et al.*, 1987), and from about 10,000 to about 6,000 years BP, the climate was very warm and dry, with summer droughts (Wright, 1983). Western red cedar is believed to have spread into B.C. from southwestern Washington from about 10,000 years ago onwards, but was only present in low amounts until about 5,500 years ago along the coast and on the Queen Charlotte Islands, when it is believed that weather patterns became cooler and moister and hence generally more favourable for this species (Barnosky *et al.*, 1987). An increase in the abundance of western red cedar has occurred over the last 3,000 years (Mathewes, 1989), with no immediate explanation.

Western red cedar's extensive range is similar to that of western hemlock. The refugial ranges of both species were believed to be similar (Baker, 1983) and both were restricted in reoccupation of previous territories until the warm, dry climatic conditions began to change (Heusser, 1983). Where western red cedar is common, its pollen forms a large percentage of the pollen cloud (Baker, 1983), and thus should be capable of rapid population expansion (Yeh, 1988).

Yeh (1988) suggested that gene flow among neighbouring populations is quite extensive, with no effective barriers; expectations would be that divergence between populations would lessen with extensive gene flow. The isozymes studied by Yeh fit Hardy-Weinberg expectations; from those results, and from the fact that this species is widespread, he surmised that severe inbreeding does not occur. Assuming a generation time of 20 years for western red cedar, he calculated that about 500 generations have occurred since a bottleneck about 10,000 years ago. However, Yeh's estimated generation time of 20 years for western red cedar is extremely questionable.

Using an approximate generation time of about 200 years (longer than that on the coast and possibly shorter than that in the interior), which is a more realistic figure when the ecology of the species is considered, and a time of about 6,000 years, which is when western red cedar became more prevalent in formerly glaciated areas of B.C., only about 30 generations would have occurred to the present time, with fewer than this on the coast, and more than this in the interior.

In summary, western red cedar has various features which distinguish it from many of its associated conifer species. Its indeterminate nature, longevity, initially high resistance to pathogens, ability to withstand flooded soil conditions and low light levels, comparative inability to withstand very low temperatures and conditions of high desiccation and sunscald, and its presumed paucity of genetic variation all contribute to the uniqueness of this species. Western red cedar is inferred to have survived in one to several refugia south of the extent of the Pleistocene ice sheets, and began reoccupying its previously held ranges from about 10,000 years ago, with the major influx into previously held territory in B.C. around 5,500 to 6,000 years ago. These ecological, silvical, and evolutionary history concepts must be understood and used as a foundation upon which knowledge of the genetics and physiology of this species should be superimposed.

The genetic architecture of western red cedar, as revealed in seedling growth, survival, and responses to environmental stress (the latter two being considered adaptive traits) were investigated. Since inbreeding depression is common in most conifers, but lethals may have been purged in this species considering its evolutionary history, the responses to inbreeding were also examined.

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2. Seedling Growth

2.1. Introduction

Genetic variation in shoot elongation has been studied in a few Cupressaceae species. Zobel (1983) studied the twig elongation patterns of Port-Orford cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.) and found little genetic variability in phenology. Harry (1987) tested shoot elongation and growth plasticity of incense-cedar (*Calocedrus decurrens* [Torr.] Florin) seedlings to age three and obtained similar results in that species. A further, recent study of this species (Rogers *et al.*, 1994) noted among-region variation in growth attributes at age 12 that had not been apparent in younger seedlings. Studies of genetic variation in yellow cedar (*Chamaecyparis nootkatensis* [D. Don] Spach) showed significant family variation in seedling height (Cherry and Lester, 1992; Russell, 1993).

There is a paucity of information on the genetics of western red cedar, although many horticultural varieties, involving crown form and foliage colouration, are known (Hillier, 1981; den Ouden and Boom, 1982; Rushforth, 1987). Polheim (1970; 1972; 1977a; 1977b) studied chlorophyll and shoot form somatic mutations and survival in haploids, diploids, and triploids of cultivated varieties. Nault (1986) found much tree-to-tree variation in thujaplicin content of old growth and second growth cedar, and suggested that variation in this trait may be influenced genetically.

A small number of disconnected studies of various characteristics, most of which included only a few populations, provide mixed conclusions about the level of genetic variation in this species. Three early studies found variation between genetic

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entries. Larsen (1953) found differences between two western red cedar clones in resistance to both the cedar leaf blight (*Keithia thujina* Durand) and to frost damage; these two traits were positively correlated in the clones tested. Søegaard (1966) tested responses to cedar leaf blight in western red cedar and Japanese thuja (*Thuja standishii* [Gordon] Carriere). He concluded that resistance to the blight was conferred mainly by a single gene, which is dominant in the *T. standishii* tested and recessive in the *T. plicata* used in the test, and suggested that resistance to frost may be inherited by a similar mechanism. Ilmurzynski *et al.* (1968) noted that growth of an Alaskan provenance was inferior to one provenance from Oregon and one from Idaho when grown in Polish nurseries and plantations.

Other studies have shown evidence of little genetic variation among measured traits. Von Rudloff and Lapp (1979) obtained no significant difference in leaf oil terpene composition between coastal and interior provenances, and concluded that this species has one of the lowest degrees of variability in North American conifers investigated to date, along with red pine (*Pinus resinosa* Ait.). Von Rudloff *et al.* (1988) reanalyzed their leaf oil terpene data nearly a decade later using discriminant analysis, and this time found minor differences among coastal provenances.

Copes (1981) investigated isozyme variability of nine enzyme systems in seven provenances of western red cedar, while Yeh (1988) studied variation in 15 enzymes from eight provenances of this species; both found little genetic polymorphism among populations. Yeh (1988) found that 14 of 19 loci were monomorphic.

Bower and Dunsworth (1987) obtained no significant differences among three local low elevation Vancouver Island western red cedar provenances in survival or

height growth of seedlings growing at three Vancouver Island plantations.

A very recent study on inland western red cedar has just been published (Rehfeldt, 1994) involving 41 interior bulked provenances from seven major river drainages in Idaho, two drainages in Montana, and two in southern interior B.C., plus one Idaho provenance with family structure, all growing at three Idaho sites. Although Rehfeldt found both between- and within-population differences in three- or four-year height at two out of three sites, in presence of winter injury at one site, and in mortality at one site, none of the traits studied varied significantly over all test sites. Weak elevational clines were detected in four-year height at one site and in presence of winter injury at the same site, with $r^2 = 0.10$ and 0.16 respectively. A wider sampling of provenances and families, spanning more of the species' range, is needed to define the structure of genetic variation in western red cedar and to obtain a broader picture of possible geographical influences.

Early estimates of genetic variation in branch angle and in the allocation of photosynthate between the stem, branches, and root are desirable, and are known to give reasonable predictions of these traits in the mature tree. No reports have been published on the genetics of shoot morphology in western red cedar, despite the many known horticultural varieties of this species having varying shoot forms.

The purpose of this chapter was to investigate the genetic patterns and relationships that may be found in western red cedar seedling growth (as measured by seedling height, root collar diameter, and dry weight allocation), morphology (branch angle), foliar nutrient content, and survival characteristics between and within zones and provenances encompassing a wide range.

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2.2. METHODS

2.2.1. Seed Sources

The coastal range of western red cedar was represented by B.C. Ministry of Forests (Cowichan Lake Research Station (CLRS), Vancouver Island) cone collections from Prince Rupert, the Queen Charlotte Islands, Vancouver Island, and the Lower Mainland (Figure 2.1; Table 2.1). Unfortunately, seed could not be obtained from the Washington or Oregon portions of the coastal range of this species or from the coastal / interior transitional populations at the time that the study was initiated. The interior western red cedar range was represented by seed collected from Salmon Arm, B.C. (B.C. Ministry of Forests, Kalamalka Research Station, Vernon, B.C., collections) and from northern Idaho and Montana (USDA Forest Service, Intermountain Research Station, Moscow, Idaho, collections). Within a provenance, cones were collected from individual trees which were at least 100 m apart, but within a one km range, to minimize relatedness between seed sources.

Initially, ten provenances from the coastal range of western red cedar and ten provenances from the interior range were included in the study of seed and seedling traits. Due to a prohibitive number of seedlings involved, not all provenances were represented by family structure; provenances where families were bulked were included so that the coastal and interior ranges could be more fully represented. Within each range, five provenances had family structure (five families per provenance), with the remaining five provenances per zone having no family structure, for a total of 60 genetic entries. Provenance abbreviations, as used throughout the thesis text, figures, and tables, are listed at the bottom of Table 2.1.
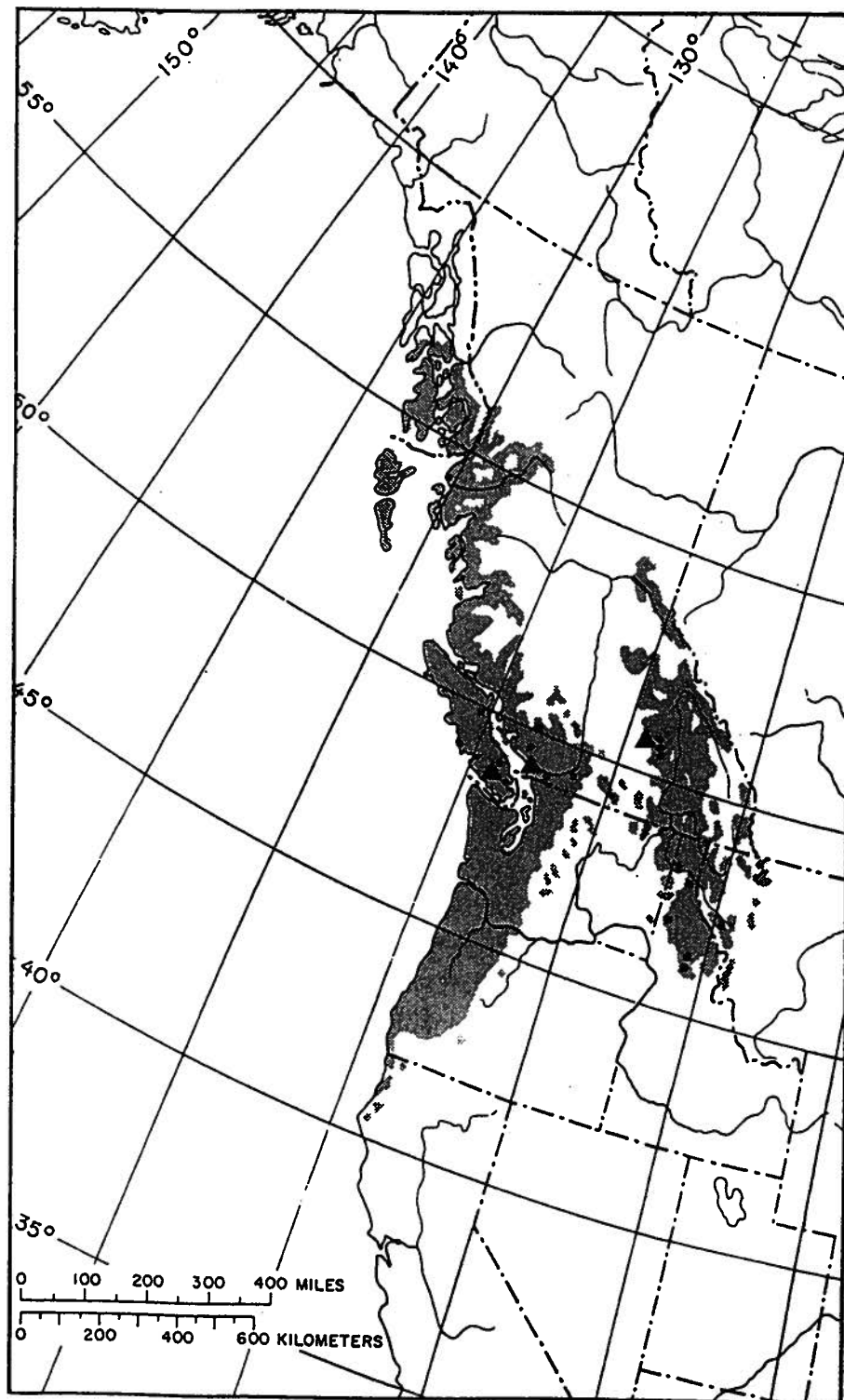
Figure 2.1. Provenances (•) and test sites (▲) within the species range (after Lowery, 1984).

Table 2.1. Geographic variables of provenances used in this study (F indicates family structure); for analysis, latitude and longitude minutes were converted to hundredths of a degree, but their whole minute values are given here.

Provenance	Elev. (m)	Latitude	Longitude	Mean # growing days	Mean annual prec. (cm)	Mean daily January temp. (°C)
Coastal						
Masset[1]	20	54°00'	132°00'	160	125	2.5
Oliver Lake	65	54°17'	130°15'	200	150	2.5
Quinsam	250	49°58'	125°32'	140	150	2.5
Tofino	50	49°01'	125°35'	230	300	5.0
Mt. Benson	700	49°08'	124°02'	180	100	2.5
Mill Bay	100	48°37'	123°33'	220	125	2.5
Squamish	100	49°47'	123°08'	220	200	0.0
Cheakamus	690	50°05'	123°03'	120	250	-5.0
Whonnock	205	49°13'	122°26'	200	175	2.5
Hope	600	49°28'	121°17'	200	125	-2.5
Interior						
Mt. Mara Low	550	50°40'	118°45'	100	50	-5.0
Mt. Mara Mid	1,100	50°43'	118°47'	80	65	-7.5
Mt. Mara High	1,950	50°45'	118°49'	60	75	-10.0
Benton Flat	686	48°21'	116°50'	120	65	-4.0
Kaniksu	1,219	48°03'	116°11'	100	90	-4.5
Lolo	945	47°09'	114°56'	100	60	-6.5
St. Joe	1,097	47°03'	116°37'	140	65	-1.5
Pierce	1,509	46°29'	115°40'	100	100	-3.5
Kooskia	411	46°08'	115°40'	130	90	-1.5

[1] The following three-letter provenance abbreviations have been used throughout the thesis:

Mas		Mib	(F)	mmL	(F)	Lol	
Oll		Squ		mmM	(F)	Stj	
Qui	(F)	Che	(F)	mmH	(F)	Pie	
Tof	(F)	Who		Bfl	(F)	Koo	
Mtb		Hop	(F)	Kan			

2.2.2. Seed Testing

In order to determine whether seed traits influenced subsequent growth, and whether variation could be detected between genetic entries even prior to the seedling stage, certain seed characteristics were investigated. Seed weights were taken on 100 cleaned nondewinged seeds per seed source.

A random sample of 100 seeds per genetic entry was X-rayed for percentage of seed containing a complete embryo (% seed fill). A Softex Supersoft X-ray Apparatus model TV-25-1 machine, set to run at 15 kVp and 3.0 mA for 7.0 seconds per exposure, was used for seed X-raying.

A subsample of one hundred seeds per seed source was tested for percentage germination. Seed from each source was placed into a plastic petri dish on a moistened Whatman filter paper and placed under broad-spectrum fluorescent grow lights under a 12-hour photoperiod at room temperature. Petri dishes were kept moist as required to allow germination. The germinants were counted as they appeared until no further germination occurred. A tally of abnormal germinants was recorded when such abnormalities were noted.

2.2.3. Greenhouse Design

The initial greenhouse design was a randomized complete block design common garden study with ten replications (reps). Each genetic entry was represented by one randomly assigned nine-seedling row per rep.

However, very little germination occurred in provenance Silver Star from

Vernon, B.C. In one Silver Star family, most of the germinants had their cotyledons, rather than their radicles, emerge first from the seedcoat; these germinants subsequently died. Silver Star could thus not be included. Only two of the Mt. Mara High elevation (near Salmon Arm, B.C.) families produced germinants. Thus the interior zone was represented by only 22 genetic entries (instead of 30) for a total of 52 genetic entries over both zones (rather than 60). Some families or bulked provenances did not have enough germinants to sow all ten replications; therefore the design contained missing cells.

2.2.4. Germination and Nursery Culture

As seed stratification is not necessary for western red cedar, seeds were stored in a cooler at about 3°C until they were utilized. All seeds were pregerminated prior to sowing. Starting on May 7, 1990, seeds were put put into plastic petri dishes on moistened Whatman filter papers and placed under a light table at the University of British Columbia (UBC), having full-spectrum fluorescent lights and a 12-hour photoperiod, at room temperature. The filter papers and lid of the petri dish were kept moist by periodically squirting them with a water bottle as needed.

When the emerging radicle of a seed was as long as the seedcoat, the germinant was dibbled into a filled styroblock cavity using tweezers and narrow cavity spoons. Blocks used were Styro Vent 91 blocks, having 7 x 13 = 91 cavities per block and a volume of 133 cc per cavity. The soil mix used was the standard mix for forest nurseries in B.C.: 2 bales peat: 1 bag vermiculite: 1.125 kg dolomite lime: 225 g trace elements per m^3 of soil. Each block could accomodate seven genetic

entries, which were planted in lengthwise rows, with one empty cavity after every two trees per row to help minimize block edge effects.

The first of the germinants were sown on May 16, 1990 and every day thereafter until all replications were filled or no more germinants were available for a particular genetic entry. Due to unusually wet weather during the spring, some seeds rotted and hence some cavities had to be resown once or twice if extra seed was available. The last germinant was planted on June 18, 1990.

Styroblocks were set up in a raised outdoor compound at UBC's South Campus Nursery in Vancouver, B.C. (elevation: 40 m; latitude: 49°15.5' N; longitude: 123°13.8' W; mean annual frost-free period: 244 days; mean annual precipitation: 128.9 cm (Environment Canada, pers. comm., 1994); biogeoclimatic subzone: CWHdm (Gordon Kayahara, UBC, pers. comm., 1994)). Circular overhead sprinklers were used for irrigation and fertilization purposes. Trees were grown under a normal container nursery fertilization regime throughout the 1990 growing season.

The seedlings were overwintered on the ground in the outdoor compound, partially covered with sawdust which was also banked around all blocks. Snowfall during the winter of 1990 / 91 was sufficient enough to blanket the seedlings and thus further insulate them.

2.2.5. Outplanting

During early to mid May 1991, seedlings were transplanted from the styroblocks. All odd-numbered replications (1, 3, 5, 7, and 9) were relocated to a

transplant bed at UBC's South Campus Nursery. Even-numbered replications (2, 4, 6, 8, and 10) were shipped to the B.C. Ministry of Forests Skimikin Seed Orchard (Skimikin) at Salmon Arm, within the interior range of western red cedar, and transplanted into a bed within the seed orchard (elevation: 540 m; latitude: 50°47' N; longitude: 119°25' W; mean annual frost-free period: 121 days; mean annual precipitation: 49.3 cm (Envir. Canada, pers. comm., 1994); biogeoclimatic variant: IDFmw2 (Keith Cox, Ministry of Forests Skimikin Seed Orchard, pers. comm., 1994)). The latter group of seedlings were trucked to Salmon Arm while still in the styroblocks to minimize handling and risk of injury.

Both fields had been plowed and harrowed prior to transplanting. Trees were planted 1/3 m apart in straight rows using a flat planting shovel. At both sites, the best eight out of nine seedlings per genetic entry per replication were outplanted in eight-tree row plots, maintaining the randomized complete block design with five replications per location.

No further fertilization treatments were given to the seedlings. However, seedlings were occasionally watered at both sites during severely dry weather to prevent severe drought stress. Occasional weeding between rows and between trees in a row was also carried out.

2.2.6. Seedling Data Collection

Seedling height measurements on all live trees were taken, to the nearest mm, on August 8 and 9, 1990 and at the end of the 1990 growing season after height elongation had stopped. Seedling height measurements to the nearest 0.5 cm were

taken after the 1991 and 1992 growing seasons at the UBC and Skimikin locations. Root collar diameter (RCD) was measured to the nearest mm on all trees at UBC and Skimikin after the end of the 1992 growing season.

Seedling dry weight measurements were taken on a subsample of trees at UBC after the end of the 1992 growing season. Initially, dry weight measurements were also planned for a subsample of trees growing at Salmon Arm. Due to a severe winter during 1991 / 92 at the latter site, virtually all seedlings underwent severe desiccation damage, and suffered either high mortality or foliage dieback and hence major shoot deformity. Thus it was decided that the dry weights of such stressed trees would not be representative of trees growing at an interior site, and so these measurements were not taken.

At UBC, trees were selected for destructive dry weight sampling after final height and root collar diameter measurements had been taken. For provenances without family structure, all eight seedlings per replication were selected for sampling, for a maximum of 40 trees over the five replications.

For provenances having family structure, four families out of the five families per replication were selected in each replication. The four families chosen rotated over each replication, so that each of the original five families was tested in four of the five replications, and skipped in one of the replications. In each replication, two trees per family were selected; wherever possible, trees #3 and #6 from the 8-tree row plots were chosen. If tree #6 was the last tree in a row, tree #5 was taken instead. Trees with double leaders or with insect damage were judged not to be truly representative and a neighbouring intact tree was sampled in its place. Thus each provenance with family structure was represented by two trees in each of four

families over five replications, for a maximum of 40 trees over all replications.

Tree samples were labelled with tags and dug up with the root system intact using a wedge-shaped planting shovel. Seedlings were then placed in plastic garbage bags and transported to the lab at UBC.

For each seedling, the number of lateral branches coming off the main stem was counted. In all trees from Replication #3, average branch angle per tree was recorded. Each tree was then cut up using garden pruners and separated into roots, main stem, and lateral branches with all of their foliage. Each of the three sections per tree was placed into a labelled paper bag. The bags were then placed into a Blue M Stabil-Therm drying oven and samples were dried for 24 hours at 65°C.

Samples were weighed immediately after being removed from the oven. Contents were removed from the paper bags and poured into a tared plastic tub on a balance. Samples were weighed to the nearest 0.01 g.

Seedling survival at both UBC and Salmon Arm was recorded for all years.

2.2.7. Foliar Nutrient Analysis

To estimate provenance level variation in foliar nutrient content of certain macro and micronutrients, and to see whether any relationships between nutrient uptake and growth could be observed, a subsample was taken. On September 30, 1992, foliage samples from nine trees in each of six provenances (Oliver Lake, Mill Bay, Tofino, Mt. Mara Low elevation, Mt Mara Mid elevation, and Benton Flat) were collected from 2-year old seedlings at UBC's South Campus Nursery transplant bed.

All provenances except for Oliver Lake and Benton Flat were represented by three trees from each of three families; the latter two provenances were represented by nine trees (no family structure). Samples were placed in labelled paper bags and put into a Blue M Stabil-Therm drying oven, where they were dried at 60°C for 48 hours. They were then ground up using a Braun coffee grinder. Each sample was then sieved through a 1 mm (#18 mesh) screen and then placed into labelled envelopes of double thickness.

Samples were shipped to the MacMillan Bloedel laboratory at Nanaimo, B.C. where foliar nutrient analysis was carried out. Samples were analyzed for % N, % P, % K, % Ca, % Mg, ppm Mn, ppm Fe, ppm Cu, and ppm Zn.

2.2.8. Data Analysis

The overall means and standard deviations were calculated for all parameters. Means were also determined by zone, provenance, and family where applicable.

Seed parameters were not replicated, so only minimal analyses could be done. Correlations were estimated on seed traits (percentage germination, percentage seedfill, and seed weight). Regressions were calculated to see whether height and root collar diameter measurements were related to seed parameters measured. In particular, seed weight can be assumed to be maternal in nature; relationships between seed weight and growth would indicate maternal influences on early growth. Germination and seedfill are indicators of seed viability, and should these traits have relationships with growth, it may be inferred that more viable seed sources also produce germinants which have superior growth capabilities, at least initially.

Regressions were carried out on the provenance least-squares means of final height measurements taken at UBC and certain geographic parameters associated with each provenance to study clinal or spatial patterns in variation which may occur.

Due to the badly unbalanced nature of the height and root collar diameter (RCD) data, a subset of provenances having family structure was used in analyses in which variability was investigated only at the zone and provenance levels, and family structure was ignored. For trees at UBC, the same individuals as chosen for dry weights were selected, as described above (Section 2.2.6). A similar selection method as described for dry weight sampling was used to choose a subsample of individuals from provenances having family structure at Salmon Arm. Thus all provenances, both those with and without family structure, were represented by a maximum of 40 seedlings over all replications per site in provenance-level analyses. Separate analyses were also carried out with only those provenances having family structure, in which all seedlings from these provenances were included, and variability at the family level was investigated.

Data were analyzed by analyses of variance (ANOVA) (SAS® PROC GLM), using Type IV Sums of Squares as the data still contained imbalances, and in particular contained some missing cells. The GLM (general linear model) procedure, using the method of least squares, allowed for the following: testing of random and mixed effect models, control over hypothesis tests, covariate analyses, specific contrasts, and analysis of unbalanced data sets; other procedures did not have these combined capabilities.

Zones were considered to be fixed effects, as all possible zones (coast, interior) for this species were included, with all other variables in the model being

considered random. Where necessary, Satterthwaite's Pseudo-F tests (Neter *et al.*, 1990) were calculated. Seedling age (in days) was used as a covariate in the models for height and root collar diameter, as sowing took such a long period to complete and thus seedlings differed slightly in age. Replication by zone and replication by provenance per zone were lumped into the replication by family per provenance per zone variable in most analyses, as these terms were not found to be significant and were not of primary importance to the analyses.

An analysis which included the variable location was carried out on heights taken at the end of 1991 at both sites; the data for 1991 were also analyzed separately per location. At the end of 1990, all seedlings were still at UBC, so location was not pertinent. The 1992 growing seasons of UBC and Salmon Arm were not comparable as the Salmon Arm site had suffered such severe desiccation damage during the winter of 1991 / 92. Hence height data were analyzed separately by site for 1992. An analysis including the variable location was also carried out for root collar diameter.

Variance components were estimated (SAS® PROC VARCOMP) for height and RCD measurements on provenances having family structure. SAS® PROC UNIVARIATE and SAS® PROC DISCRIM were also performed; the former was used to test for normality of the data while the latter tested for homoskedasticity, or equal variances of the dependent variable Lack of fit tests (residual plots) were also performed to test for linearity of the models.

From the dry weight data collected, shoot weights (foliage + stem weight), total dry weights (shoot + root weight), shoot/root ratios, stem wt./total wt. ratios, and shoot wt./total wt. ratios were calculated. Correlations were estimated between all dry weight parameters, number of lateral branches, and height and root collar

diameter of trees sampled for dry weight measurements. Analyses of variance at the provenance and family levels were carried out on all parameters, and variance components were determined at the family level for these traits. Univariate and discriminant analyses and residual plots were also carried out to test for normality and heteroskedasticity of the data. Separate correlations and analyses of variance were estimated on samples from Replication 3 in which branch angle had been measured and was included in the analyses.

Correlations were estimated between all macro and micronutrients analyzed. Analyses of variance at the provenance and family levels were also estimated.

Correlations were estimated on the amount of foliage per tree escaping desiccation damage during the winter of 1991 / 92 at Salmon Arm and tree heights at the end of 1991 and 1992 and RCD after the 1992 growing season. Correlations were also carried out on mean height and survival after 1991 and 1992 and on mean height after 1991 and survival after 1992 by site; a regression was also estimated to determine whether survival in 1992 was dependent on mean height at the end of 1991 at Salmon Arm. Genetic covariances between 1992 height and survival per location were calculated.

Regressions were estimated between the mean percentage of trees planted in 1990 still alive after 1992 and the mean percentage of live foliage after 1992 per provenance at Skimikin and certain geographic parameters associated with each seed source, again to investigate clinal or spatial trends in the variation of these traits.

Analyses of variance were performed on foliage escaping desiccation per tree after the 1991 / 92 winter, number of trees alive in 1991 and in 1992, percentage of

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trees living in 1991 that were still alive at the end of 1992, and the percentage of trees initially planted in 1990 still surviving after the 1992 growing season at each location. The latter trait was also tested in an analysis which included both locations. Variance components were estimated for provenances with family structure for the amount of foliage surviving desiccation, number of trees living in 1991 and in 1992, and percentage of trees alive after 1991 still living after 1992.

Narrow-sense individual heritabilities were calculated for traits which gave some evidence of family variation. The purpose of calculating heritabilities was not to reflect an accurate estimation for the species; rather, it was to give an indication as to whether earlier claims of little to no variation present in this species were supported by this research. Thus it was considered of little importance to look at the effects of zone, provenance, and location in these determinations, and so these effects were not included in the model.

It is suspected that some unknown degree of relatedness, in particular some self-pollination, may be a factor in natural stands of western red cedar. To be conservative, it was arbitrarily decided that the σ^2Family estimated $3/8$ of the additive variance rather than $1/4$ of the additive variance as is normally used in half-sib analyses. The ratio of $3/8$ was thus midway between half-sib and full-sib estimations. The general form of heritability calculations was:

$$h^2 = \frac{2.7 * \sigma^2 F(P\ Z)}{\sigma^2 E + \sigma^2 R(L)*F(P\ Z) + \sigma^2 F(P\ Z)}$$

where: F = family; P = provenance; Z = zone; R = replication; L = location;
          E = error

Standard errors of the Family variance components were calculated.

For seed data, 1992 height and RCD data, and dry weight data, provenances were split into five regions: the north coast of B.C., Vancouver Island, the lower mainland of B.C., the B.C. interior, and Idaho / Montana populations. For each region, overall means and standard deviations were calculated. These statistics were also determined for the seed data by seed collection source. Analyses of variance were conducted in which region was a variable in the model. The proportion of σ^2Provenance out of the σ^2Total was estimated from variance component estimates.

Hierarchical cluster analysis for provenance groups was carried out on UBC final height, Skimikin survival, and Skimikin live foliage after winter desiccation damage for comparison with the five geographic regions, using SAS® PROC CLUSTER with SAS® PROC TREE used to draw the output.

2.3. RESULTS

2.3.1. Seed Traits

Expected mean square equations that were used to determine the appropriate error terms to be used in F tests for each factor are given in Appendix 1. Mean squares and significance levels (*** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05) as estimated by analysis of variance (ANOVA) are given in Appendix 2 through Appendix 7. Where analysis of covariance was performed (denoted as β*covariate term in the linear model), the covariate term was adjusted to its mean (often shown as $\beta*(X_{ij} - \overline{X})$ in the literature). Table 2.2 summarizes the overall means plus or minus the standard deviation, variables found to be significant at provenance and family levels by ANOVA, narrow-sense individual heritabilities, and the family variance component plus or minus standard error for measured growth traits.

ANOVA showed that zonal differences could be detected in percentage seed fill and seed weight, and provenance differences were evident in percentage germination for those with family structure (Appendix 2.1). All seed parameters were correlated with each other to some degree. Germination varied widely between seed provenances. Cones were collected in different years, and by different people. Also, evidence of insect damage to a few seed lots was found. It is possible that a few weeks of stratification may have aided in causing better uniformity in speed of germination. Although it is believed that differences between provenances in seed viability are real, further analysis is needed for rigid conclusions regarding the viability of seed sources to be drawn.

Table 2.2. Summary of measured growth traits; see text for a full description of variables and the units they were measured in.

Variable	Overall Mean	± st. dev.	ANOVA results Prov. level	Fam. level	h^2	$\sigma^2 F$	± s.e.
Seed							
% Fill	68.8	20.53	Z		-	-	-
Weight, g	0.0013	0.00023	Z		-	-	-
% Germ.	55.5	23.43		P	-	-	-
Height							
'90 Plugs	12.5	2.93	Z P	Z P F	0.28	0.681	0.201
'91 UBC	37.4	8.06	P	P F	0.33	6.732	2.255
'92 UBC	72.6	14.74	P	P F	0.38	24.795	8.250
'91 Skim	29.0	7.45	P	P F	0.12	3.048	1.167
'92 Skim	29.2	9.90	P	Z F	0.12	3.856	2.486
'91, 2 Loc.	33.3	8.84	P	P F	0.20	3.738	1.360
RCD							
'92 UBC	9.93	1.98	P	F	0.16	0.181	0.078
'92 Skim	9.56	2.06	P	F	0.23	0.330	0.163
'92, 2 Loc.	-	-	P		0	0	0
Dry Weight							
Br. angle	42.6	8.60			-	-	-
#laterals	18.7	3.31	Z		0.06	0.221	0.426
Stem wt	8.4	3.58	P	P	0.17	0.653	0.566
Foliar wt	15.5	7.87	Z P	P	0.25	4.209	2.584
Shoot wt	23.9	10.98	Z P	P	0.22	7.523	5.136
Root wt	7.5	3.09	Z	P	0.12	0.435	0.513
Total wt	31.4	13.46	Z P	P	0.20	10.845	7.934
Shoot/Root	3.21	0.90	P	P	0.29	0.065	0.037
Shoot/Total	0.75	0.055	P	P	0.20	0.0002	0.0001
Stem/Total	0.27	0.049	P	P F	0.34	0.0002	0.0001

Table 2.2 continued...

<u>Nutrients</u>

% N	1.25	0.131	P			F	-	-	-
% P	0.26	0.022	P		P		-	-	-
% K	0.97	0.160	P		P	F	-	-	-
% Ca	0.95	0.123	P		P		-	-	-
% Mg	0.21	0.032	P				-	-	-
ppm Mn	114.46	23.345	P				-	-	-
ppm Fe	48.02	20.043				F	-	-	-
ppm Cu	7.32	2.629	P				-	-	-
ppm Zn	15.89	7.500	P				-	-	-
P/N	0.21	0.024	P		P		-	-	-
K/N	0.78	0.149	P		P		-	-	-
K/Ca	1.05	0.278	P		P	F	-	-	-

<u>Survival</u>

UBC:

% 90/92 [1]	91.99	11.09	P	Z	-	-	-

Skim:

% 90/92 [1]	41.89	26.97			P	-	-	-
% 91/92 [2]	42.7	27.47			P	0	0	0
# Live' 92 [3]	2.9	1.91			P	0	0	0
Fol. des. [4]	16.52	28.55	P	Z	P	F	0.05	11.340	8.170

'92, 2 Loc.:

% 90/92 [1]	-	-			-	-	-

[1] Percentage of trees planted in 1990 still alive at the end of 1992
[2] Percentage of living trees at the end of 1991 still alive at the end of 1992
[3] Number of trees alive at the end of 1992
[4] Percentage of foliage per crown surviving desiccation damage of winter 91 / 92

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However, differences between seed sources were detected in the amount and type of abnormal germinants. Table 2.3 lists the abnormalities which were noted during the first growing season. Cotyledon morphology differences between provenances could be seen. For example, germinants from Mill Bay had very short, thin cotyledons while germinants from Hope uniformly exhibited long cotyledons. These observations were obvious, and further analysis was deemed unnecessary.

2.3.2. Height and Root Collar Diameter

Height and RCD measurements were not found to be dependent on seed parameters, except in a few cases where the coefficient of determination values were very low (all r^2 were < 0.12). These weak relationships were found for RCD with germination and seed weight, and for heights measured in August 1990 and at the end of 1990 with seed weight. Thus maternal influences and growth advantages of genetic entries having greater seed viability did not materialize, and seed level effects could be ignored.

Stepwise regressions of final height of seedlings grown at UBC on elevation, latitude, longitude, estimated average annual days in the growing season, annual precipitation, and daily temperature in January indicated that elevation was the most influential factor of those tested on height, alone accounting for 30.9 % of the variation in height between provenances (Figure 2.2). An elevational cline determined through simple linear regression found that 1 cm in height gain occurred for every 167 m decrease in elevation. However, the best model fitted, according to the stepwise procedure and to Mallow's C_p statistic, using a significance level of

Table 2.3. Germinant abnormalities noted during the first growing season.

Abnormality	Provenance	Family	# counted
albino	Squamish	-	2
"	Oliver Lake	-	2
"	Masset	-	1
"	Mill Bay	1	1
"	Mt. Mara Mid elev.	4	1
chlorotic	Mill Bay	1	5
"	Squamish	-	1
double leader	Cheakamus	3	1
"	Mill Bay	2	1
"	Mt. Mara Mid elev.	4	1
two radicles	Whonnock	-	1
adventitious lateral	Quinsam	5	1
cotyledons emerged 1st	Silver Star Low elev.	2	most
single fused cotyledon	Whonnock	-	1
three cotyledons	Kooskia	-	6
"	Squamish	-	4
"	Masset	-	3
"	Mill Bay	1	2
"	Mill Bay	4	2
"	Tofino	1	2
"	Tofino	5	2
"	Oliver Lake	-	2
"	Mill Bay	2	1
"	Quinsam	3	1
"	Quinsam	5	1
"	Cheakamus	1	1
"	Cheakamus	4	1
"	Mt. Mara Low elev.	4	1
"	Mt. Mara Mid elev.	5	1
"	Hope	5	1
"	Benton Flat	2	1
"	Lolo	-	1
"	Pierce	-	1
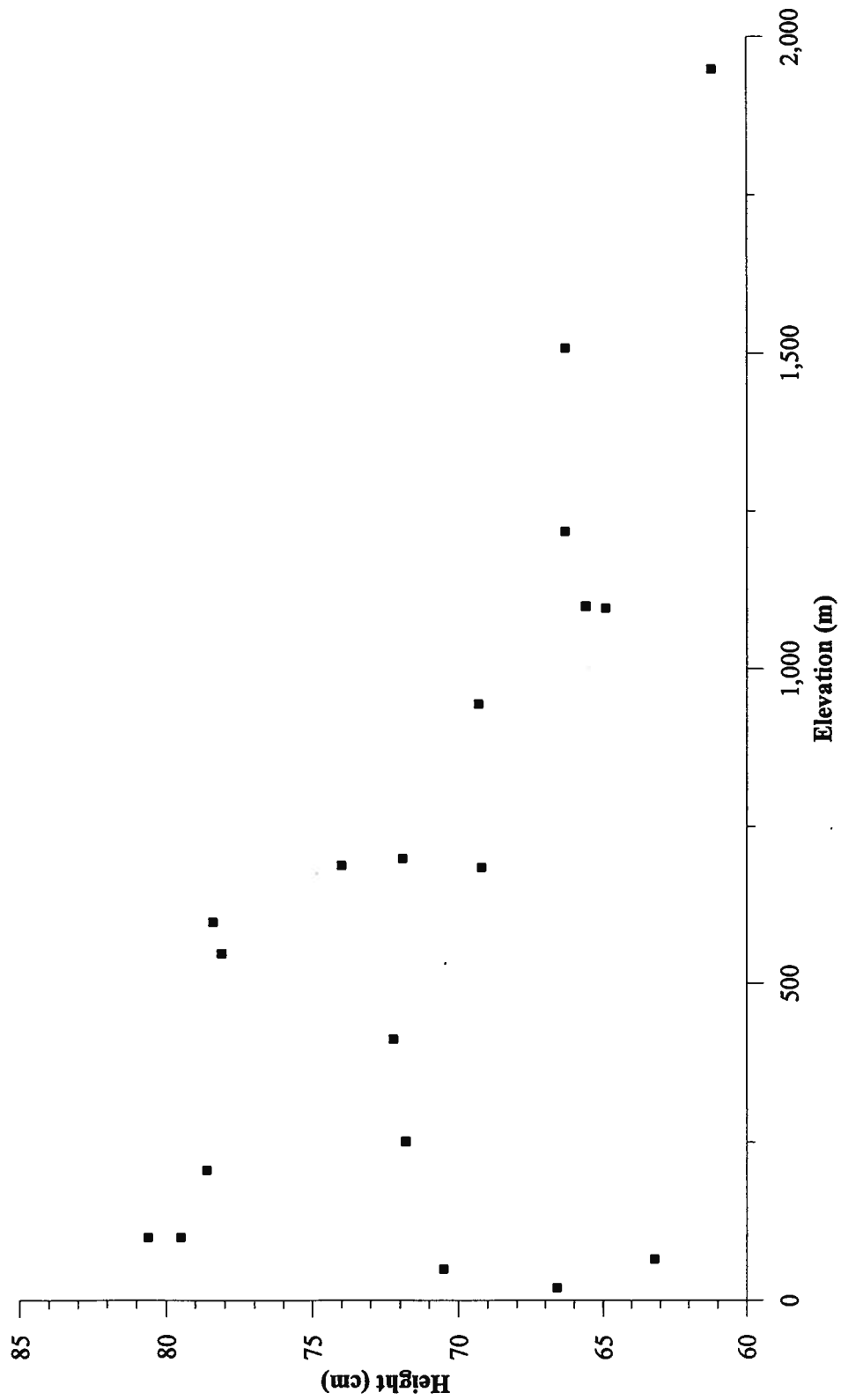
Figure 2.2. Mean provenance final heights of trees growing at UBC contrasted with provenance elevation.

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$\alpha = 0.05$ for entry into and staying in the model, was:

$$HtUBC = 156.545 - 0.010*Elev. - 0.652*Longitude; \quad R^2 = 0.466; \quad P = 0.0066$$

For all height and RCD measurements, provenance differences were evident for provenances without family structure (Appendix 3.1), and provenance (except in final height at Skimikin) and family differences occurred in provenances with family structure (Appendix 3.2; Figure 2.3). Zonal differences were only observed in first year heights and in final heights at Skimikin.

Location differences were obvious when 1991 heights at UBC and Salmon Arm were analyzed together, but were not evident in root collar diameter (Appendix 3.3; Appendix 3.4). The seedlings growing on the coast were able to take advantage of a much longer growing season. After 1991, trees growing at UBC were about 23 % taller than those at Skimikin; after 1992, trees at UBC were more than double in height compared to trees at Skimikin. Replication and rep by family interactions were significant, indicating probable microsite differences. Genotype by environment interactions were noted at the family level for 1991 height (for location*family, $P = 0.0208$) and at the zonal level for RCD ($P = 0.0072$).

The trees at Salmon Arm which were still alive to be measured by the end of 1992 were virtually no taller than they had been one year earlier, due in part to foliage dieback over the previous winter and in part to the fact that the trees had been severely stressed and grew in height little or not at all in the season after damage had occurred. Root collar diameters measured at Salmon Arm after 1992 were about the same as those measured at UBC during the same year. It is believed that the trees increased in caliper in 1991 even after seedling height growth had ceased. Caliper
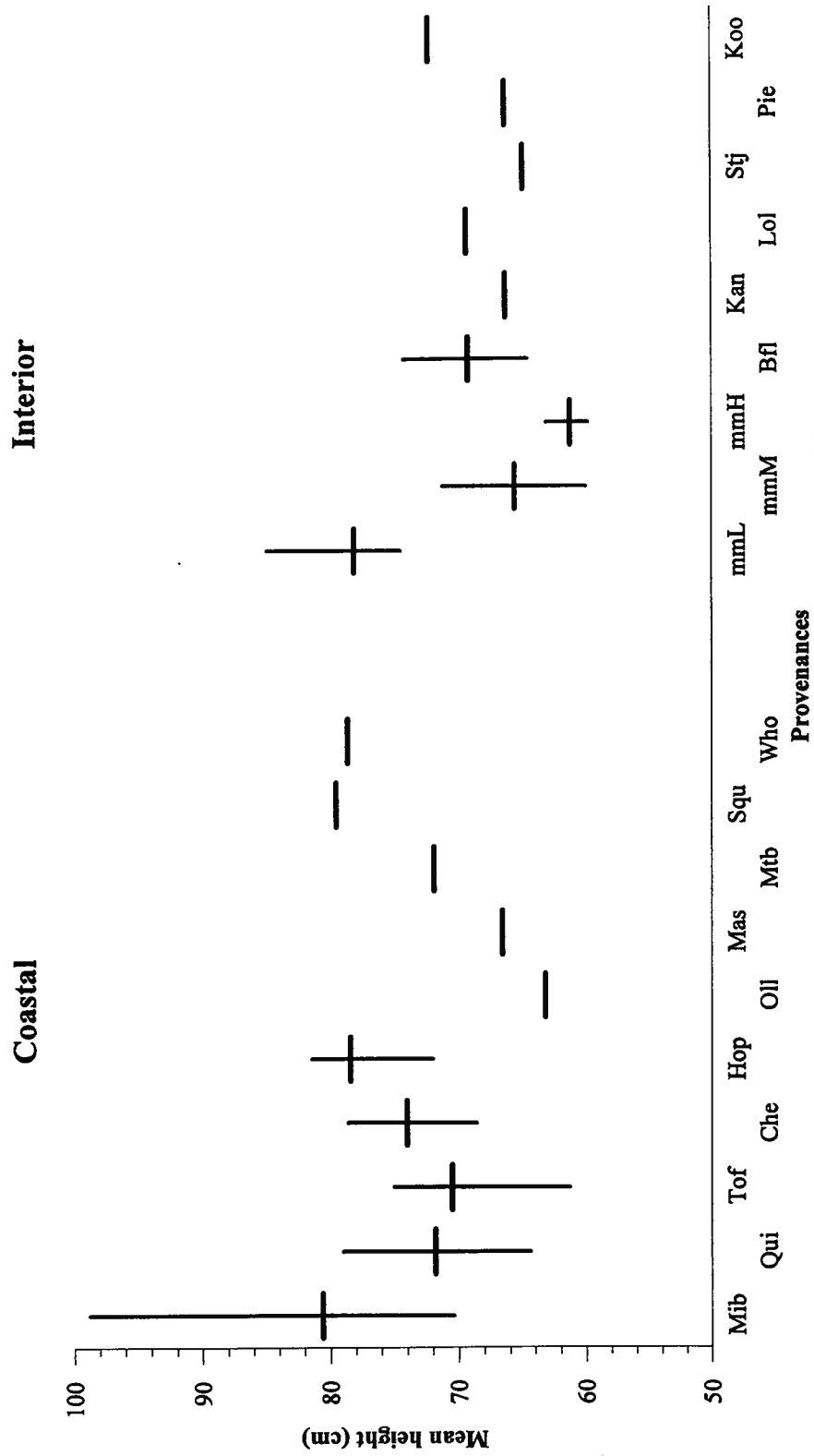
Figure 2.3. Mean provenance final heights (horizontal bars) and range of family / provenance means (vertical bars) of trees growing at UBC.

growth could have occurred in 1992, at least in the base of the stem, if the lower cambium was not damaged.

Table 2.4 shows the apportionment of total variance attributed to provenance and to family at the family level of analysis. After one growing season, the plug seedlings showed a higher provenance variance component for heights than family component, but after three seasons, the transplanted seedlings at both sites displayed a greater proportion of variance between families within provenances than between provenances. Root collar diameters measured after 1992 at UBC showed a greater percentage of variance attributable to family than to provenance, although the opposite was true at Salmon Arm.

Analyses were carried out on each provenance separately to compare the amount of within-provenance variation in height and root collar diameter of UBC trees between the provenances (Appendix 3.5; Appendix 3.6). The three Vancouver Island provenances contained family variation in height (Table 2.5), while family variation in RCD was evident at one Vancouver Island provenance (Quinsam), at Cheakamus, and at Mt. Mara Mid elevation.

Narrow-sense individual heritabilities of seedling height calculated on trees growing at UBC increased yearly from 0.28 after one year to 0.38, with a standard error of 0.13, after three years. Although these numbers cannot be treated as absolute indications of the amount of heritable variation in western red cedar, they do indicate that heritability in this species is not zero. The heritability of height at Skimikin was much lower, at 0.12 for both 1991 and 1992. Heritabilities of RCD at both locations after 1992 were lower than those of UBC heights, but large enough to indicate real variation.

Table 2.4. Percentage of the total variance attributable to provenance and to family within provenance for measured growth traits.

Trait	% $\sigma^2P(Z)$		% $\sigma^2F(PZ)$
Height			
'90 plug	17.77	>	8.23
'91 UBC	11.95	>	10.09
'92 UBC	9.06	<	10.66
'91 Skimikin	8.57	>	5.23
'92 Skimikin	2.55	<	3.88
'91, 2 Locations	6.50	>	3.84
RCD			
'92 UBC	3.08	<	4.57
'92 Skimikin	10.37	>	7.34
Dry Weight			
# laterals	1.83	<	2.08
Stem wt	9.54	>	4.56
Foliar wt	11.72	>	6.67
Shoot wt	9.19	>	5.94
Root wt	5.37	>	4.07
Total wt	8.67	>	5.87
Shoot/Root	8.80	>	6.89
Shoot/Total	8.11	>	5.40
Stem/Total	18.18	>	9.09
Survival			
Foliar desiccation	14.90	>	1.44

Table 2.5. Results of individual provenance analyses[1] of measurements taken at UBC after the 1992 growing season on provenances having family structure (values appear in brackets where Family was not significant).

Region	Prov.	Fam. signif.	σ^2F	s.e. σ^2F	% σ^2F/σ^2Tot
Height					
Van. Is.	Quinsam	F	22.812	21.712	12.8
	Tofino	F	24.275	19.841	14.1
	Mill Bay	F	93.024	63.772	36.2
Mainland	Cheakamus		(11.718)	(13.582)	(5.8)
	Hope		(1.388)	(8.061)	(0.7)
B.C. Inter.	Mt. Mara Low		0	0	0
	Mt. Mara Mid		(8.742)	(10.609)	(4.3)
	Mt. Mara High		0	0	0
Idaho	Benton Flat		(6.910)	(9.552)	(4.5)
Root Collar Diameter					
Van. Is.	Quinsam	F	0.645	0.498	16.2
	Tofino		0	0	0
	Mill Bay		(0.182)	(0.219)	(5.1)
Mainland	Cheakamus	F	0.403	0.355	9.2
	Hope		0	0	0
B.C. Inter.	Mt. Mara Low		0	0	0
	Mt. Mara Mid	F	0.229	0.213	7.1
	Mt. Mara High		0	0	0
Idaho	Benton Flat		(0.086)	(0.144)	(2.9)

[1] $\text{Provenance}_{rfn} = \mu + \text{Rep}_r + \text{Family}_f + R^*F_{rf} + \varepsilon_{(rf)n}$

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2.3.3. Dry Weights

Branch angle was highly variable, even within a tree (Appendix 4.3). No differences were noted in branch angle at any level through ANOVA. This may only be true for juvenile trees though. The number of lateral branches per stem varied between the coastal and interior zones, but no differences were evident between provenances or families.

Correlations between dry weight parameters of all test seedlings were all highly significant, with the only negative relationship between root dry weight and $shoot/root$ dry weight ratio. For provenances without family structure, most dry weight parameters differed at either the provenance or zonal level or at both levels when analyzed by ANOVA (Appendix 4.1; Appendix 4.2). For provenances having family structure, all dry weight traits differed at the provenance level. Family differences occurred only in the $stem/total$ dry weight ratio. Heritabilities ranged from 0.12 to 0.34 on these variables. Standard errors were calculated for the heritability of the $stem/total$ ($h^2 \pm$ s.e. $= 0.34 \pm 0.11$) and $shoot/root$ ($h^2 \pm$ s.e. $= 0.29 \pm 0.11$) ratios, indicating that heritabilities were significantly greater than zero.

2.3.4. Foliar Nutrient Analysis

ANOVA of the foliar nutrient analysis of all provenances showed provenance differences for all macro and micronutrients analyzed except for Fe (Appendix 6.1). For provenances having family structure, family differences were apparent in N, K, Fe, and the K/Ca ratio, while provenance differences were noted in P, K, Ca, and the ratios of P/N, K/N, and K/Ca. Nine pairs of nutrients were correlated, with the

significant correlations ranging from r = -0.53 to r = 0.40.

2.3.5. Seedling Survival

Although little mortality to seedlings occurred over the duration of the study at the UBC site, significant differences in survival at the zonal level were detected in the ANOVA of seedlings growing at UBC on provenances having family structure, and provenance differences were noted in provenances without family structure (Appendix 7.1; Appendix 7.2).

At the Salmon Arm site, differences existed between provenances having family structure in the number of living trees at the end of 1992, percentage of germinants sown in 1990 still alive after 1992, percentage of trees living at the end of 1991 (before the winter where major damage occurred) which were still alive by the end of 1992, and in the percentage of foliar damage occurring to a tree's crown over the winter of 1991 / 92. For foliar desiccation damage, zonal and family differences could also be detected (Appendix 7.3; Appendix 7.4). Although a higher amount of the variation was found at the provenance level than at the family level (Table 2.4), a heritability of 0.05 was estimated for foliar damage.

When an analysis of the percentage of germinants sown in 1990 still alive after 1992 was carried out in which both locations were included (Appendix 7.5; Appendix 7.6), genotype by environmental interactions were noted at bothe the zonal (P = 0.0462) and provenance (P < 0.0001) levels.

Foliage escaping desiccation damage per tree in 1992 at Salmon Arm was

negatively correlated to tree height at the end of 1991 ($r = -0.19$; $P < 0.0001$) and positively correlated to height ($r = 0.58$; $P < 0.0001$) and RCD ($r = 0.47$; $P < 0.0001$) after the 1992 growing season, after damage had occurred. The percentage of the original germinants still surviving after 1992 was negatively correlated to the mean height in 1991 at Skimikin ($r = -0.12$; $P < 0.0389$) but positively correlated at UBC ($r = 0.26$; $P < 0.0001$). Genetic correlations of seedling height and survival at UBC and Skimikin turned out to be meaningless due to negative variance components. Table 2.6 compares provenance rankings for the following traits: height at UBC, survival at Skimikin, and % live foliage at Skimikin.

Stepwise regressions of mean percentage survival of the original number of trees planted still alive after 1992 and of mean percentage of live foliage per provenance after the winter of 1991 / 92 at Skimikin with elevation, latitude, longitude, estimated average annual days in the growing season, annual precipitation, and daily temperature in January indicated that elevation was the most important of the independent variables on both traits. For survival, elevation alone accounted for 51.6 % of the variation (Figure 2.4); for live foliage, elevation was associated with 38.9 % of the variation. Elevational clines estimated from simple linear regression showed that 1 % increase in survival at Skimikin occurred for every 43 m increase in elevation, while 1 % increase in live foliage occurred for every 58 m increase in elevation. The best models which could be fitted, based on stepwise regression and Mallow's C_p statistic, using a significance level of $\alpha = 0.05$ for entry into and staying in the model, were:

Skimsurv = $-153.556 + 0.028*$Elev. $+ 3.567*$Latit.; $R^2 = 0.682$; $P < 0.0001$

Livefol $= -211.126 + 0.023*$Elev. $+ 4.310*$Latit.; $R^2 = 0.726$; $P < 0.0001$

Table 2.6. Provenance rankings of final height at UBC (in cm) and % live foliage and % survival at Skimikin; ranking of height is from largest to smallest, while ranking for live foliage and survival is from smallest to largest mean value.

Height (cm)		Live foliage (%)		Survival (%)	
Mib	(80.6)	Mib	(2.3)	Mib	(16.9)
Squ	(79.5)	Squ	(3.2)	Mas	(19.8)
Who	(78.6)	Tof	(4.5)	Squ	(21.8)
Hop	(78.4)	Who	(5.5)	Koo	(22.5)
mmL	(78.1)	Stj	(5.6)	Tof	(24.1)
Che	(74.0)	Koo	(8.5)	Stj	(28.4)
Koo	(72.2)	Kan	(10.4)	Who	(32.5)
Mtb	(71.9)	Qui	(10.4)	Qui	(36.6)
Qui	(71.8)	Che	(11.3)	Pie	(38.3)
Tof	(70.5)	Pie	(14.1)	Che	(39.9)
Lol	(69.3)	Mas	(16.2)	Oll	(45.0)
Bfl	(69.2)	Lol	(17.2)	Mtb	(48.2)
Mas	(66.6)	Hop	(17.3)	Kan	(48.3)
Pie	(66.3)	mmL	(19.6)	mmL	(48.4)
Kan	(66.3)	Mtb	(21.5)	Bfl	(49.8)
mmM	(65.6)	Oll	(23.9)	Lol	(52.1)
Stj	(64.9)	Bfl	(25.5)	Hop	(57.6)
Oll	(63.2)	mmM	(34.0)	mmM	(58.3)
mmH	(61.2)	mmH	(68.3)	mmH	(90.6)
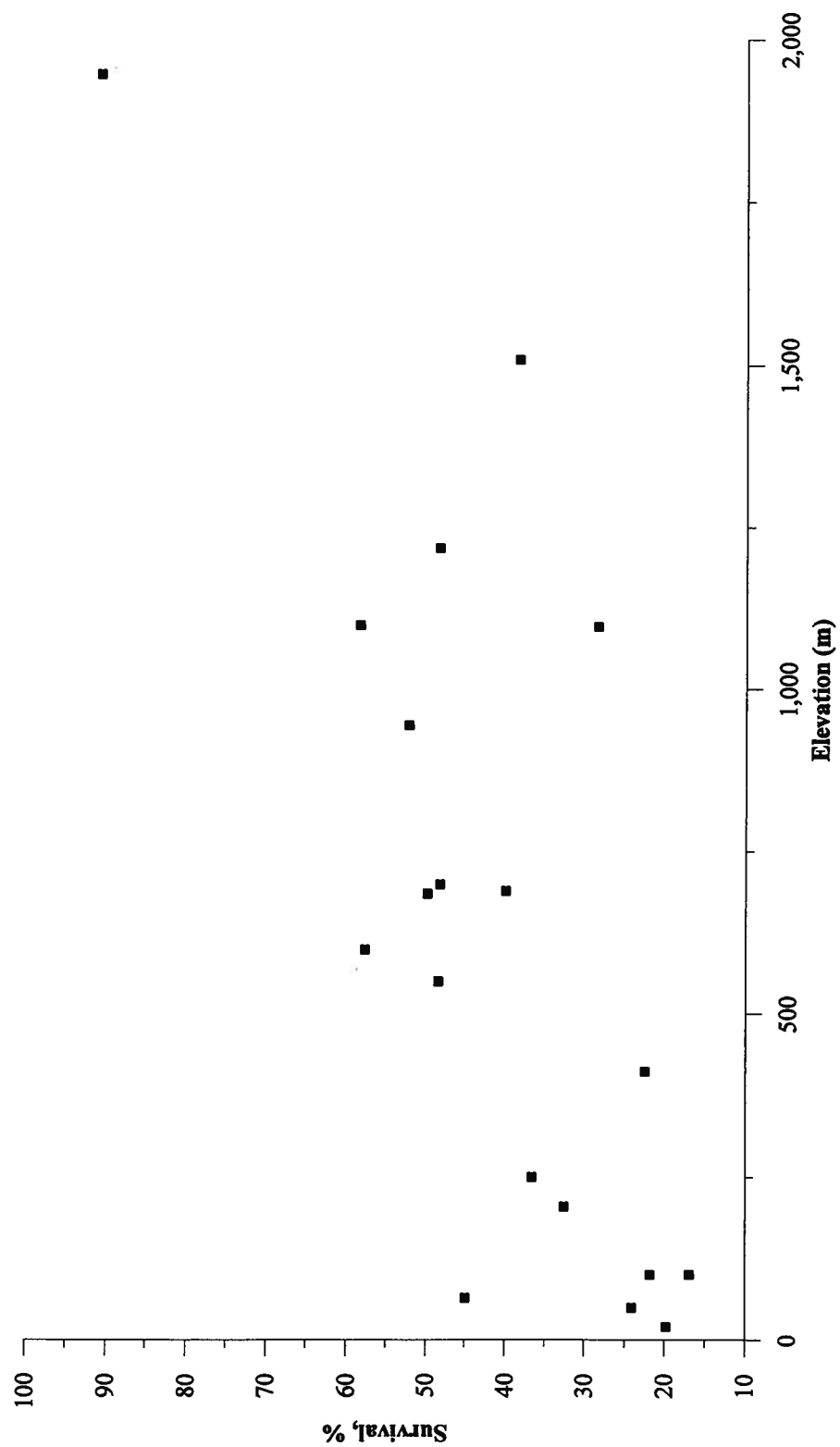
Figure 2.4. Mean provenance survival after the winter of 1991 / 92 of trees growing at Salmon Arm contrasted with provenance elevation.

2.3.6. Analysis by Seed Collection, Geographical Region, and by Cluster

When the seed data were separated into the three seed collections that made up this study, differences between collections in germination, percentage seed fill, and in seed weight were found in the ANOVA when all lots were looked at (Appendix 2.2). However, when provenances not having family structure were removed and the data reanalyzed, no significant differences were evident between collections in any of the seed parameters.

The same data were regrouped into broad geographical regions, which consisted of splitting the coastal seed collection into three regions. Thus the five regions were: northern B.C. coast, Vancouver Island, B.C. mainland, B.C. interior, and Idaho / Montana. ANOVA results showed that, as found for collections, regions differed in all seed parameters when all provenances were analyzed but did not differ in any seed trait when analyzing only those provenances with family structure (Appendix 2.3).

Dry weight parameters plus heights and RCD taken at UBC after the 1992 growing season were grouped into the same five regions. ANOVA was performed on all provenances by region (Appendix 5.1). Regions varied significantly in stem dry weight, foliar weight, shoot (foliage + stem) weight, root weight, total dry weight, stem/total dry weight ratio, and in RCD at UBC after 1992. The shoot/root and shoot/total dry weight ratios and height after 1992 at UBC did not vary significantly by region, although a north / south trend in the shoot/root ratio was noted. However, as the representation of provenances by region was unbalanced, and not abundant in one case, these results should be viewed with caution.

Table 2.7 lists the parameter means plus or minus the standard deviation, percentage of the total variance (σ^2T) attributed to provenance (σ^2P) , and the standard error of estimate (s.e.e.) on the provenance variance component per region. The regions were represented respectively by 2, 4, 4, 3, and 6 provenances.

The percentage of variation attributed to provenances and s.e.e. of the σ^2P appeared to vary greatly from region to region. The highest percentage of σ^2P and s.e.e. of the σ^2P for all parameters occurred in the B.C. interior region. No provenance variance or s.e.e. of the σ^2P was found in any parameter except the shoot to root dry weight ratio in the northern B.C. coastal region. However, this region was represented by only two provenances, neither of which had family structure, so these results probably do not reflect the true amount of provenance variance in this region. Although these results cannot be taken as absolute, they do indicate that real differences do occur between regions for western red cedar.

Hierarchical cluster analysis produced somewhat different clusters than the above geographically based regions. Table 2.8 presents the clustering of provenances by different variables and Duncan analysis of mean differences in UBC final height. When UBC final heights were clustered into four groups, all provenances within a geographical region except for the Mt. Mara provenances were clustered within two consecutive clusters. The same did not hold true for the other traits.

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Table 2.7. Final UBC height (cm), RCD (mm), and dry weight (g) means ± standard deviation, percentage of the total variation attributable to provenance, and standard error (s.e.e.) of the provenance variance component by region.

	Region				
Trait	1 N coast	2 Van. Is.	3 Mainland	4 BC inter.	5 Idaho
$\overline{X} \pm$ s.d.					
Ht '92	64.54±12.85	74.19±14.67	76.70±13.89	70.43±16.18	68.54±12.26
RCD '92	9.44±1.88	10.34±1.90	10.42±2.01	9.55±2.00	9.11±1.70
Stem wt	6.87±2.95	9.08±3.70	9.83±3.71	8.22±3.74	7.15±2.81
Foliar wt	19.16±8.48	17.74±8.17	18.10±7.76	14.93±7.70	10.93±5.02
Shoot wt	26.03±11.25	26.82±11.26	27.93±11.09	23.14±11.15	18.07±7.57
Total wt	33.70±13.68	34.87±13.71	36.81±13.54	30.47±13.51	24.17±9.38
Shoot/Root	3.41±0.84	3.41±1.00	3.22±0.83	3.15±0.87	3.01±0.86
% $\sigma^2 P/\sigma^2 T$					
Ht '92	0	8.30	1.37	20.03	1.54
RCD '92	0	1.95	2.04	5.64	3.01
Stem wt	0	4.46	1.63	8.68	2.74
Foliar wt	0	12.36	6.97	21.88	2.59
Shoot wt	0	6.08	4.87	22.37	2.91
Total wt	0	4.14	4.24	22.05	3.42
Shoot/Root	2.46	5.12	5.09	21.92	6.41
s.e.e. of $\sigma^2 P$					
Ht '92	0	14.435	5.003	47.774	3.612
RCD '92	0	0.099	0.104	0.278	0.093
Stem wt	0	0.811	0.629	2.627	0.246
Foliar wt	0	7.868	4.019	12.157	0.832
Shoot wt	0	9.331	6.908	26.171	1.934
Total wt	0	12.124	9.390	37.473	3.211
Shoot/Root	0.106	0.056	0.044	0.172	0.049
# trees, Ht / RCD	59	569	431	391	320
# trees, dry wt	55	151	155	98	195

Table 2.8. Comparison of geographical regions with groupings of provenances based on cluster analysis of certain traits; UBC height is ranked from tallest to shortest provenance mean, while survival and living foliage at Skimikin are ranked from smallest to largest percent, due to expected negative correlations with height; regions are ranked by average group height.

Geographical region	UBC final height	Duncan, UBC ht.	Skimikin survival%	Skimikin live foliage%	UBC ht., Skim. surv., live fol.
B.C. Mainland	Mib	a	Mib	Mib	Mib
Squamish	Squ	a b	Mas	Squ	Squ
Whonnock	Who	a b	Squ	Tof	Who
Hope	Hop	a b	Koo	Who	
Cheakamus	mmL	a b	Tof	Stj	
		b			Koo
Vancouver Island		b			Tof
Mill Bay	Che	c b	Stj	Koo	Mas
Mt. Benson	Koo	c d	Who	Qui	Stj
Quinsam	Mtb	c d e	Qui	Kan	
Tofino	Qui	c d e	Pie	Che	
	Tof	c d e f	Che		Che
B.C. Interior	Lol	c d e f g			Qui
Mt. Mara Low	Bfl	c d e f g		Pie	Pie
Mt. Mara Mid		d e f g	Oll	Mas	Kan
Mt. Mara High		d e f g	Mtb	Lol	
	Mas	h d e f g	Kan	Hop	
Idaho, Montana	Pie	h d e f g	mmL		Hop
Kooskia	Kan	h d e f g	Bfl		mmL
Lolo	mmM	h e f g	Lol	mmL	Mtb
Benton Flat	Stj	h f g		Mtb	Lol
Pierce		h g		Oll	Bfl
Kaniksu		h g	Hop	Bfl	mmM
St. Joe	Oll	h g	mmM		Oll
	mmH	h			
Northern B.C.				mmM	
Masset			mmH		mmH
Oliver Lake					
				mmH	

2.4. DISCUSSION

It is apparent that significant levels of genetic variation exist in *Thuja plicata* seedling growth attributes. Although only a sampling of populations could be tested and only two test locations were used, and hence heritability estimates were only representative of measured seedling traits at these specific sites, it is safe to infer that significant variation in this species exists. Heritability estimations were conservative to guard against the possibility of inbreeding, although indications are that adult western red cedar populations may not be inbred to a great degree. In the unlikely event that the estimates were still not conservative enough, it can still be said with a high amount of confidence that the heritabilities are not zero. These heritabilities certainly fall within the normal range for growth traits of other coniferous trees, roughly 0.2 to 0.3 (J.S. Brouard, pers. comm., 1994).

Differences between populations from the coastal versus the interior ranges existed in initial plug seedling height (during the early seedling establishment stage), in height and amount of crown dieback at the interior location after a severe winter, and in some dry weight parameters. Regional differences appeared in many dry weight parameters and in RCD measured at the coastal site. While survival and dry weights showed mainly between-population differences, the trend in height at the mild coastal site appeared to be towards more within- than between-population differences. Broken down by provenance, the Vancouver Island populations showed family (within-population) differences in height; root collar diameter showed family differences at Cheakamus, Quinsam, and Mt. Mara Mid elevation.

As gene flow seems to be extensive in this species, and outcrossing is assumed to be the normal reproductive strategy, one would expect to find greater

within- than between-population differences. This seems to be the case for height and root collar diameter.

For the traits where between-population differences are greater than those within populations, broad environmental selection pressures may be the most important factors shaping the genetic structure of this species since the last glacial retreat, in contrast to mutation and random drift. As greater between-population differences were exhibited in survival traits, this would lend credence to the above theory. The separate analyses by provenance also seem to support this theory. If disruptive (diversifying) selection is occurring, then variation would be increasing in those characteristics that were affected.

Within-population differences were noted on Vancouver Island and near the southern mainland coast where environments are most favourable to western red cedar growth, and less limited by environmental (i.e. temperature, moisture) extremes. Gene flow may be higher in these locales.

As the apical meristem is not protected by bud scales and foliage has little cutin and wax for protection from excessive transpiration, it would be expected that western red cedar would be quite susceptible to frost damage, transpirational stress, and foliar sunburn, and would be expected to be opportunistic as far as shoot expansion. Thus the most probable selective pressures are those imposing conditions of transpirational stress, very low winter temperatures, and foliar sunscald. If climatic conditions are not limiting, western red cedar could be expected to undergo rapid expansion due to extensive gene flow.

The severe winter damage to trees growing at Salmon Arm during the winter

of 1991 / 92 may have been so devastating in part because the seedlings were growing under full sunlight without the benefit of protection from an overstory. Thus the amount of damage and mortality, and major growth reduction the following growing season, may not truly represent conditions of a naturally regenerated or planted forest stand in the interior (the former becoming established below a protective overstory, and the latter probably having some degree of brush which would partially shade seedlings, at least during part of the year).

An increase in elevation appears to be the most important geographical element of those studied in limiting western red cedar growth. This concurs with the findings of Rehfeldt (1994), although the current study observed much stronger elevational relationships than those found by Rehfeldt. The discrepancy would most likely be due to the far greater diversity and much wider range of locales sampled in the current research. The Mt. Mara elevational cline near Salmon Arm in the interior of B.C. gives evidence of the degree to which an interior population source will vary by elevation.

Besides the elevational cline at Mt. Mara, a latitudinal cline occurred in Idaho, unfortunately confounded by the difference in elevation between the two populations. Pierce is 21' north of Kooskia (and 1,098 m higher in elevation). Pierce was the shorter of the two at the UBC site but was taller at Skimikin after 1992, and survival at Skimikin was slightly better for Pierce than for Kooskia.

The populations from the U.S. are the only ones of the current study to have possibly escaped glaciation. The locations where the Benton Flat and Kaniksu populations come from in northern Idaho were almost certainly glaciated. St. Joe in western Idaho and Lolo in western Montana were near the inferred glacial boundary

and so it is unknown whether these areas would have been covered by ice or not. Pierce and Kooskia, however, were probably not glaciated, although they may have undergone severe flooding during deglaciation. It is impossible to tell at this time whether sources of western red cedar trees reoccupying formerly glaciated areas in Idaho and trees coming from nonglaciated regions had the same ancestral origin. It is also unknown if coastal and interior populations migrated from the same refugium.

One baffling question to be addressed is the apparent discrepancy between the amount of variation evident in quantitative traits as observed in this study and the lack of variation seen in former studies involving isozymes, leaf extractives, or a very small, or nonrangewide, sampling of populations.

Studies have often found metric traits of silvicultural importance to be uncorrelated with allozyme variation. Geographic clines may differ in location or direction among loci or traits; thus factors other than migration must be influential in such cases (Namkoong and Kang, 1990). Many studies have suggested that quantitative traits are much more differentiated between populations than are isozymes (e.g. see Muona, 1988).

Only about 0.1 % of all nucleotide substitutions in the total genome, or about 20 % of the 0.5 % of the genome which codes for all proteins in a eukaryotic organism, can be detected by electrophoresis (Powell, 1975, as cited in El-Kassaby, 1991). No study ever examines all of the proteins in any species; thus only a small portion of the total variation present is ever investigated with this method. Enzymes surveyed are usually those found in high concentrations in tissue, and excluded are the products of regulatory loci and loci which code for ribosomal proteins and transfer RNA (Falkenhagen, 1985). Poor laboratory technique will limit even further the

detection of isozyme variation. Between DNA and the protein are the steps of transcription and translation; isozymes are thus phenotypes, and the genotypes can only be inferred from them, as they are not directly observed (Hattemer, 1991).

Lewontin (1984) also showed that the power of statistical tests discriminating between quantitative traits of populations and species and those discriminating between gene frequencies at individual loci are vastly different, and thus direct comparisons between the two should not be made. He stated that the probability of finding differences between populations and species in quantitative traits is much higher than that of gene frequences.

The variability of quantitative traits is governed more by selection pressures than is the variability of isozymes (Muona, 1988). Isozyme variation gives little insight into adaptive patterns of variation in quantitative characters and hence should not be used to make inferences about them, or to determine seed transfer and breeding zone delineations.

Assuming a mutation rate of about 10^{-6} per gamete per generation for a single locus, on the order of 100 to 1,000 generations are required after reclaiming territory after a genetic bottleneck for the heritable variance in quantitative traits of a species to be restored to previous levels, while 10^5 to 10^6 generations would be necessary to restore neutral alleles such as those studied by isozyme analysis (Lande, 1988). Western red cedar is believed to be still undergoing this process of restoration of former variability, so some level of variability must have been retained through the ice age.

None of the abovementioned ideas could be conclusively proven to be the

governing factor in why the results of this current study would indicate that much more variation is present in western red cedar than was inferred by previous isozyme studies of this species, but perhaps certain inferences might be suggested. It is also not known why many other associated coniferous species exhibit greater levels of isozyme variation than western red cedar, although perhaps other species of indeterminate nature may show similar trends. Less variation in neutral alleles may have been present in western red cedar even prior to glacial retreat. Alternatively, this species may have been reduced to lower levels of neutral alleles during glaciation, from which it is still recovering, as relatively few generations have occurred since the last ice age. Western red cedar may be slower to adapt to environmental changes than other species exhibiting higher heritabilities in quantitative traits, or else may be more plastic in certain traits, with less specialized genetic variation in these traits.

All but one of the populations tested in this study were part of large contiguous ranges (either coastal or interior), so random drift probably does not affect these provenances. However, isolated patches of western red cedar do occur between the the coast and interior ranges and along the eastern edge of the range; random drift may affect such populations to some degree in the distribution of variation within the species. The provenance from Lolo in Montana is situated on a noncontiguous patch, but is close to the Idaho range and so some degree of gene flow from the main range probably occurs.

A paper on *Picea omorika* (Panc.) Purk., a species also previously believed to be genetically depauperate with a high degree of self-fertility, reported much genetic variation in both enzyme and quantitative loci, and high levels of inbreeding depression in 24-year old trees of this species; the authors theorized that the onset of

selection against inbreds occurs later than for other species (Kuittinen *et al.*,1991). They concluded that neither post-glaciation mutation rates nor random drift could have accounted for the levels of variation that were observed in their study.

Some evidence of genotype by environment interaction, at the family level for height and the zonal level for RCD, and at the zone and provenance levels for seedling survival, was found in measured traits between two very different sites. At the coastal location, with a high number of frost-free days and an average amount of annual precipitation in comparison with provenances used in the study, the three best performers in height growth were the three closest provenances, also with long growing seasons, having average or above average annual precipitation levels, and coming from similar biogeoclimatic units. From this it can be stated that provenances from the southern coast probably perform best in relatively unstressful environments such as that found at the UBC site.

The interior test site was characterized by less annual precipitation than any of the provenances tested except for the nearest low elevation provenance, and a low number of frost-free days in comparison to most provenances tested. After the 1991 growing season, the provenance with the best average height was Mt. Mara Low elevation, the closest provenance at low elevation. The local mid and high elevation provenances, Mt. Mara Mid and Mt. Mara High, were ranked 16th and 19th respectively out of 19 provenances. Provenances ranked 2nd and 3rd at this time were Squamish (coastal) and Hope (eastern edge of the coastal range).

After the 1992 growing season, after experiencing severe desiccation the previous winter, Mt. Mara Low elevation was still ranked the tallest. However, Lolo from Montana and Pierce from Idaho (all interior wet belt provenances) were now

ranked 2nd and 3rd. The local mid and high elevation Mt. Mara provenances were now ranked 4th and 8th in height. Survival was greatest in Mt. Mara High, followed by Mt. Mara Mid with Mt. Mara Low ranked 6th after the severe winter damage at Skimikin. Generally provenances where the mean January temperature was comparatively low survived the best.

Specialist species are those with between-population differences, which are adapted to current specific environments (Rehfeldt, 1984). The genotype is expressed phenotypically, so selection acts on the genotype. Specialists may not have the ability to adapt quickly to catastrophes, due in part to coadapted gene complexes which may exist. Generalist species are those exhibiting plasticity (the ability of an organism to alter its phenotype in response to changes in environmental conditions) or homeostasis (canalization), which can function under a broad range of conditions, with many genotypes potentially being expressed as similar phenotypes. As selection would act against the phenotype, the genotype should be conserved over time. Generalists can sometimes undergo rapid gene flow and are more resilient to environmental fluctuations, particularly in time, and hence strong clinal variation should not occur (Rehfeldt, 1984).

Western red cedar seems to lie somewhere between a specialist and a generalist. Timing of shoot initiation and cessation is extremely plastic; in that sense, western red cedar is an opportunist, taking full advantage of favourable growth conditions. Seasonal height growth in general followed the pattern of a generalist, as within-population differences seemed to be greater than those between populations.

Survival under stressful conditions and dry weight traits were more specialized in nature. Population differences were apparent in these traits, whereas no

significant within-population differences were detected. Foliar desiccation at Salmon Arm following the severe winter showed both between- and within-population differences, but between-population differences were much greater than within-population differences.

Western red cedar showed similar plastic shoot elongation patterns to those of other indeterminate conifer species studied (Zobel, 1983; Harry, 1987; Cherry and Lester, 1992; Russell, 1993). Variation levels appeared to be not unlike those of other associated conifer species. For example, apportionment of variation between and within populations for first year container seedling heights of this study was very similar to that of western hemlock container seedlings of about the same age (Kuser and Ching, 1981), the latter species having a very similar species range and presumed recent refugial history to that of western red cedar. Response of western red cedar to harsh environments seemed to be severe when compared to seedlings of other species undergoing similar conditions.

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3. RESISTANCE TO ENVIRONMENTAL STRESSES

3.1. INTRODUCTION

The ability of trees to withstand cold temperatures and associated stresses such as desiccation during the winter without being damaged (to the extent that subsequent growth is adversely affected) or killed is one measure of adaptability. Winter conditions would be expected to impose selective pressures upon populations, and damage and mortality at any one locale might be expected to be related to the geographic origin of the tree seed. However, the physiology of frost hardiness is quite complex, and direct responses of trees to their environs may not be obvious.

The terms stress resistance, frost hardiness, and dormancy are often incorrectly used interchangeably. Resistance to stress, often termed hardening off or acclimation, is acquired by northern temperate forest tree species in a gradual process at the end of every growing season. In the spring gradual dehardening, or deacclimation, of the tree occurs. Hardy trees are more resistant to many forms of stress, not just that of cold temperature; stress resistance refers to all portions of the organism (Lavender, 1985). Frost hardiness is the ability of a tree to withstand subfreezing temperatures without damage, and is often expressed as the minimum temperature at which 50 % of a group of seedlings are killed or injured, the "lethal" temperature 50 (LT_{50}) (Glerum, 1985). Dormancy is simply the cessation of shoot growth in tissues; this refers only to the shoot apical meristem, as diameter growth may still occur. Roots, however, do not become dormant; feeder roots may grow when temperatures are not too severe (Perry, 1971).

The onset of dormancy occurs in mid-July in B.C., and may be induced by moisture stress (Lavender, 1985), although dormancy can be broken at this point if environmental conditions are favourable. Shoot elongation ceases and terminal buds form in determinate species. Eventually, during late summer, the tree becomes unresponsive to favourable environmental conditions, and the shoot and then the cambium will no longer begin growing in response to external stimuli.

The shortening photoperiod after the summer solstice is commonly the first stimulus acting on most temperate tree species to induce hardiness (Weiser, 1970), usually around early September in B.C. Trees at this stage become more responsive to temperatures at and just below freezing; such temperatures initiate a second stage of acclimation, in which large increases in degree of hardiness take place (Glerum, 1985). A third acclimation stage may be reached in very hardy woody species (Weiser, 1970). This stage is induced by prolonged exposure to very low (i.e. -30°C to -50°C) temperatures, and this stage is quickly lost.

In the northern hemisphere, temperate forest trees are generally most hardy during January. After a certain chilling requirement has been met, they begin to lose deep dormancy. The environmental cues which act to initiate deacclimation are not known, although Silim and Lavender (1991) obtained strong correlations in white spruce (*Picea glauca* [Moench] Voss) seedlings between exposure to temperatures of about 5°C and both the release of bud dormancy and development of dehardening potential. Dehardening lags behind release of dormancy (Lavender, 1985). Dehardening can occur very rapidly once it has been initiated (Glerum, 1985), although trees have been known to deepen in hardiness if adverse environmental conditions are imposed at this time (R. Guy, UBC, pers. comm., 1994).

Under freezing conditions, intracellular and / or extracellular freezing occurs in plants. Intracellular freezing, which occurs when freezing is rapid, is essentially always fatal, probably due to physical injury to the cell, as in cell membrane rupture by ice crystals (Weiser, 1970).

Extracellular freezing results when freezing is slow, and is sometimes, but not always, damaging (D. Lavender, UBC, pers. comm., 1987). The mechanisms of injury are not fully understood for this type of freezing, although hypotheses abound. During gradual freezing, water is drawn out from the inside of cells, and freezes in the intercellular spaces (Levitt, 1980). Tree cells are known to increase their cell membrane permeability during acclimation (Weiser, 1970), thus allowing water to exit the cell more easily during the period when cold temperatures are expected.

Cell dehydration occurs progressively as water becomes frozen externally. Plasmolysis, in which the protoplast withdraws away from the cell wall, occurs as cellular water moves outward. During deplasmolysis, water reentering the cell upon thawing may cause cell lysis, which is the most probable cause of death and injury in nonhardened tissue (Steponkus, 1984). Hardened plant cells may form exocytotic extrusions which can be reincorporated without lysing during rehydration (Thomashaw, 1990), but rapid temperature changes could cause hardy cells to lyse.

Risk of frost injury varies with stem position and tissue type. Basal stems have been found to be less hardy than upper stems of forest tree species, with the difference between stem position lessening with increasing hardiness (Sakai and Okada, 1971). In a test comparing various portions of one western red cedar tree, foliage samples were more resistant than twigs, which in turn were slightly hardier than buds [sic] (Sakai and Okada, 1971). Douglas-fir stem tissues were more

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sensitive to subfreezing temperatures than buds during the periods of hardening and dehardening, but were less sensitive than buds during the time of peak hardiness (Ritchie, 1991). Sakai and Okada (1971) found that cambial cells and surrounding phloem cells were the least frost sensitive; cambial cell resistance increased with age in Japanese larch (*Larix leptolepis* [Sieb. and Zucc.] Gordon) seedlings.

Frost damage may be compounded by desiccation due to an imbalance between water loss and absorption. Wind may increase transpiration by sweeping away the thin surface boundary layer of water vapour from the foliage; this effect is compounded in bright conditions when stomata are open and thus offer little resistance to transpiration (Kramer and Kozlowski, 1979; Salisbury and Ross, 1978; Sakai, 1970). When the tree roots or the stem below the snowline are very cold or frozen, water cannot be absorbed at a fast enough rate to replenish that which is lost due to transpiration; thus shoots become desiccated and may be killed (Kozlowski, 1971; Miller, 1978).

Metabolic changes have been noted in trees during hardening, especially during the second and third stages of acclimation. Growth inhibitors, anthocyanins, fats, and phenols are accumulated, while growth hormones (indole acetic acid, IAA, and the gibberellins, GA's) are at low levels in midwinter (D. Lavender, UBC, pers. comm., 1987; Perry, 1971). Changes have been noted in sugar, amino acid, nucleic acid, lipid, and protein levels (Glerum, 1985; Weiser, 1970), and in configurations of the latter two (Blum, 1988); some of these changes may confer cryoprotectant properties (Ritchie, 1991).

It is generally believed that the nutrient status of a plant affects its ability to harden, but how this occurs is ambiguous. Many contradictory results have been

published, probably because nutrients affect growth rate and hence only indirectly affect hardiness (Glerum, 1985). Of importance is undoubtedly the amount of nutrients available (adequate levels being better than deficient or excessive levels), and their relative proportions (Glerum, 1985; Pellett and Carter, 1981). Plant genotypes have been found to differ in the uptake and utilization of mineral nutrients (Fisher and Mexal, 1984). Schaedle (1991) discussed heritable traits which could be selected for in relation to nutrient uptake and utilization.

The stimuli inducing dormancy in trees apparently turn some genes on and others off (Perry, 1971), affecting the synthesis of certain enzymes. Thomashaw (1990) noted the expression of certain genes (COR, or cold-regulated, genes) in plants after exposure to cold temperatures, whose functions are as yet unknown but whose expression seems to parallel freezing tolerance. He speculated that levels of abscisic acid (ABA) increase in response to cold, which in turn could regulate the expression of COR genes.

Trees may be genetically adapted to withstand cold damage by having an earlier or greater response to the stimuli influencing cold hardiness in the autumn, by having a delayed response to favourable growing conditions in the spring where there is a threat of late spring frosts, by having a greater degree of hardiness which can be attained, by having a greater phenotypic plasticity to environmental conditions, or by differences in the synthesis of enzymes (such as by the COR genes mentioned above).

Generally, more northerly provenances, or those from higher elevations or more exposed or windswept sites, would be expected to have the greatest frost hardiness and slowest growth rates. Such was found to be the case for interior Douglas-fir (Rehfeldt, 1978) and lodgepole pine (Rehfeldt, 1980).

The range in frost hardiness among provenances seems to vary with the stage of acclimation. The greatest differentiation between populations in hardiness level occurred during the autumn, before trees were able to withstand very cold temperatures, for lodgepole pine (Rehfeldt, 1980) and for Sitka spruce (Cannell and Sheppard, 1982). In the latter study, more northerly provenances became hardy sooner, but began to deharden later in the spring, than more southerly provenances.

Knowledge about frost hardiness patterns of western red cedar has only recently begun to emerge. It is known that this species is not very frost resistant in comparison with other coniferous species that grow in the same range (Krajina *et al.*, 1982; Minore, 1983). In the interior, western red cedar becomes established mainly in areas where snowcover occurs before the ground becomes solidly frozen (Krajina *et al.*, 1982). The primordia of western red cedar, as with all Cupressaceae species, are surrounded by only a few scalelike tissues, without the benefit of the resin covering found on buds of determinate species, and hence have little protection from water loss and thus from desiccation damage (Sakai, 1983). Sunscald may occur when a period of subfreezing temperatures is followed by sunny days, where the leader and youngest branches above the snowline may die, especially lateral branches on the southern side of the crown (Miller,1978).

It has been repeatedly demonstrated that short photoperiods and moisture stress have little effect in inducing cold hardiness in western red cedar (Vaartaja, 1959; Silim, 1991; Krasowski and Owens, 1991; and Folk *et al.*, 1994). The main factor found to date which seems to induce hardiness in this species is cold, in particular subfreezing, temperatures.

Weger *et al.* (1993) observed that synthesis of the carotenoid pigment

rhodoxanthin, and to a lesser degree lutein, was correlated to hardiness in western red cedar, and was largely responsible for the colour change observed in foliage during the winter. They proposed that the pigmentation reduces the light intensity reaching the photosynthetic apparatus and hence helps to prevent winter photodamage. Their results coincide with those of Columbo and Raitanen (1991) for *Thuja occidentalis*, who found that seedlings whose foliage had turned brownish were hardier than trees whose foliage had remained green.

Genetic testing of cold hardiness in this species has been very limited. An inland Idaho western red cedar provenance was found to be more frost hardy than two coastal provenances from Corvallis, Oregon and Seattle, Washington (Sakai and Weiser, 1973), presumably in response to differences in climatic conditions.

Methods of assessing cold hardiness in plants are numerous. The most direct method is to freeze whole intact plants and then visually assess the amount of damage to tissues that shows up over a period of a few weeks after thawing. Drawbacks of this method include the subjectivity of ocular assessment of discolouration and tissue flaccidity, the necessity of destructively sampling whole seedlings, and the lengthy period it takes for damage to be expressed.

The electrical conductivity method, to be explained in detail in Section 3.2.4.2, is based on the fact that when injury such as frost damage occurs, the cell membrane loses its selective permeability, and thus electrolytes in the cytoplasm leach out of injured tissues when submersed in water in proportion to the severity of the injury. The electrical conductivity of a sample can easily be measured. This method utilizes tissue segments, not the whole plant.

Very good correlations between the whole tree browning method and electrical conductivity testing (estimated LT_{50}'s: -18.5°C ± 0.5°C with electrical conductivity; -18.9°C ± 1.2°C with cambial assessment) were found for western red cedar, with visual assessment of the cambium being more closely related than foliar browning (Silim, 1991). Even if not as accurate compared to testing whole plants, this test should be the more precise of the two, and it is precision which is desired for detecting genetic differences.

Other methods sometimes used to assess cold hardiness are variable chlorophyll fluorescence, calorimetry, and nuclear magnetic resonance (NMR), the first two methods which will be described below. NMR and calorimetry both measure cellular ice formation. NMR is a type of spectroscopy which measures spectral differences between frozen and liquid water. However, it is expensive, and it has been reported (Burke et al., 1976) that attempts to correlate the quantity of bound unfreezable water with cold hardiness have been unsuccessful.

When foliage is exposed to light, normally some of the light energy which is absorbed by the chlorophyll pigments is used in photochemical water splitting reactions which lead to carbon assimilation, with some of the excess energy being dissipated as heat, and some reemitted as fluorescence (Vidaver et al., 1991; Taiz and Zeiger, 1991). The variable chlorophyll fluorescence test measures the fluorescence emitted in response to light exposure, but is only indirectly linked with frost hardiness.

The initial baseline fluorescence emission, F_o, represents the amount of fluorescence prior to any light excitation, and so indicates those processes which are independent of any photochemical events. F_o has been found to increase with plant

hardiness in one seedlot of western red cedar (Weger *et al.*, 1993). When a pulse of high-intensity saturating light is given, the fluorescence level rises to a maximum (F_m). The difference between F_m and F_0 is the variable fluorescence, F_v, which occurs only in photosynthetically active tissue (Vidaver *et al.*, 1991). The ratio of F_v/F_m, or sometimes F_v/F_0, is used as a probe for photoinhibition, indicating stressful conditions, with a lower ratio indicating a greater amount of photoinhibition.

Plants under duress such as low temperatures are sensitive to photoinhibition, even at moderate light levels (Öquist and Huner, 1991). Current theory holds that photoinhibition of photosynthesis has two components (R. Guy, UBC, pers. comm., 1994): the first has photoprotective effects, while the second, photooxidation, is detrimental to the plant. The first photoinhibitory response is a reduction in photosynthetic capacity occurring when light levels exceed photosynthetic requirements (Ögren, 1991), preventing electron buildup. However, this photoprotective mechanism does not work well under cold conditions.

If carbon assimilation is halted due to low temperatures, but an excess of unutilized electrons are present, photooxidation damage occurs (Öquist and Huner, 1991). Resulting toxic oxygen species may cause injury to the chloroplast membranes, photosynthetic pigments, lipid membranes, and electron transport chain proteins (Blum, 1988; Ritchie, 1991; Powles, 1984). Photodamage may be irreversible and even lethal.

Calorimetry, or thermal analysis, measures the latent heat of fusion occurring when water freezes within a plant tissue by using a single thermocouple attached to the plant as the test chamber is progressively cooled (Salisbury and Ross, 1978). Temperature exotherms are produced at each freezing event. One or two large

exotherms are produced when normally noninjurious extracellular water freezes, somewhere around -2°C to -10°C (Weiser, 1970).

Deep supercooling occurs when water molecules have no nucleus around which to crystallize, and hence ice formation cannot occur. Hardy plants are believed to be able to deep supercool to about -38°C in the absence of heterogeneous nucleating agents in the tissue environment (George *et al.*, 1974). This temperature corresponds with the point to which pure water can be supercooled if ice nucleation can be prevented, after which it spontaneously nucleates (Burke *et al.*, 1976; Salisbury and Ross, 1978). Ice is most likely then formed within living cells, killing them. Low temperature exotherms (LTE's), associated with the point where injury or death occurs, have been documented by calorimetry in some cold-hardy species down to -40°C, which coincides with the range limit of many of the species tested (George *et al.*, 1974; Ritchie, 1991). Deep supercooling may be an avoidance mechanism for surviving freezing injury in some tissues (Salisbury and Ross, 1978).

The purpose of this chapter was to explore the genetic relationships in cold hardiness of western red cedar, in particular genetic variation in and correlations of traits related to cold hardiness. Variation was studied at three levels: between zones, provenances, and families within provenances.

Specific objectives of cold hardiness research were:

- to estimate the extent and allocation of genetic variation found in cold hardiness traits of western red cedar between coastal and interior zones and between and within provenances and families using quantitative genetic analyses

- to investigate the influence of environmental factors on levels of cold hardiness and on rates of acclimation and deacclimation

- to compare different methods of assessing cold hardiness

The seasonal stages of hardening, attaining maximum hardiness, and dehardening were investigated over two winters and at two locations, one relatively mild and one relatively harsh. Hardiness attributes (index of injury, LT_{50}, frost hardiness curves, base, variable, and maximal fluorescence levels, low temperature exotherms, and influence of foliar nutrient status on hardiness) in this species were studied, using a number of available techniques: electrical conductivity testing, variable chlorophyll fluorescence, and calorimetry, and by comparing nutrient analyses with the LT_{50}, as found by electrical conductivity, within the same tree.

3.2. METHODS

3.2.1. Frost Hardiness Profile over a Broad Range of Temperatures

To test for differences between provenances in the electrical conductivity test index of injury (I_t) of acclimating western red cedar seedlings over a broad range of temperatures, a test was carried out on December 19, 1991. The frost hardiness of six provenances (the same as those used in pretesting; see Section 3.2.4.1) at -5°C intervals from -10°C to -50°C was monitored. Four seedlings per provenance were tested at each of the nine test temperatures.

Samples were frozen normally (see Section 3.2.4.2) over the range of temperatures, and electrical conductivity tests carried out. The I_t values per provenance were plotted against each temperature to produce profile curves. These graphs were scrutinized for pattern and degree of change with temperature decrease in the six provenances. An analysis of variance was carried out on the I_t values. Nonlinear regression, using the logistic function due to the sigmoid nature of the frost curve profiles, was carried out following the methods of Sit and Poulin-Costello (1994), using the following functional form:

$$Y = \frac{a}{1 + e^{b-cX}}$$

The solutions found for a (index of injury at -50°C), b (intercept), and c (slope), where $Y = I_t$ and X = temperature, for each tree per provenance were then analyzed by analysis of variance and multivariate analysis of variance.

3.2.2. Effect of Crown Position on Frost Hardiness

To determine whether within-tree differences in degree of frost hardiness were apparent, a study was set up to compare branch samples from different positions within the seedling crown. The same six provenances as used in pretesting were selected for this study (Section 3.2.4.1). Four seedlings were tested per provenance; seedling samples were kept separate from each other.

Branches were taken from the upper third and lower third of each tree. Samples were placed into labelled plastic bags and processed as per regular frost test samples, with the exception of keeping separate jars for branches from the upper crown and from the lower crown.

Seedling samples were frost tested on December 2, 1991 to three test temperatures: -12°C, -20°C, and -28°C; temperatures were selected based on the knowledge of how cold hardy these provenances were at that time according to the regular frost tests. A date near to when maximum cold hardiness could be expected was desired, but because of freezer scheduling constraints, this test was performed a little prior to the desired time frame.

An electrical conductivity test was done as per usual (Section 3.2.4.2), and analysis of variance was used to determine whether differences in hardiness occurred between the upper and lower crown.

3.2.3. Effect of Rate of Freezing on Measures of Cell Injury

A frost test was carried out in which samples were frozen at a rate of at least

twice as fast as normal frost tests to see whether a difference in degree of damage was noted. A higher degree of damage at faster rates of freezing would indicate damage such as cell lysis or intracellular freezing.

Six provenances were chosen for this study, the same provenances as used in pretesting (Section 3.2.4.1). Four trees per provenance were tested at three temperatures: -15°C, -22°C, and -29°C. Temperatures were based on the degree of hardiness found in the normal frost tests at that time. The test was performed on February 24, 1992.

Freezing rates were limited by the ability of the freezing unit to cool down. Between +2°C and -15°C, the freezer cooled at a rate of -11.86°C per hour. Between -15°C and -22°C, the cooling rate was -12.73°C per hour. Between -22°C and -29°C, the cooling rate was only -9.77°C per hour. Thus the overall cooling rate averaged out to about -12°C per hour.

An electrical conductivity test was conducted on the samples. Estimated LT_{50}'s were compared to those of the normal frost tests on adjacent dates.

3.2.4. Frost Testing over Two Winters

3.2.4.1. Greenhouse Design and Nursery Culture

Of the ten provenances per zone used in the western red cedar growth studies, four provenances per zone were chosen for studying seedling frost hardiness over two winters. Half of the provenances per zone maintained family structure (three families per provenance) while the other half of the provenances per zone had no family

structure. Provenances and families used for frost testing were as follows:

Coast	Interior
Tofino 1, 3, 5	Mt. Mara Low elev. 2, 3, 4
Mill Bay 1, 2, 4	Mt. Mara Mid elev. 2, 4, 5
Oliver Lake	Benton Flat (families bulked)
Squamish	Kooskia

Entries were chosen to give a broad representation of each zone; however, provenance selection was limited to those that had adequate seed germination to provide enough seedlings for the study.

Seedling germinants were taken from the petri dishes full of germinants used in the growth studies. Germinants were dibbled into Styro Vent 91 blocks, filled with normal nursery soil mix as used in the growth studies, using tweezers and a cavity spoon. Two blocks per genetic entry were sown for frost test purposes. Blocks sown for frost hardiness testing were grown along with and under the same cultural regime as the blocks grown for growth studies.

For frost hardiness pretesting prior to every frost test, six genetic entries were chosen, three from the coast and three from the interior: Oliver Lake, Squamish, Mill Bay (families bulked), Mt. Mara Low elev. 3, Mt. Mara Mid elev. 2, and Kooskia. Ten styroblocks were split into thirds and sown for pretesting samples. Each provenance was sown into $1/3$ of each of five blocks. Seedlings were sown and grown as described above for normal frost test seedlings. The first winter of frost testing, 1990 / 91, used samples from these containerized plug seedlings.

Seedlings to be used for pretests and frost tests during the winter of 1991 / 92 were outplanted into a transplant bed adjacent to the transplant bed used for growth studies at UBC's South Campus Nursery. A subset of trees, for frost hardiness testing on three test dates, was transplanted at Skimikin Seed Orchard; the same eight provenances, half having family structure, were used as per the samples at UBC. At both locations, seedlings were planted in whole unreplicated blocks at $1/3$ m spacing.

3.2.4.2. Frost Hardiness Tests

One week prior to running a frost test, a pretest run was carried out on six provenances to give a rough estimate of the weekly rate of hardening and to estimate a range within which the LT_{50}'s of the different provenances fell. These pretest results were used in estimating the range of temperatures to be used in the larger frost tests.

Pretesting was done at four test temperatures, with an equal interval between all temperatures. Samples from four trees per provenance were used. The main frost tests used four trees per genetic entry and three test temperatures; the remaining methodology was the same for both tests. However, only three samples per genetic entry from Skimikin were used, due to limitations in material available at that site.

For each sample, lateral branches from the upper crown were cut off of the trees at the stem, bagged, and brought back to the lab for immediate processing. In the lab, all samples were stored in a cooler ($\sim +3°C$) for the short period (a matter of minutes to an hour) until they were processed.

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During 1990 / 91, the first winter in which frost testing was done, the material within each genetic entry was mixed together and the bulked foliar material divided into four replicates. During 1991 / 92, the second winter of cold hardiness monitoring, the tree samples were kept separate so that individual analyses for each tree could be estimated.

Frost testing was performed using the electrical conductivity test which was modified from the methods of Glerum (1985) and Silim (1991). Upon removal from the cooler, branches were rinsed with distilled, deionized water. The tips of all foliage to be used were snipped off and discarded. The remaining foliage was cut into pieces about 5 mm long. Each piece had two cut ends; this would facilitate an easier flow of electrolytes during testing.

Approximately five foliage pieces were placed into labelled 20 ml glass scintillation vials which had been previously washed and rinsed with distilled, deionized water. A small amount of silver iodide and a small squirt of distilled, deionized water were added to the jars prior to adding sample pieces to act as an ice nucleator to aid the onset of freezing. Four replicated jars were used per genetic entry per test temperature. Vials to be frozen to different test temperatures were given washed, colour-coded plastic lids with plastic inner liners. Samples for each test temperature were placed into matching colour-coded test tube racks which would allow for unrestricted air circulation between vials.

Frost testing was carried out in a programmable semi-customized Forma Scientific Biofreezer unit. Control trees which were not frozen were kept in a darkened cooler at ~ +3°C while the tests were being run. Test trees were put into the freezer and kept at +2°C for one hour to allow the freezer to stabilize prior to

beginning the test.

The temperature was then decreased at a rate of -5°C per hour until the first desired test temperature was reached. The freezer was held for one hour at that temperature, and then the samples which were to be tested only to that temperature were removed with all overhead lights turned off. The removed vials within their racks were placed into a dark plastic garbage bag to minimize photoinhibitory effects. The vials were placed in the darkened cooler and allowed to thaw gradually overnight at ~ +3°C.

The remaining vials in the freezer were cooled at -5°C an hour until the next test temperature was reached, held at that temperature for one hour, and then the next group of samples was removed. This process was repeated until all test temperatures had been reached and all vials were removed from the freezer and placed into the cooler.

The following morning all vials, including those of the unfrozen controls, were removed from the cooler. Fifteen ml of distilled, deionized water was added to each jar. The vials were then left at room temperature for 24 hours.

The next morning all jars, including controls, were then shaken, and the electrical conductivity measured with a Cole-Parmer 1481-60 conductivity meter. Samples were then completely killed by placing the vials, still in their racks, into a 60°C Fisher Scientific waterbath for 45 minutes. Jars were then left at room temperature for a further 24 hours; a second conductivity measurement was then taken on each jar.

The amount of injury due to freezing to a certain temperature was calculated

for each sample using the following formula (Columbo *et al.*, 1984):

$$I_t = \frac{RC_{frozen} - RC_{control}}{1 - (RC_{control} / 100)}$$

where:

I_t = index of injury (0 - 100 %)

RC_{frozen} = Relative Conductivity of frozen sample

= $\frac{\text{Electrical Conductivity of frozen sample}}{\text{Electrical Conductivity of frozen killed}}$ * 100

$RC_{control}$ = Relative Conductivity of control sample

= $\frac{\text{Electrical Conductivity of control sample}}{\text{Electrical Conductivity of control killed}}$ * 100

The I_t values were plotted against test temperatures. From these graphs, the temperature at which 50 % of injury would occur (LT_{50}, lethal temperature 50) was estimated. Figure 3.1 illustrates one such graph used in the interpolation of LT_{50}'s.

Trees at UBC were frost tested ten times over each of two winters. Trees at Skimikin were frost tested three times over the winter of 1991 / 92. For each of the latter tests, snipped branches were bagged, placed into a box containing frozen icepacks, and shipped to the lab at UBC via Greyhound bus for testing.

3.2.4.3. Data Analysis

Analysis of variance (SAS® PROC GLM) was carried out on the results of all
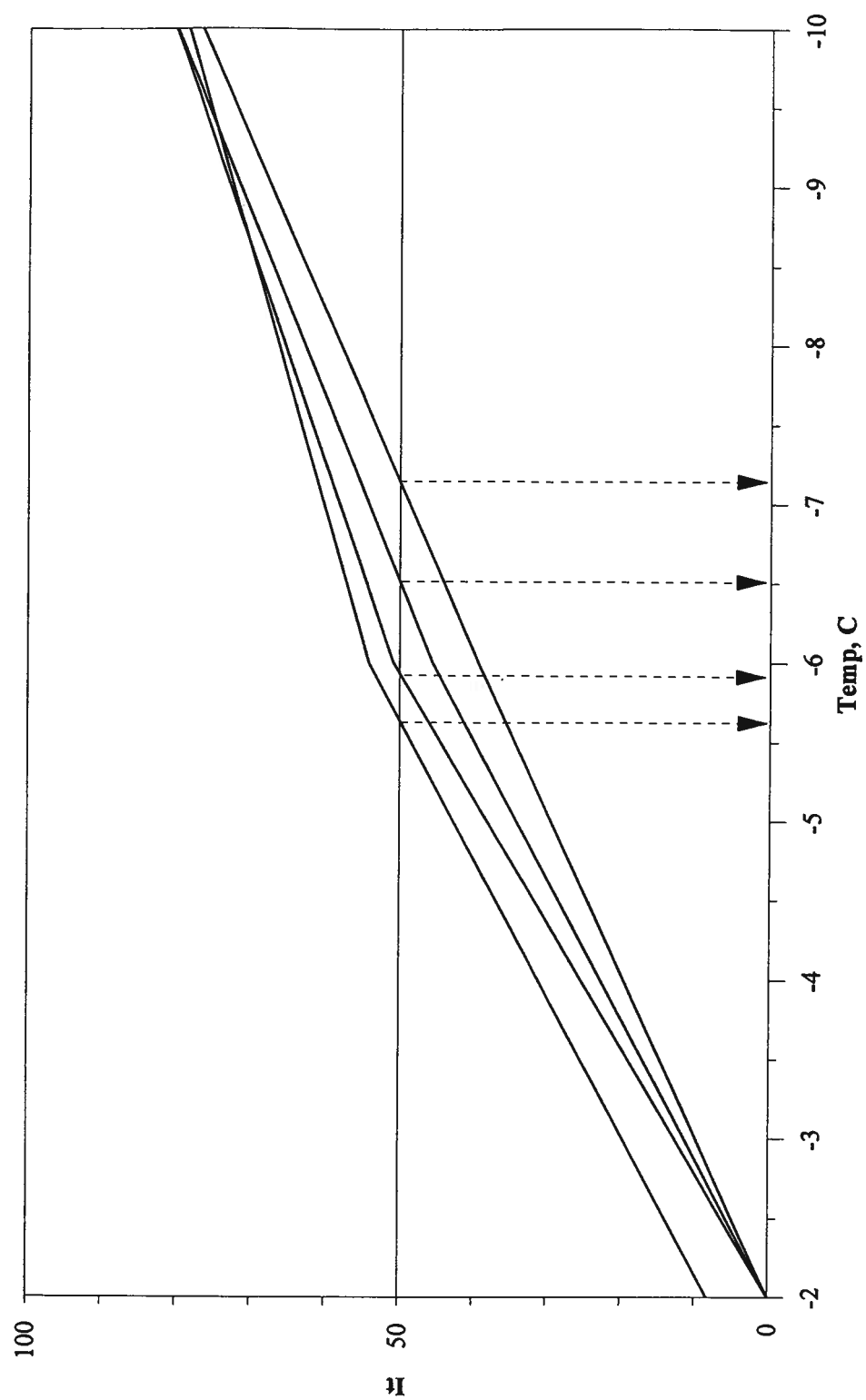
Figure 3.1. Example of plotted index of injury vs test temperature used to estimate LT_{50}'s per sample per test date.

tests separately for each date. All provenances were tested by the following model per date:

$$I_{t\text{ tpzn}} = \mu + \text{Temperature}_t + \text{Zone}_z + T*Z_{tz} + \text{Prov}(Z)_{p(z)} + T*P(Z)_{tp(z)} + \varepsilon_{(tpz)n}$$

Provenances having family structure were also analyzed by the following model:

$$I_{t\text{ tfpzn}} = \mu + T_t + Z_z + T*Z_{tz} + P(Z)_{p(z)} + T*P(Z)_{tp(z)} + \text{Family}(P\ Z)_{f(pz)}$$
$$+ T*F(P\ Z)_{tf(pz)} + \varepsilon_{(tfpz)n}$$

Temperature and zone were treated as fixed effects, while provenance and family were treated as random variables, with the appropriate F tests being constructed accordingly (Appendix 1.9 and Appendix 1.10). Variance components were determined (SAS® PROC VARCOMP) for the purposes of heritability estimation where family was a significant effect.

Curvilinear regressions were estimated on the curves produced by graphing estimated LT_{50} values over each season. Due to their shape, LT_{50} curves were analyzed by weighting models by the number of weeks from the beginning of the testing season and by weeks^2, which describe a general parabolic curve (second degree polynomial). The log of LT_{50} was used as the dependent variable. During the first winter of frost testing, samples were bulked together for each genetic entry, so only simple analyses could be attempted due to nonreplications. The models tested for 1990 / 91 were:

$$\text{Log } LT_{50zn} = \mu + \text{Week} + \text{Week}^2 + Z_z + \varepsilon_{(z)n}$$
$$\text{Log } LT_{50pn} = \mu + \text{Week} + \text{Week}^2 + P_p + \varepsilon_{(p)n}$$

The former model was used on all provenances, with the latter being used on

provenances having family structure. Provenance was not nested within zone in the latter model, as only two provenances per zone having family structure remained. The models tested for the 1991 / 92 season were:

$$\text{Log } LT_{50zpn} = \mu + \text{Week} + \text{Week}^2 + Z_z + P(Z)_{p(z)} + \varepsilon_{(zp)n}$$
$$\text{Log } LT_{50pfn} = \mu + \text{Week} + \text{Week}^2 + P_p + F(P)_{f(p)} + \varepsilon_{(pf)n}$$

with the former model being used with all provenances, and the latter model being used with provenances having family structure.

Analysis of variance was also done on the estimated LT_{50}'s per date. For tests done in 1990 / 91, as seedling samples per genetic entry were bulked to give four mixed replications, family level analysis was not possible. Zone was left out of the model because only four provenances, the ones with family structure, had replicates for the purposes of LT_{50} analysis. During 1991 / 92, the seedlings were kept separate and each of four seedlings per genetic entry were treated as replicates, so analysis could be done at the family level as well as at the provenance level, and provenances not having family structure could be included in the latter analysis.

To investigate possible relationships between the geographical distribution of provenances and frost hardiness, hierarchical cluster analysis was carried out on the LT_{50} values for January 21, 1991 and January 27, 1992, the dates per year closest to the maximum hardiness reached. Correlations were also estimated comparing the LT_{50}'s from these dates with environmental attributes of the provenances. Stepwise multiple linear regressions were also done to determine whether the maximum LT_{50} per year was dependent on certain environmental conditions related to each provenance.

Frost tests of seedlings from Salmon Arm were analyzed by ANOVA per test date as per the trees from UBC that were tested. An analysis of variance was done for each of the dates when trees from Skimikin were tested, in which the LT_{50} of trees from Skimikin was compared to that of trees from UBC at the same time period. Thus the effect location (treated as a fixed effect) and its interactions were added to the standard model. The expected mean squares were determined as shown in Appendix 1.7 and Appendix 1.8 by substituting Location for Temperature.

3.2.5. Variable Chlorophyll Fluorescence and Frost Hardiness

To determine whether damage to the photosynthetic apparatus (i.e. to the chloroplast) due to cold temperatures paralleled the results obtained using the electrical conductivity method, six genetic entries (the same as those used for pretesting, Section 3.2.4.1) were tested by variable chlorophyll fluorescence at two test dates. Four trees per genetic entry per temperature per test date were used. Trees were tested at three temperatures on January 13, 1992 and April 1, 1992; test temperatures were chosen based on how hardy the normal frost tests showed these provenances to be. In January, seedlings were tested to -15°C, -25°C, and -35°C while in April, seedlings were tested to -2°C, -6°C, and -10°C.

Seedlings which had been grown for one year in a styroblock Vent 91 and then one year in a transplant bed at the UBC forest nursery were snipped off at the root collar on a cloudy afternoon and brought directly into the lab. The cut portion of the stem was wrapped in wet cotton wool. Trees were placed into large size plastic freezer bags.

Frost testing was carried out at night using the Biofreezer. Control seedlings were dark adapted (left at ~ +3°C in total darkness for over two hours) prior to measuring. Sample trees were frozen to the desired temperatures under dark conditions. After being maintained for one hour at the appropriate temperature, trees of each treatment were removed from the freezer in the dark. Samples were placed on a lab bench and given 20 minutes of bright light. To accomplish this, an overhead projector was turned on its side on the bench so that the light was pointing towards the seedlings which were fanned out, leaders towards the lamp, around the circle of the beam. A light meter was used to check that seedlings were exposed to similar irradiance. A rectangular glass tank was filled with water and placed directly in front of the projector bulb to reduce exposure to infrared light. After the light treatment, trees were given 20 minutes of total darkness.

The variable and maximum fluorescence were then measured in the dimmed lab using a pulse modulated Heinz Walz PAM 101 chlorophyll fluorometer. The 1 cm diameter fiberoptic probe was placed on the tree foliage, trying to cover as much leaf surface area as possible. The optical tip initially shone red light onto the foliage; this gave a reading for the minimal chlorophyll fluorescence level, F_o. A Xenon high intensity lamp was then activated for one second of illuminating saturating flash of about 3400 μmol m^{-2} s^{-1}; this gave $F_{maximal}$ (F_m) for the tree sample.

$F_{variable}$ (F_v) was calculated as the difference between F_m and F_o ($F_v = F_m - F_o$). The fluorescence ratios of F_v/F_m and F_v/F_o were calculated for all samples. Analysis of variance was carried out on these ratio values, as well as on F_o, F_m, and F_v individually (Appendix 1.7).

3.2.6. Calorimetry

Thermal analysis, or calorimetry, was carried out to see whether the temperatures at which extracellular freezing and internal supercooling occurred could be detected, and if so, whether there were differences between provenances in the temperature at which these exotherms occurred. Two seedlings from each of four provenances (Oliver Lake, Mill Bay, Kooskia, and Mt. Mara Mid elev.) were selected. Testing was done in mid-February, when trees were starting to deacclimate.

Seedling branch samples were collected from UBC's transplant bed on February 15, 1992 and brought into the lab. A datalogger was set up with one thermocouple probe attached to each of nine separate channels. The probe of each of eight channels was embedded into the foliage of a sample so that the foliage touched both sides of the thermocouple. The probe was taped into place, ensuring that the portion with the probe tip was not obstructed.

Samples were placed into the freezer. One channel was not hooked up to any seedlings, but measured ambient air temperature (the control). The cooler was programmed to run for one hour at +2°C and then to steadily decrease in temperature at a rate of -5°C per hour until -50°C was reached.

Once the temperature of the freezer reached 0°C, the datalogger was started; temperatures were sampled on all nine channels every five seconds for ten hours. Temperatures were recorded throughout the test using a connected laptop computer. The freezer was only able to reach -49°C, as its compressors could not maintain a decrease in temperature of -5°C / hour as the temperature approached -50°C. Data were later transferred to a Sun computing system. For each sample, graphs of foliage

temperature over time and sample temperature minus control temperature were developed. Analysis of variance was carried out to test for provenance differences in the temperatures at which the first and second exotherms occurred, the size in degrees C of the two exotherms, and the temperature to which each sample rose to after each exothermic event.

3.2.7. Foliar Nutrient Analysis and Frost Hardiness Testing

A study was done to determine whether there was any correlation between frost hardiness of a seedling and foliar nutrient content of certain macronutrients (% N, % P, % K, % Ca, and % Mg).

Five seedlings from each of eight provenances were tested for both frost hardiness and for foliar nutrient analysis, using the same seedlings for both tests in order to observe any relationships from the individual tree level and upwards. Provenances tested were: Oliver Lake, Squamish, Mill Bay, Tofino, Mt. Mara Low elev., Mt. Mara Mid elev., Benton Flat, and Kooskia.

Foliage samples were collected from sample trees on January 25, 1993, placed into individual labelled bags, and brought into the lab. Portions of the branch samples were processed as per usual for frost testing (Section 3.2.4.2). Remaining portions were processed for foliar nutrient analysis, as described previously (Section 2.2.7).

Frost testing was done at four temperatures (-28°C, -36°C, -44°C, and -52°C), based on how hardy the trees were deemed to be at the time. Foliar nutrient analysis was carried out at the MacMillan Bloedel lab in Nanaimo, B.C.

As provenance Tofino was found to be far less hardy than anticipated, its LT_{50} could not be estimated from this test. Therefore Tofino was retested the following week on February 2, 1993, using test temperatures of -20°C, -28°C, and -36°C.

Correlations were conducted between the macronutrient concentrations and the estimated LT_{50} of the same tree. A regression and an analysis of variance were also performed, with LT_{50} as the dependent variable (Y value) in both cases.

3.3. RESULTS

3.3.1. Temperature Profiles

Analysis of six provenances frost tested on December 19, 1991 at -5°C intervals between -10°C and -50°C showed differences between provenances, but not between zones, in index of injury (I_t). A nonsignificant interaction term of provenance by temperature, where temperature was used as a covariate, indicated that the slopes of provenance curves did not differ when provenance I_t was plotted against temperature.

Nonlinear regressions of the frost profile curves were estimated based on the logistic function (Sit and Poulin-Costello, 1994), as plotted curves were sigmoid in shape (Figure 3.2). For this analysis, a few obvious outlier points on a small number of the individually plotted tree curves were manually smoothed (i.e. a few of the I_t values at -10°C were higher than those at -15°C). Values obtained per tree per provenance were then used in an analysis of variance. Provenances did not differ significantly in index of injury at -50°C, curve slopes, or intercepts, although the least squares mean a (I_{t-50}) of Mt. Mara Mid elevation differed significantly from those of Mt. Mara Low elevation and Oliver Lake according to pairwise t-tests. The inflection points of the curves ($X = b/c$) did differ between provenances, with mean provenance inflection points ranging from -26.5°C to -32.6°C.

Examination of the curves showed all provenances approaching the upper horizontal asymptote between -35°C and -40°C, regardless of what the index of injury was. For the majority of all provenance profiles, between the test temperatures of about -20°C and -40°C, the curves were linear. Thus it was hoped that by careful
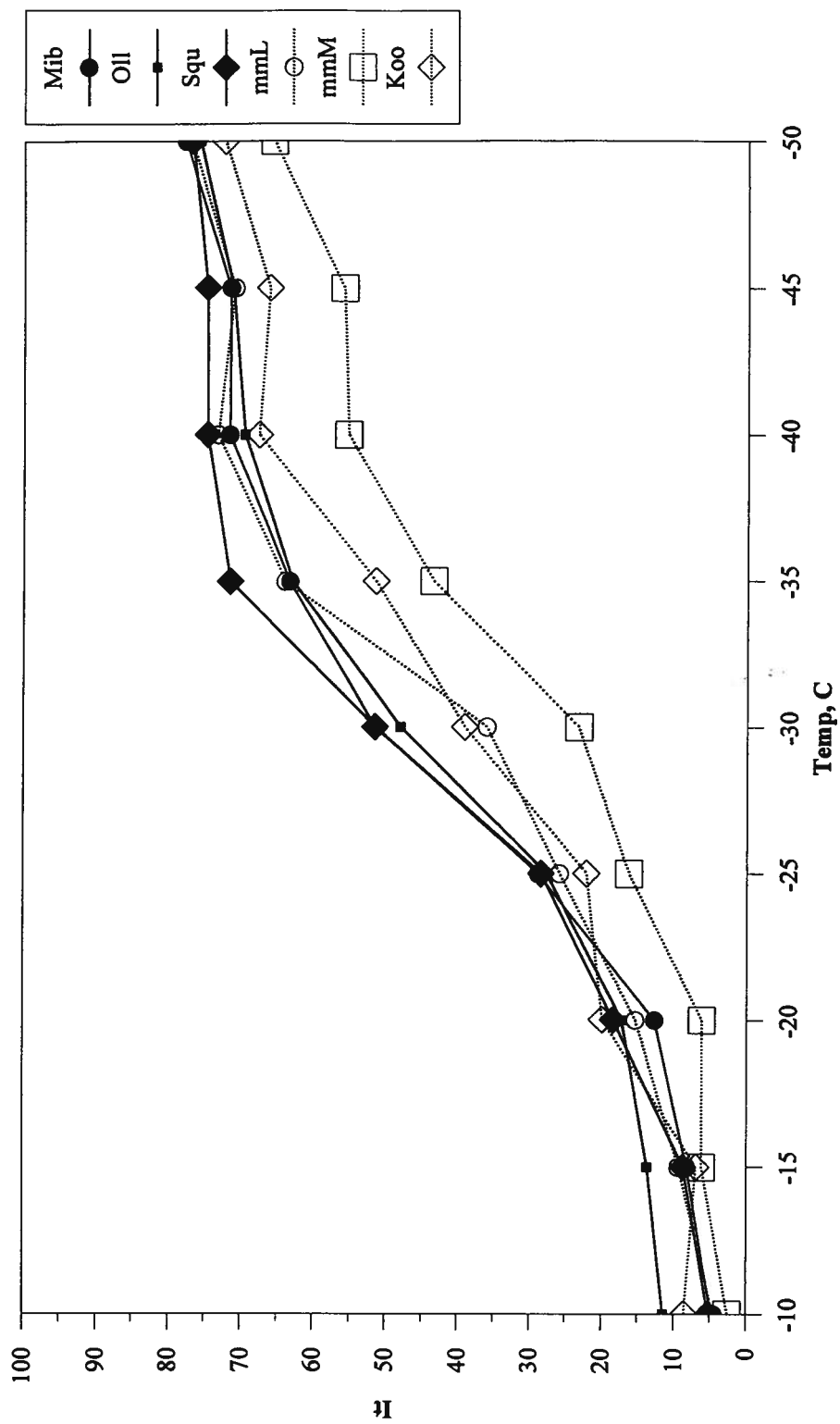
Figure 3.2. Mean provenance index of injury profile curves resulting from frost testing between -10°C and -50°C on Dec. 19, 1991.

selection of frost test temperatures to include all provenance LT_{50}'s but which were not much broader in range than necessary, only the linear portion of the curves would be sampled. Thus pretest results obtained the week prior to every frost test were carefully heeded as a check of the current status of seedling cold temperature susceptibility.

3.3.2. Crown Position

Analysis of variance on six provenances tested for differences in hardiness from different crown positions within a single tree indicated that the upper third of a crown was significantly less hardy than the lower third of a crown, although the mean difference in index of injury was only 2.8 %. A paired t-test comparing the upper and lower crown confirmed that crown position was significant in its effect on frost hardiness.

Subsequent foliage sampling for frost tests was always done from the same area within a crown, namely the upper third of the crown but not the uppermost branches.

3.3.3. Freezing Rate

Figure 3.3 shows the estimated LT_{50} values of the six provenances tested at about twice the freezing rate as normal tests, compared to estimated LT_{50} values as determined from freezing tests the week prior to (Feb.17, 1992) and the week following (Mar. 2, 1992) the doubled freezing rate test (Feb. 24, 1992). An estimate
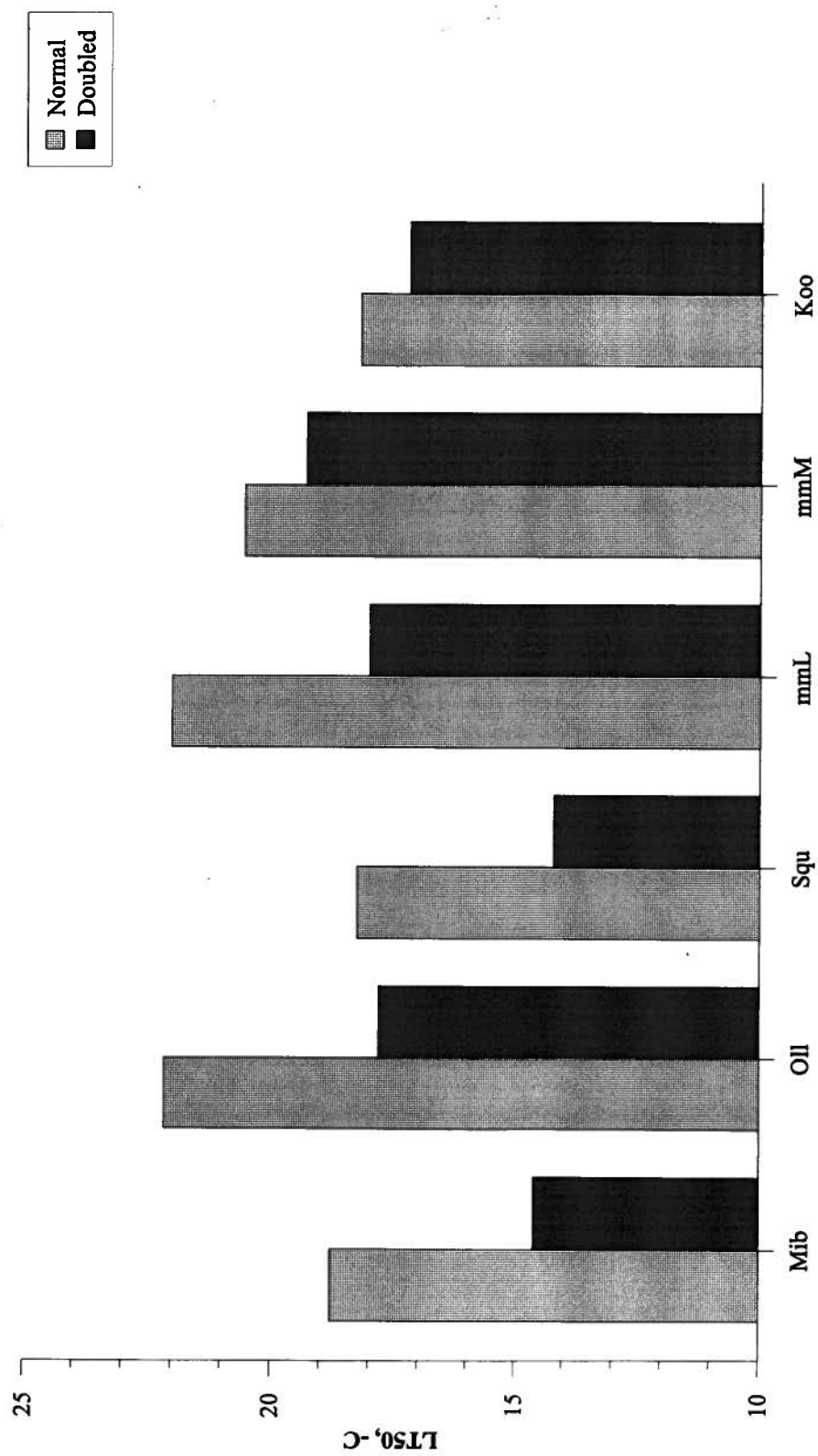
Figure 3.3. Mean provenance LT_{50}'s of seedlings tested at about twice the standard freezing rate compared to seedlings tested at the standard rate of freezing.

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of the hardiness of trees frozen at the normal -5°C / hour was obtained by extrapolating LT_{50} values from lines drawn between the Feb. 17 and Mar. 2 test results.

A t-test to test for differences in LT_{50}'s between the two freezing rates showed that differences were significant (P < 0.0098). Samples frozen at twice the normal rate had LT_{50}'s about 3.2°C warmer than trees frozen at the normal rate; i.e. damage to 50 % of the cells occurred 3.2°C higher for samples frozen at the faster rate.

Winter frost testing over two seasons was constantly done at a temperature decrease of -5°C / hour so that freezing rate did not adversely affect test result interpretations.

3.3.4. **Midsummer Resistance to Cold Temperatures**

A frost hardiness test carried out in midsummer when the trees were actively growing and were deemed to be in their least cold hardy state found an overall mean LT_{50} of -4.1°C, with family / provenance means ranging from -2.8°C in a Mill Bay family to -6.4°C in a Mt. Mara Low elevation family. The coastal average LT_{50} was -4.2°C, while the interior average LT_{50} was -4.1°C. Analysis of variance of midsummer I_t values found no variation at the zonal, provenance, or family levels, or in any of the interaction terms; however, as would be expected, test temperature was significant.

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3.3.5. Coastal Hardiness Cycles

Frost hardiness curves from the main frost tests conducted over two winters on seedlings growing at UBC, depicting estimated LT_{50} over the whole winter seasons, are shown for zonal means for both winters, provenance means over both winters, family means for 1990 / 91, and family means for 1991 / 92 in Figure 3.4, Figure 3.5, Figure 3.6, and Figure 3.7 respectively. Curves indicated that differences in hardiness between genetic entries are maximal when trees are most hardy, and nonexistent when trees are least hardy during the summer.

Daily maximum and minimum temperatures at UBC for the winters of 1990 / 91 and 1991 / 92 are given in Figure 3.8 and Figure 3.9. Notably, the first below-freezing temperatures of the season occurred earlier in the year in the fall of 1991 (at the end of October) than in 1990 (late December).

In the curvilinear regression analyses of LT_{50} curves over each test season, weighting terms were significant in all models tested (P < 0.0001). Zonal differences were apparent during 1990 / 91, but not during 1991 / 92. Provenance differences in LT_{50} curves were seen during both seasons (P < 0.0001). Family differences were not evident during the second season of testing.

Variance components were estimated for provenances having family structure on the dates where the family term was found by analysis of variance to be significant. Unless significant (in which case they remained in the model), interactions were dropped from the model for the purposes of variance component determination to increase the power of the error term. Figure 3.10 compares the percentage of the provenance and family variance components between the two test
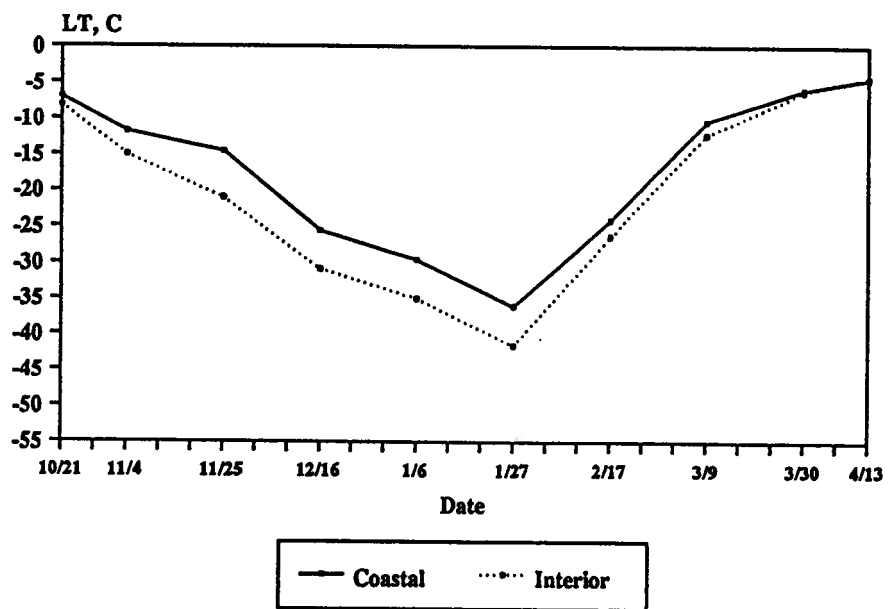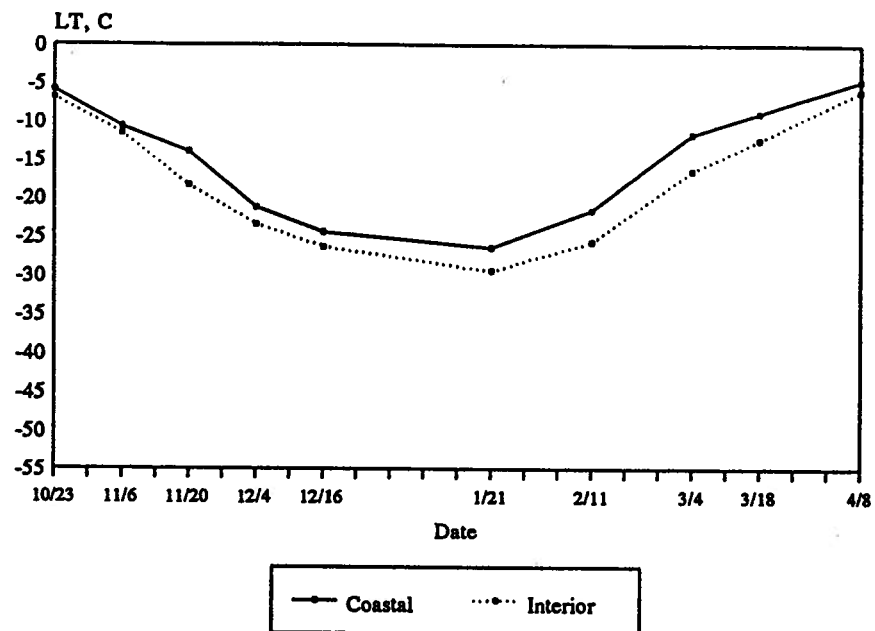
Figure 3.4. Mean zonal LT_{50}'s of trees growing at UBC over two winters of testing (top: 1990 / 91; bottom: 1991 / 92).

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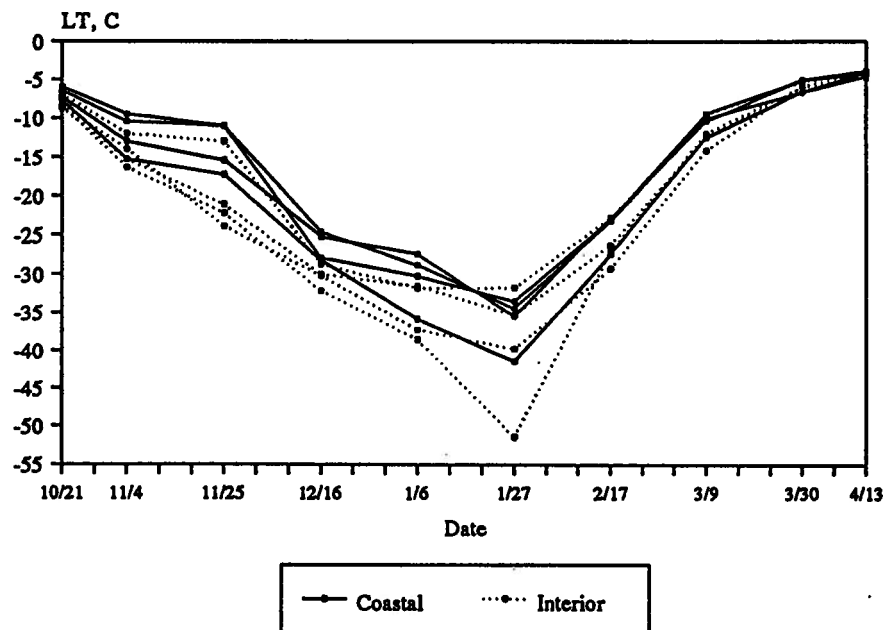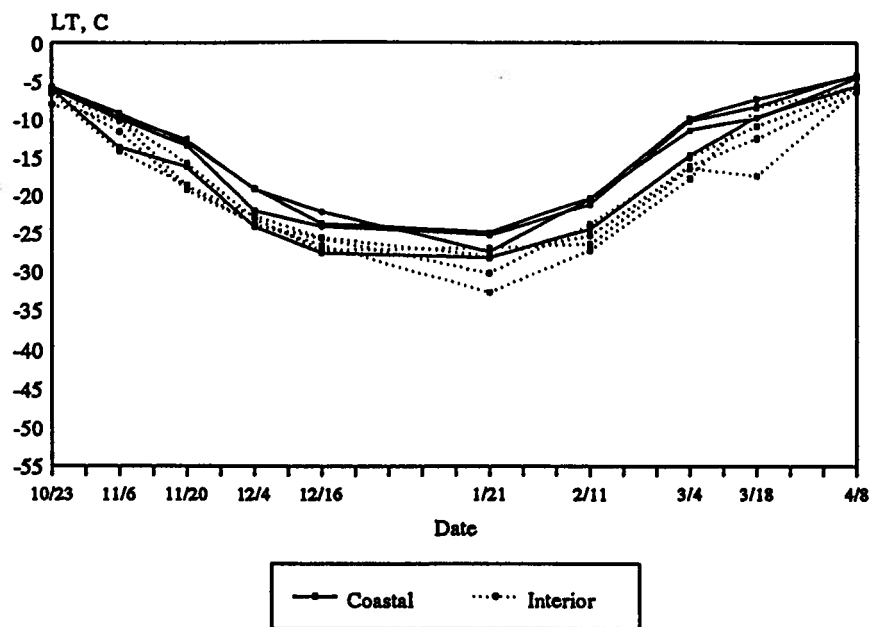
Figure 3.5. Mean provenance LT_{50}'s of trees growing at UBC over two winters of testing (top: 1990 / 91; bottom: 1991 / 92).
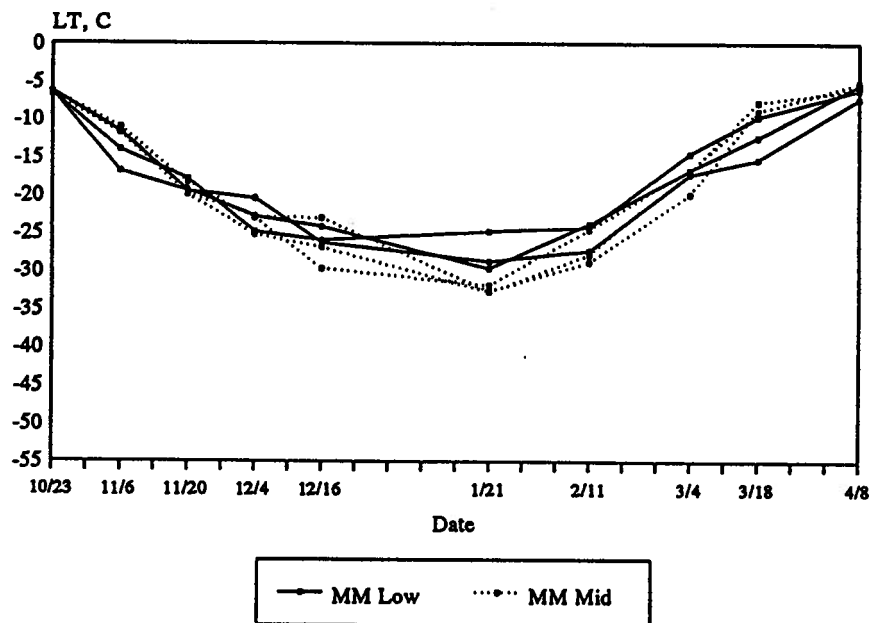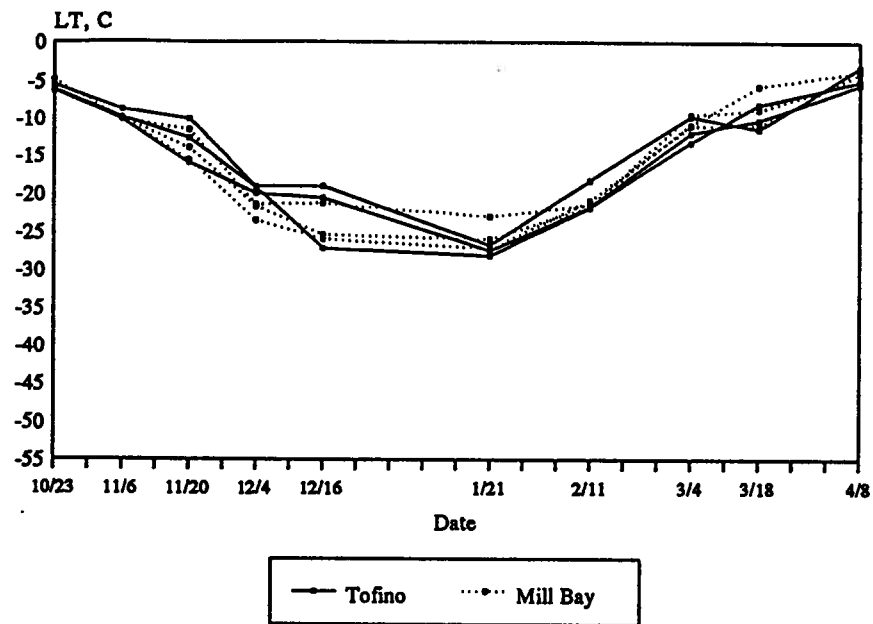
Figure 3.6. Mean family LT_{50}'s of trees growing at UBC during 1990 / 91 (top: coast; bottom: interior).
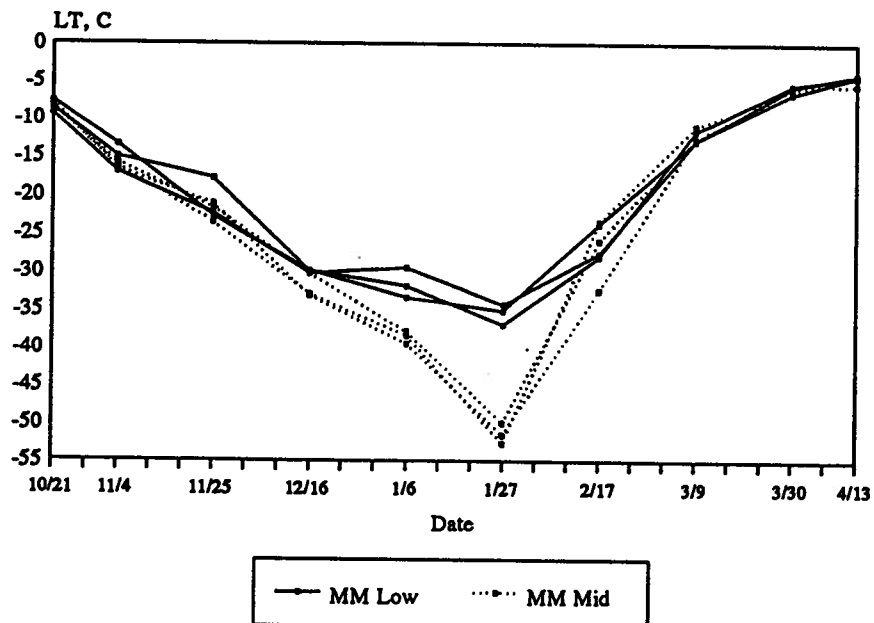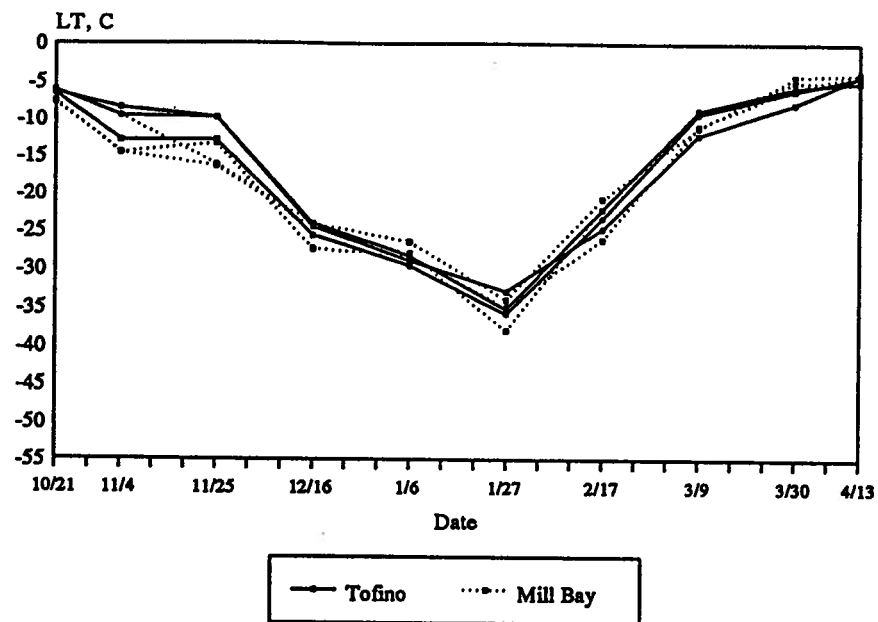
Figure 3.7. Mean family LT_{50}'s of trees growing at UBC during 1991 / 92 (top: coast; bottom: interior).
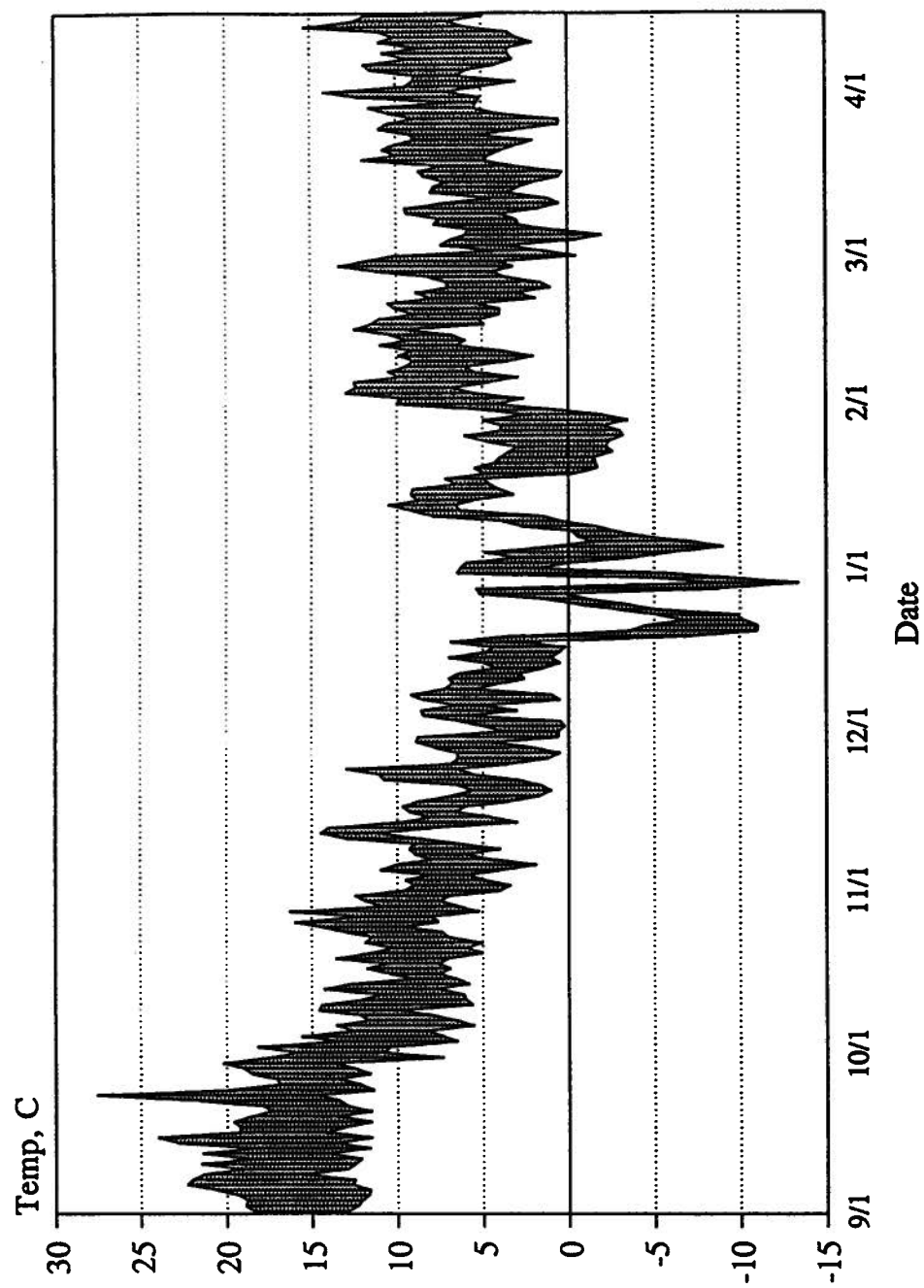
Figure 3.8. Daily maximum and minimum temperatures at UBC during 1990 / 91.
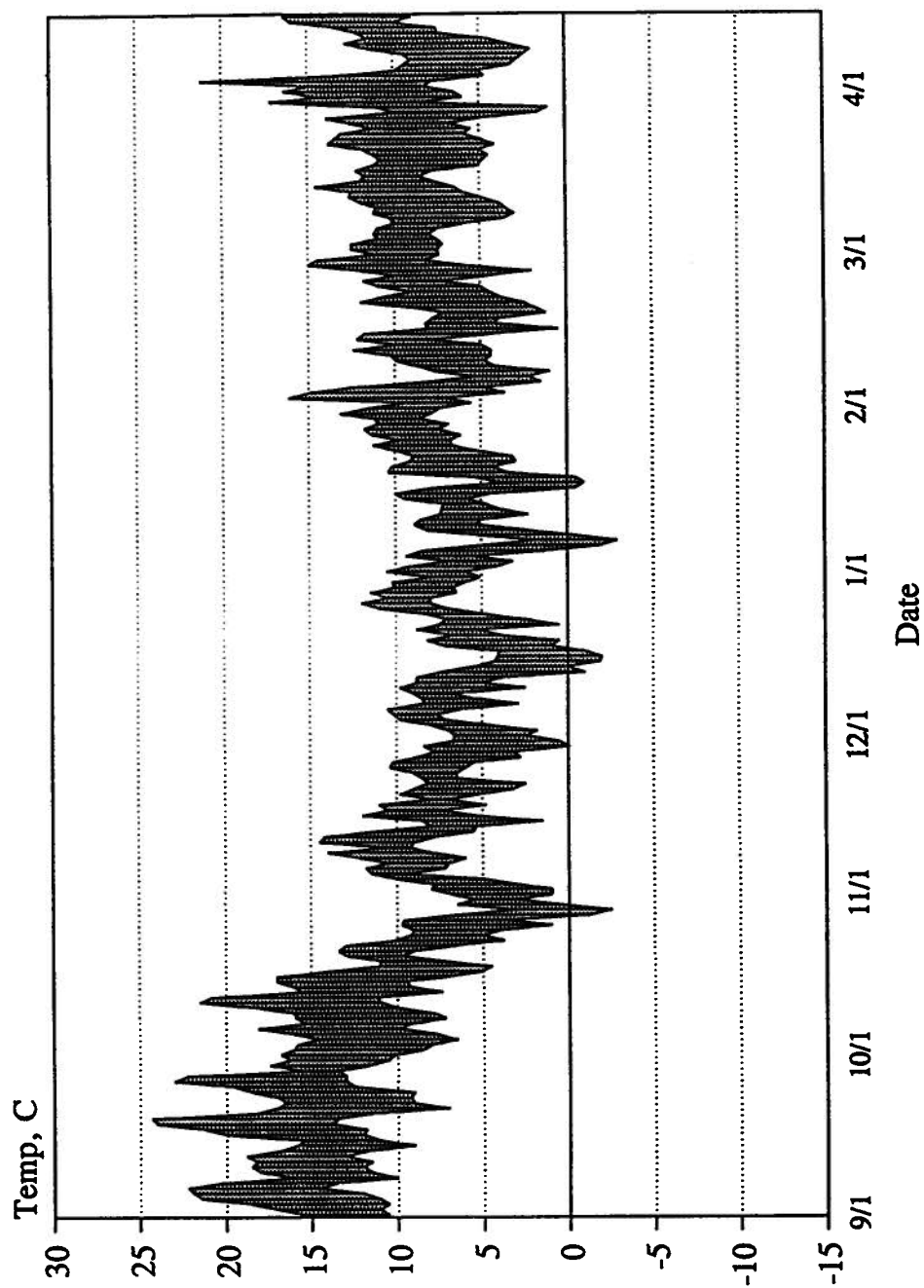
Figure 3.9. Daily maximum and minimum temperatures at UBC during 1991 / 92.

101
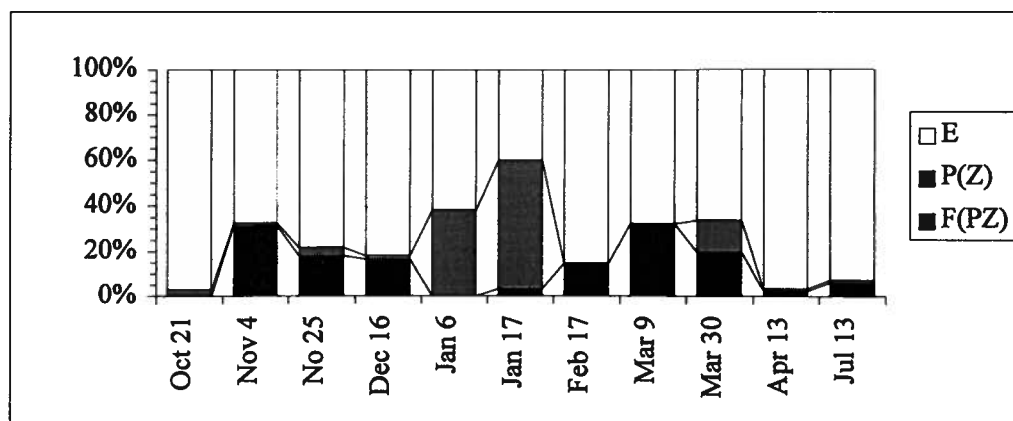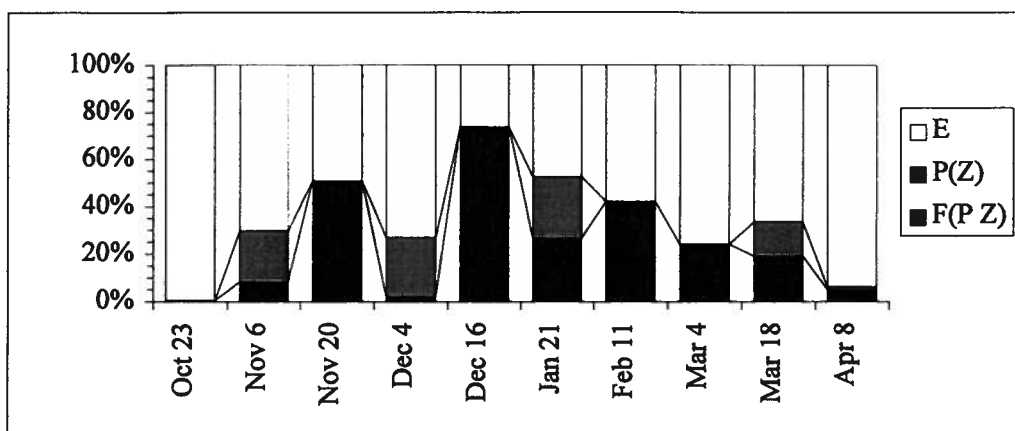
Figure 3.10. Apportionment of σ^2Provenance and σ^2Family of index of injury for each of ten test dates per year over two years of frost testing (top: 1990 / 91; bottom: 1991 / 92).

years, and Figure 3.11 compares the % σ^2F for each of the provenances over two test seasons. Variance components, and heritability estimates, were highly variable from test to test; they were presumed to be imprecise due to the low number of provenances having family structure and the low number of families per provenance, so heritabilities are not included.

3.3.6. Acclimation

Prior to the onset of hardening, no variability occurred in the level of hardiness maintained by a genetic entry; differences did not appear until around late October. While testing did not commence until after acclimation had already begun, acclimation appeared to commence at the same time at the coast for all genetic entries during both test years. Once the hardening process had begun, interior provenances acclimated at a faster rate than coastal provenances (Figure 3.5); this was particularly noticable during the second test year.

ANOVA results of the frost index of injury for all provenances (Table 3.1) and for only those provenances with family structure (Table 3.2) showed that zonal differences were apparent on some dates during acclimation. Provenance differences were evident when all provenances, irrespective of family structure, were analyzed. However, when only those provenances with family structure were investigated, most of the variation appeared to be within provenances during acclimation. While temperature was significant on all test dates, temperature interactions occurred in a few tests during the time when trees were actively hardening.

ANOVA results of the LT_{50} per test date are shown in Table 3.3. Except for
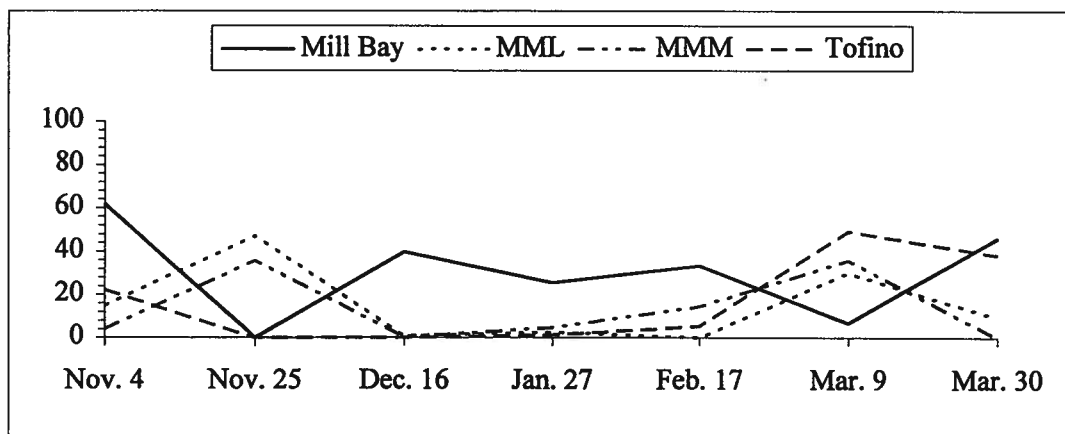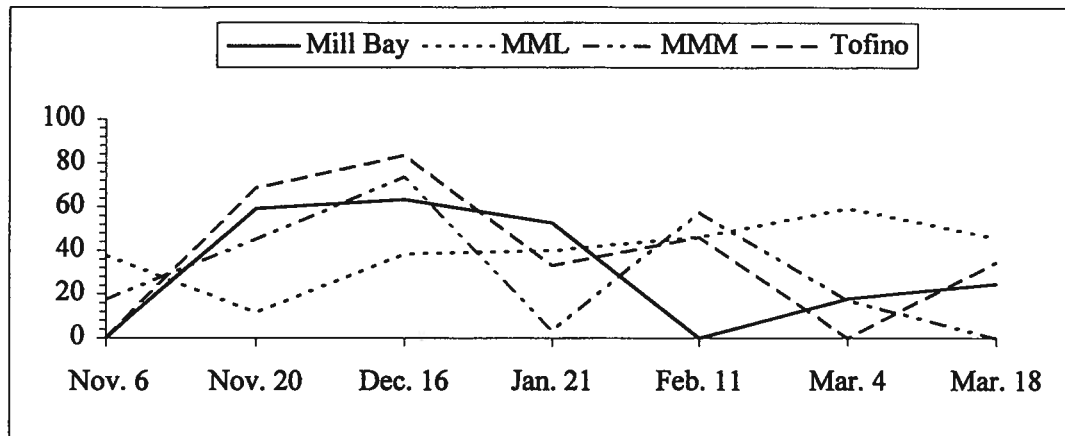
Figure 3.11. Family variance components of frost test index of injury over two years for each provenance having family structure (top: 1990 / 91; bottom: 1991 / 92).

Table 3.1. Analysis of variance[1] results of frost hardiness testing index of injury over two winters, plus one summer test, for all provenances, where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Date	Z	P(Z)	T	T*Z	T*P(Z)
Oct. 23/90	ns	*	***	ns	*
Nov. 6/90	ns	***	***	ns	ns
Nov. 20/90	**	***	***	ns	ns
Dec. 4/90	ns	***	***	ns	ns
Dec. 16/90	ns	***	***	ns	ns
Jan. 21/91	ns	***	***	ns	ns
Feb. 11/91	*	***	***	ns	ns
Mar. 4/91	**	***	***	**	ns
Mar. 18/91	ns	***	***	*	ns
Apr. 8/91	*	ns	***	*	ns
Oct. 21/91	*	*	***	ns	ns
Nov. 4/91	ns	***	***	ns	ns
Nov. 25/91	*	***	***	ns	ns
Dec. 16/91	*	***	***	ns	ns
Jan. 6/92	ns	***	***	ns	ns
Jan. 27/92	ns	***	***	ns	ns
Feb. 17/92	*	ns	***	ns	ns
Mar. 9/92	ns	***	***	ns	ns
Mar. 30/92	ns	***	***	ns	ns
Apr. 13/92	ns	ns	***	ns	ns
July 13/92	ns	ns	***	ns	ns

[1] The full linear model tested was as follows:

$$It_{tzpn} = \mu + T_t + Z_z + T*Z_{tz} + P(Z)_{p(z)} + T*P(Z)_{tp(z)} + \varepsilon_{(tzp)n}$$

where: It = index of injury, T = temperature, Z = zone, and P = provenance. The interaction terms on most test dates were not significant, so those nonsignificant terms were subsequently eliminated from such models.

Table 3.2. Analysis of variance[1] results of frost hardiness testing index of injury over two winters, plus one summer test, for provenances having family structure, where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Date	Z	P(Z)	F(P Z)	T	T*Z	T*P(Z)	T*F(P Z)
Oct. 23/90	*	ns	ns	***	ns	ns	ns
Nov. 6/90	ns	*	*	***	ns	ns	ns
Nov. 20/90	*	ns	***	***	ns	ns	*
Dec. 4/90	ns	**	ns	***	ns	ns	***
Dec. 16/90	ns	ns	***	***	ns	ns	ns
Jan. 21/91	ns	ns	***	***	ns	ns	ns
Feb. 11/91	*	ns	***	***	ns	*	ns
Mar. 4/91	*	ns	***	***	*	ns	ns
Mar. 18/91	ns	ns	***	***	ns	ns	ns
Apr. 8/91	ns	ns	ns	***	ns	ns	ns
Oct. 21/91	ns	ns	ns	***	ns	ns	ns
Nov. 4/91	ns	ns	***	***	ns	ns	ns
Nov. 25/91	*	ns	***	**	ns	**	ns
Dec. 16/91	*	ns	***	***	ns	ns	ns
Jan. 6/92	ns	***	ns	***	ns	ns	ns
Jan. 27/92	ns	***	*	***	ns	ns	ns
Feb. 17/92	*	ns	**	***	ns	ns	ns
Mar. 9/92	*	ns	***	***	ns	ns	*
Mar. 30/92	ns	ns	***	***	ns	ns	*
Apr. 13/92	ns	ns	ns	***	ns	ns	*
July 13/92	ns	ns	ns	***	ns	ns	ns

[1] The full linear model tested was as follows:

$$It_{tzpfn} = \mu + T_t + Z_z + T*Z_{tz} + P(Z)_{p(z)} + T*P(Z)_{tp(z)} + F(P\ Z)_{f(pz)} + T*F(P\ Z)_{tf(pz)} + \varepsilon_{(tzpf)n}$$

where: It = index of injury, T = temperature, Z = zone, P = provenance, and F = family. The interaction terms on most test dates were not significant, so those nonsignificant terms were subsequently eliminated from such models.

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Table 3.3. Analysis of variance significance levels of LT_{50} by test over two seasons (test dates per year are given in Table 3.2) where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Test	1990/91 [1] P	1991/92 Model 1 [2] Z	1991/92 Model 1 [2] P(Z)	1991/92 Model 2 [3] P	1991/92 Model 2 [3] F(P)
1	ns	ns	ns	*	ns
2	**	ns	**	*	*
3	**	*	***	**	*
4	*	*	**	***	ns
5	ns	ns	***	***	ns
6	**	ns	***	***	ns
7	**	ns	ns	ns	ns
8	***	ns	**	ns	*
9	ns	ns	*	ns	ns
10	ns	ns	ns	ns	ns

[1] 1990 / 91 Model: $LT_{50pn} = \mu + P_p + \varepsilon_{(p)n}$
[2] 1991 / 92 Model 1: $LT_{50pzn} = \mu + Z_z + P(Z)_{p(z)} + \varepsilon_{(pz)n}$
[3] 1991 / 92 Model 2: $LT_{50fpn} = \mu + P_p + F(P)_{f(p)} + \varepsilon_{(fp)n}$

where: Z = zone; P = provenance; and F = family

the first test per year, provenance differences occurred in LT_{50} estimates. Zone and family differences also were found during acclimation in the second year except on the first test in late October.

Frost testing of trees growing at Skimikin showed similar ANOVA results in index of injury to those found with trees growing at UBC during the same test year (Table 3.4). In November, frost index of injury differed at the provenance and family levels; while zones did not differ, a zone by temperature interaction occurred. The LT_{50} differed between provenances, but not between zones or families.

A comparison of LT_{50} values from Salmon Arm and trees at UBC during the same year (Figure 3.12) indicated that trees at Skimikin became hardier much quicker than trees growing at UBC. By comparing the daily temperatures at Skimikin (Figure 3.13) with those at UBC (Figure 3.9), it is evident that below-freezing temperatures occurred much earlier at Salmon Arm (late September) than at Vancouver (end of October), although warm daytime temperatures were occurring at both locations during the fall. By the first testing of trees from Skimikin, Salmon Arm had just experienced temperatures down to about -15°C.

Analysis of variance of the LT_{50} values obtained from tests of Skimikin trees vs tests of UBC trees at the same time are given in Table 3.5, and confirm the observations made regarding Figure 3.12. During November, location differences were obvious. Provenance differences were apparent when all provenances were analyzed, but when only those provenances having family structure were investigated, variation appeared to reside mainly within provenances. Location interactions did not occur during the period of acclimation.

Table 3.4. Analysis of variance results of frost testing index of injury on three dates of seedlings growing at Salmon Arm, where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Variable	Nov. 8 / 91	Jan. 23 / 92	Mar. 25 / 92
All Provenances[1]			
Zone	ns	ns	ns
Prov(Z)	***	***	***
Temprature	***	***	***
T*Z	ns	ns	ns
T*P(Z)	ns	ns	ns
Provenances with Family Structure[2]			
Zone	ns	ns	ns
Prov(Z)	*	**	**
Family(P Z)	**	*	ns
Temperature	***	***	***
T*Z	*	ns	ns
T*P(Z)	ns	ns	ns
T*F(P Z)	ns	ns	ns

[1] Linear model: $It_{tpzn} = \mu + T_t + Z_z + T^*Z_{tz} + P(Z)_{p(z)} + T^*P(Z)_{tp(z)} + \varepsilon_{(tpz)n}$

[2] Linear model: $It_{tfpzn} = \mu + T_t + Z_z + T^*Z_{tz} + P(Z)_{p(z)} + T^*P(Z)_{tp(z)} + F(P\ Z)_{f(pz)} + T^*F(P\ Z)_{tf(pz)} + \varepsilon_{(tfpz)n}$

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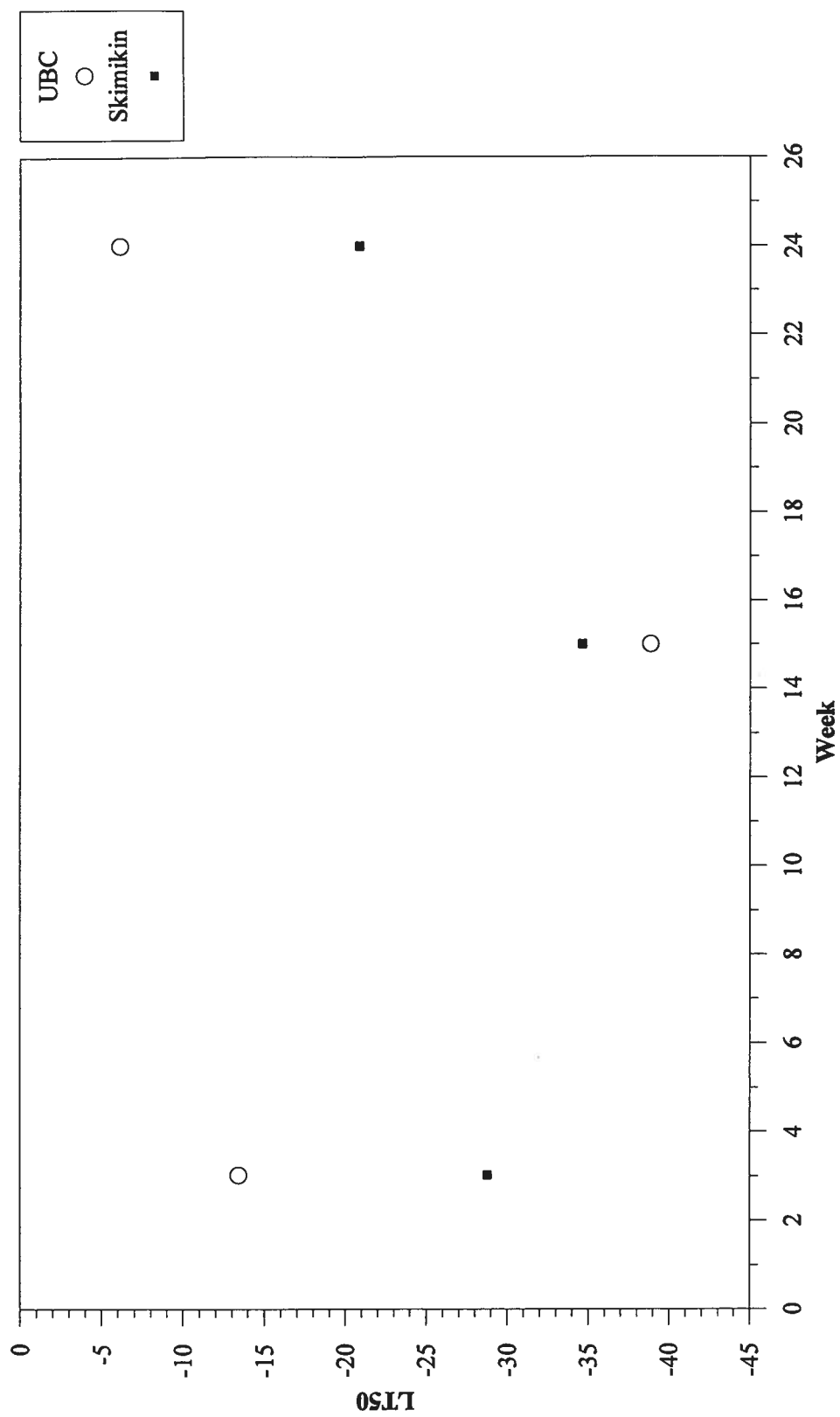
Figure 3.12. Mean LT_{50}'s of trees growing at Salmon Arm compared to trees at UBC during 1991 / 92.
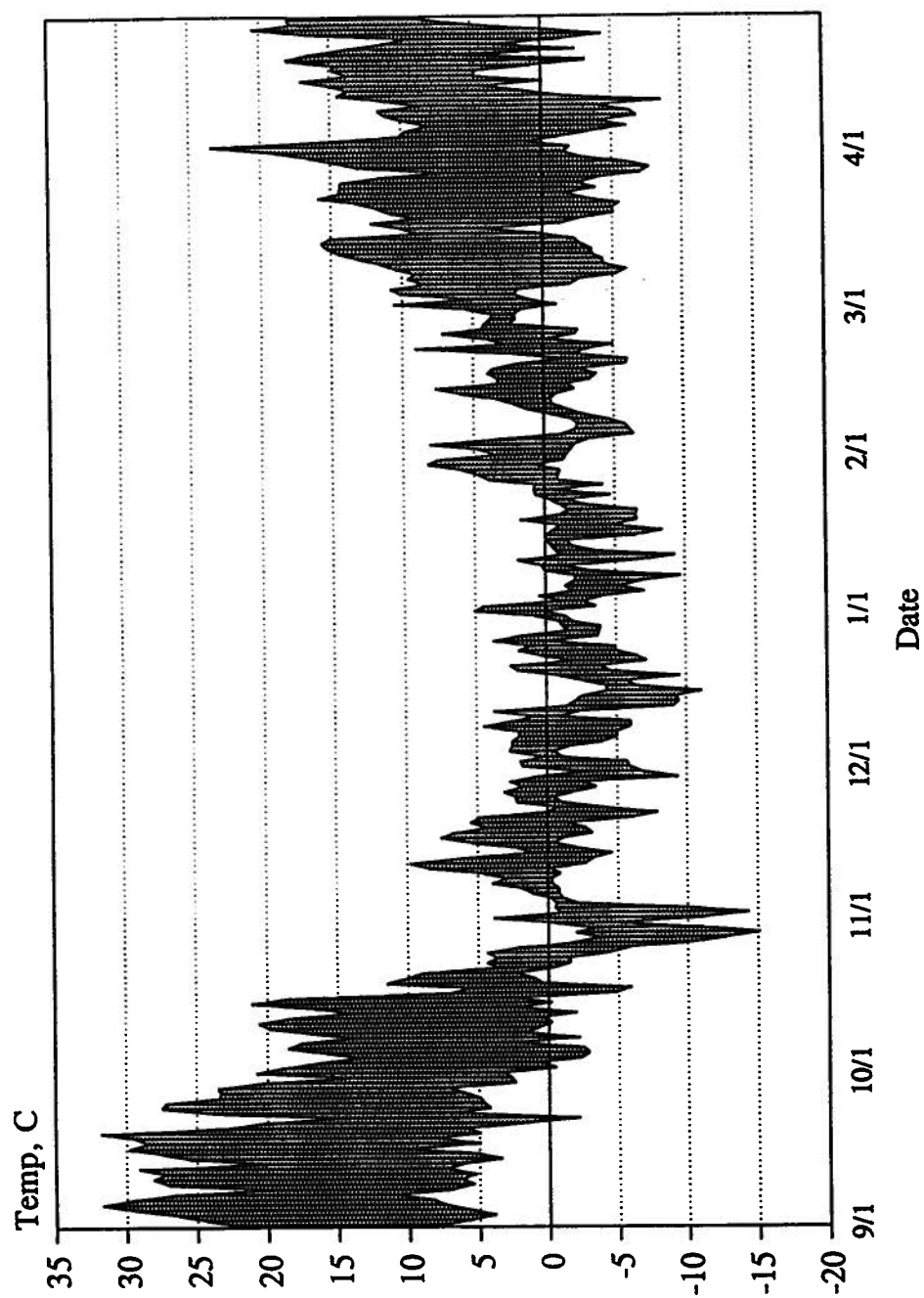
Figure 3.13.   Daily maximum and minimum temperatures at Salmon Arm during 1991 / 92.

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Table 3.5. Analysis of variance results of frost LT_{50} of trees grown at Skimikin compared to trees grown at UBC, where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

| | Test Date: UBC / Skimikin | | |
Variable	Nov. 4/8, 91	Jan. 27/23, 92	Mar. 30/25, 92
All Provenances[1]			
Zone	ns	ns	ns
Provenance(Z)	***	***	***
Location	***	*	***
L*Z	ns	ns	ns
L*P(Z)	ns	*	***
Provenances with Family Structure[2]			
Zone	ns	ns	ns
Provenance(Z)	ns	***	*
Family(P Z)	*	ns	ns
Location	***	ns	**
L*Z	ns	ns	ns
L*P(Z)	ns	*	ns
L*F(P Z)	ns	ns	ns

[1] Linear model: $LT_{50_{lpzn}} = \mu + L_l + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + \varepsilon_{(lpz)n}$

[2] Linear model: $LT_{50_{lfpzn}} = \mu + L_l + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + F(P\ Z)_{f(pz)}$
$+ L*F(P\ Z)_{lf(pz)} + \varepsilon_{(lfpz)n}$

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3.3.7. Maximal Hardiness

During midwinter (mid-December to mid-February), when the trees were at or near their maximum levels of frost hardiness, analysis of all provenances showed that variation in I_t between provenances occurred on almost all test dates (Table 3.1). When provenances having family structure were analyzed (Table 3.2), it appeared that most of the variation associated with provenances occurred at the family level. On the one midwinter date in the first year when family was not significant, provenances and the temperature by family interaction terms were both significant, and on the one midwinter date in the second season where no family variation occurred, strong provenance differences were observed. Zonal differences did not occur at the time when trees were most hardy.

Analysis of LT_{50} values indicated provenance differences during both midwinters except in mid-December of the first test season (Table 3.3). Figure 3.5 shows slightly less divergence in the LT_{50} curves on that date; the rate of acclimation may have been slowing considerably at that time. Zonal differences did not occur at maximal hardiness. Family variation was also not evident during midwinter in estimated LT_{50}.

Lower maximum LT_{50}'s were obtained during the second year of frost testing at UBC. Provenance rankings over the two seasons were similar, but the degree of hardiness was deeper for 1991 / 92 in all cases. The date when maximum hardiness occurred each season was the same for all provenances.

Midwinter I_t (Table 3.4) and LT_{50} at Skimikin varied between and within provenances, but not between zones. In comparing midwinter tests at UBC with

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Skimikin (Table 3.5), location effects were significant when all provenances were examined, but not when only provenances with family structure were analyzed. Provenance differences were apparent in both analyses, and zone did not vary in either case. A significant location*provenance interaction indicated that provenance rankings changed somewhat between locations, causing genotype*environment interactions. Testing in late January showed that trees from Salmon Arm no longer had a lower LT_{50} than trees from UBC; in fact the latter had a slightly lower LT_{50}.

Hierarchical cluster analysis was carried out on the provenance mean LT_{50}'s found on Jan. 21, 1991 and Jan. 27, 1992 at UBC, the tests closest to the times of maximum hardiness. Arbitrarily grouping these two traits into three clusters, the least hardy cluster consisted of Kooskia, Squamish, Mill Bay, Tofino, and Mt. Mara Low elev., with Oliver Lake and Benton Flat forming the middle cluster and Mt. Mara Mid elev. forming the most hardy cluster. When survival at Skimikin following winter desiccation, foliar damage at Skimikin, and the final height of trees growing at UBC were added to the midwinter LT_{50}'s, clustering into three groups produced the same results as above with the exception of Mt. Mara Low elev., which moved to the middle cluster.

The mean UBC midwinter LT_{50}'s were correlated to final height at UBC (in 1991, r = -0.700; in 1992, r = -0.598), mean survival (r = 0.787 for 1991; r = 0.668 for 1992) and foliar desiccation (r = 0.829 and 0.750 for 1991 and 1992 respectively) at Skimikin, elevation (r = 0.800 and 0.711 for the two years), average number of growing degree-days (r = -0.680 and -0.523 for 1991 and 1992), and mean January daily temperature (r = -0.651 and -0.564), while the mean UBC midwinter LT_{50} for 1992 was also correlated to latitude (r = 0.429) and mean annual precipitation

($r = -0.358$). Stepwise regressions, using a significance level of $\alpha = 0.05$ for entry into and staying in the model, found the following models to be the best:

$$LT_{50}Jan91 = 13.823 - 0.008*Elev. - 0.318*Longit.; \quad R^2 = 0.729; P < 0.0002$$

$$LT_{50}Jan92 = 109.359 - 0.028*Elev. - 1.061*Longit. - 0.048*Growing\ days;$$
$$R^2 = 0.759; P < 0.0001$$

Hierarchical cluster analysis of the estimated January LT_{50} of Skimikin trees into three clusters placed Tofino alone in the least hardy group, Benton Flat and Mt. Mara Mid elev. in the most hardy group, with the middle group being comprised of the other five provenances. When survival at Skimikin following winter desiccation, foliar damage at Skimikin, and the final height of trees growing at UBC were added to the midwinter Skimikin LT_{50}, clustering was identical to that for the two midwinter LT_{50}'s for UBC, that is Mt. Mara Mid elev. in the hardiest group, Benton Flat, Mt. Mara Low elev., and Oliver Lake in the middle group, and Kooskia, Squamish, Mill Bay, and Tofino in the least hardy group.

The mean midwinter LT_{50} of trees at Skimikin was correlated most of the same variables as the trees at UBC during 1992 (height: $r = -0.343$; survival: $r = 0.583$; foliar desiccation: $r = 0.644$; elevation: $r = 0.685$; growing days: $r = -0.575$; mean January temperature: $r = -0.622$; longitude: $r = -0.415$; and mean annual precipitation: $r = -0.509$). A stepwise multiple regression, using a significance level of $\alpha = 0.05$ for entry into and staying in the model, found the following model to be the best:

$$LT_{50}JanSkim = -30.379 - 0.010*Elev.; \quad r^2 = 0.469; P < 0.0001$$

As with growth traits of this study, elevation was the most important independent variable affecting hardiness in all cases. Elevational clines estimated by simple linear regression for the January LT_{50}'s of UBC trees during 1991 and 1992 and Salmon Arm trees during 1992 showed that 1°C of hardiness would be obtained for every 175 m, 79 m, and 98 m increase in elevation respectively.

Calorimetry results are presented in Table 3.6. An example of a graph of foliage temperature over time produced from one tree sample, plus the difference between this same sample and the ambient temperature during the period when the exotherms were observed, is given in Figure 3.14.

Two exotherms were detected for each tree sample; the first exotherm occurred between -3.6°C and -11.3°C, while the second exotherm occurred between -7.4°C and -13.5°C. No exotherms were seen at temperatures approximating the LT_{50} of these provenances at that particular time (which ranged from about -23.0°C to -27.7°C) as estimated from electrical conductivity frost testing. The mean temperatures per provenance at which the first and second exotherms occurred were in general ranked similarly to the LT_{50} rankings per provenance, with the hardier provenances having slightly lower temperatures at which exothermic events took place and slightly larger spikes at each exothermic occurrence.

No provenance differences were detected in ANOVA of the temperatures at which either of two exotherms occurred, the size (°C) of the two exotherms, and the temperature to which each sample rose to after each exothermic event, although a test involving a larger sample size might have been able to detect stronger trends.

The first and largest exotherm per sample seen in the current study would

Table 3.6. Temperatures at which exotherms were observed during calorimetry experimentation on Feb. 15, 1992 in two trees / provenance, plus provenance mean (size of exotherm in °C is given in brackets) compared to LT_{50} of these provenances as determined in normal frost testing.

Provenance	Tree	LT_{50}	1st Exotherm		2nd Exotherm	
Oliver Lake	A	-27.7	-8.89	(1.77)	-10.41	(4.75)
	B		-11.30	(5.86)	-13.46	(4.30)
	X̄		-10.09		-11.93	
Mill Bay	A	-23.1	-4.10	(1.14)	-8.07	(1.78)
	B		-5.19	(1.92)	-8.04	(3.32)
	X̄		-4.64		-8.05	
Kooskia	A	-23.0	-3.61	(1.52)	-9.49	(4.69)
	B		-7.13	(0.31)	-8.66	(4.51)
	X̄		-5.37		-9.07	
Mt. Mara Mid	A	-27.3	-5.41	(1.88)	-12.08	(4.78)
	B		-6.00	(1.94)	-7.42	(3.02)
	X̄		-5.70		-9.75	
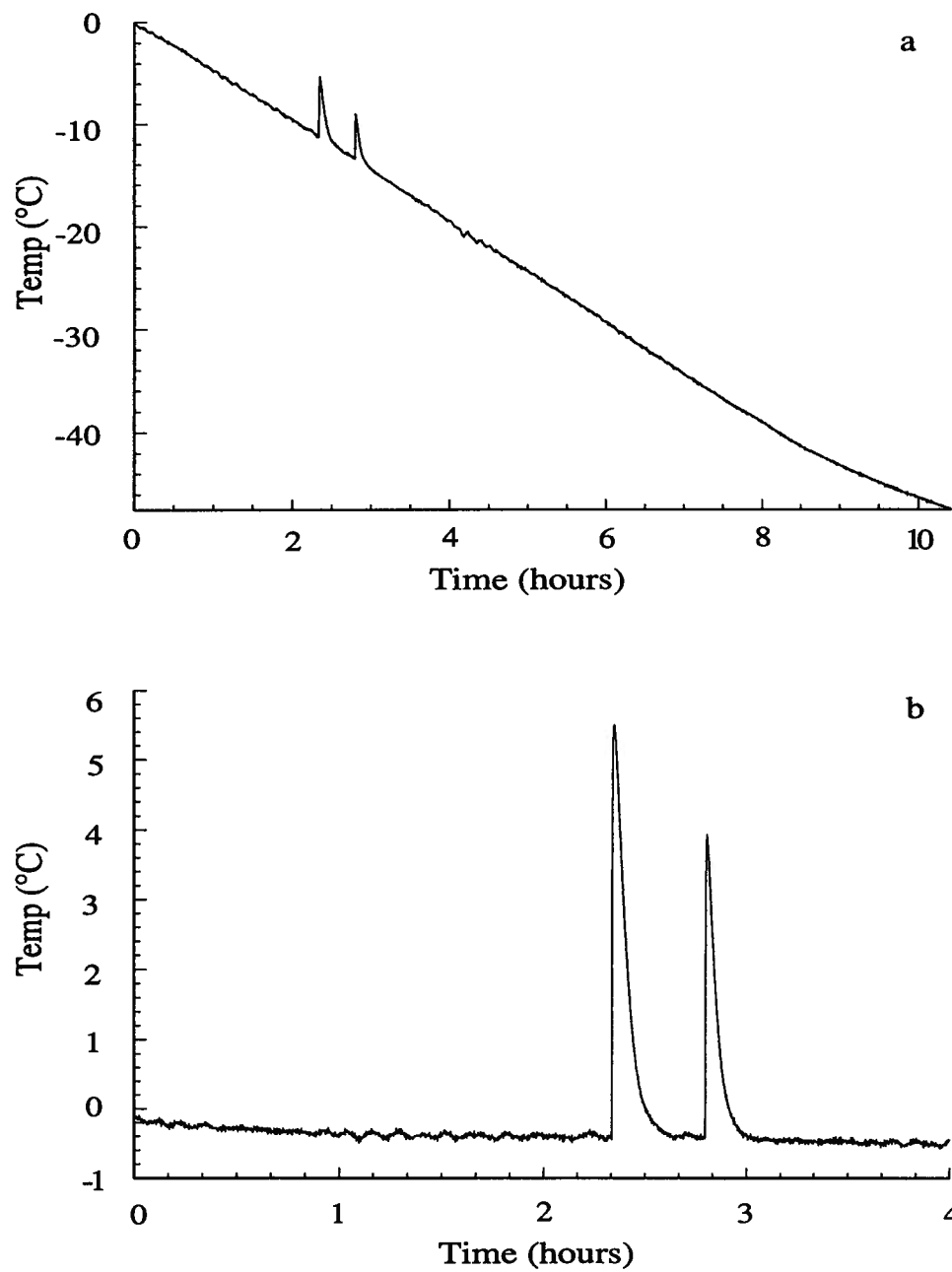
Figure 3.14. Foliage temperature, as monitored by calorimetry, plotted against ambient test temperature for one tree sample (a); the same sample minus the control channel (b).

correspond to when readily available water is frozen extracellularly. The second exotherm was probably due to a particular chamber or compartment of the cell freezing, and not due to intracellular water migrating out of the cell and freezing extracellularly, as the latter occurs gradually, not suddenly (R. Guy, UBC, pers. comm., 1994). Alternatively, two exotherms may have represented the two foliage pieces sandwiching each probe undergoing extracellular freezing at different times.

The lack of a low temperature exotherm in this study could be attributable to insufficient detection by the apparatus. Alternatively, Ritchie (1991) has suggested that some boreal and high elevation species do not supercool, although he suggested that species which can reach low levels of hardiness without supercooling are able to harden because of an ability to tolerate extreme cytoplasm dehydration, which would intuitively seem unlikely for western red cedar. However, these results do echo those of George *et al.* (1974) who found no low temperature exotherm for *Thuja occidentalis.*

A summarization comparing results of the various tests used to investigate maximal frost hardiness is given in Table 3.7. For this summary, where seasonal testing was carried out, the tests done nearest to the time of maximum hardiness were used.

A joint study of foliar nutrient analysis and frost hardiness of the same sample trees found that the LT_{50} of seedlings was not correlated to any of the macronutrients analyzed (N, P, K, Ca, and Mg) or to the ratios of P/N, K/N, or K/Ca. However, when separate correlations were done by zone, trees from the interior zone showed correlations between the LT_{50} and N, K, and K/Ca. When correlations were carried out by provenance, the LT_{50} was correlated to N, Ca, and P/N at Tofino, to P and P/N

Table 3.7. Summary of analyses from the various tests used to assess frost hardiness, using the date closest to maximum hardiness where seasonal monitoring was done, where - indicates that means were not estimable because values occurred over more than one temperature, or values were not estimable at the family level; see text for definitions of abbreviations.

Variable	Overall Mean	±	standard dev.	All Prov.	Prov. with Fam. structure	%σ^2P	%σ^2F
UBC I_t 91	-		-	P	F	25.65	26.73
UBC I_t 92	-		-	P	P F	56.58	3.10
Skim I_t 92	-		-	P	P F	39.94	10.24
UBC LT_{50} 91	-28.19		2.89	P	-	73.29	-
UBC LT_{50} 92	-38.84		7.04	P	P	82.20	0.87
Skim LT_{50} 92	-34.62		5.89	P	P	52.92	0
Jan. Fv/F_m[1]	0.49		-	P	-	-	-
Jan. Fv/F_o	1.00		-	P	-	-	-
Calorimetry[2]	-		-	all ns	-	-	-

[1] Fluorescence ratio means are based on values obtained at -35°C, the test temperature closest to the estimated LT_{50} at the time.

[2] Calorimetry refers to the temperatures at which the two exotherms per sample occurred, the size in degrees C of the exotherms, and the difference between the temperatures at which exothermic reactions occurred and the size of the exotherm produced.

at Mill Bay, and to Ca at Benton Flat. A stepwise elimination regression of LT_{50} on the macronutrients and ratios found none of the variables significant to the model at the 0.05 level; N was significant at $P = 0.11$ with $r^2 = 0.067$.

Analysis of variance of the frost hardiness / nutrient status test found that the LT_{50} varied between zones but not between provenances per zone. All macronutrients and tested ratios varied between provenances but not between zones except for Ca, which did not vary significantly at either level. These results are similar to those obtained in the nutrient study covered in Chapter 2.

3.3.8. Deacclimation

All provenances at UBC appeared to begin dehardening around the same date, and are presumed to have completed deacclimation at the same time. Deacclimation on the coast seemed to occur at the same rate for all genetic entries.

Patterns of variation during deacclimation seemed similar to those during acclimation. During deacclimation, differences between zones in I_t were observed. While variation occurred at the provenance level when all provenances were investigated (Table 3.1), most of this variation appeared within populations when only provenances with family structure were analyzed (Table 3.2). For LT_{50} analysis, zones did not vary; provenances varied on a few dates, and families were significant only in early March of the second test year (Table 3.3).

The nonsignificance of families and provenances near the end of the hardening cycle can easily be explained, as it is evident that significant differences do not occur

while trees are nonhardy throughout the summer, and variation in LT_{50} is low. The nonsignificance of provenances on Feb. 17, 1992 when all provenances were looked at is harder to explain, but frost curves show slightly less divergence on that date, maybe because the rate of dehardening was accelerating; zones varied on that date.

At Salmon Arm, provenance differences in I_t and in LT_{50} were evident in late March (Table 3.4), but zone and family differences were not. At this time, trees from UBC were dehardening much more rapidly than trees at Skimikin (Figure 3.12).

When comparing Vancouver and Salmon Arm in late March, locations varied significantly, as did provenances. A location*provenance (genotype*environment) interaction was significant in March where all provenances were examined.

3.3.9. Variable Chlorophyll Fluorescence

Variable chlorophyll fluorescence analysis results were obtained at test temperatures similar to expected LT_{50} values on two dates, once close to maximum seedling hardiness and once when seedlings were almost completely dehardened. Figure 3.15 shows the ratios of Fv/F_m and Fv/F_o respectively plotted against test temperatures on January 13, 1992, while Figure 3.16 shows the same ratios as obtained on April 1, 1992.

The expected trend for the fluorescence graphs would be decreasing ratios with lower temperatures, and for the nonfrozen controls to have the highest ratios, indicating that as temperatures decrease, the photoprotective effects of photoinhibition are less apparent, and more damage is possible to the plant. This was
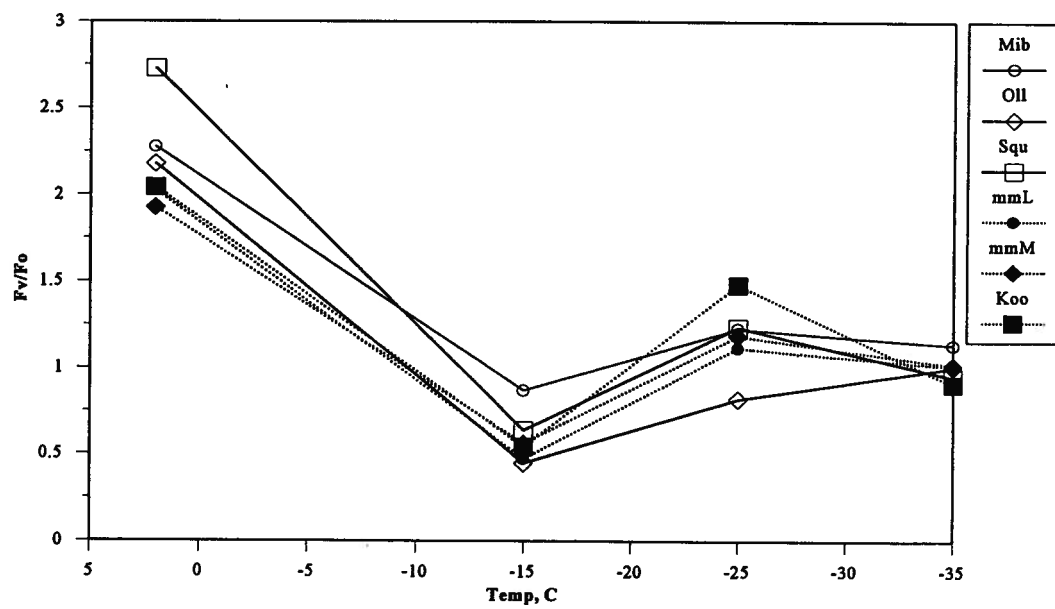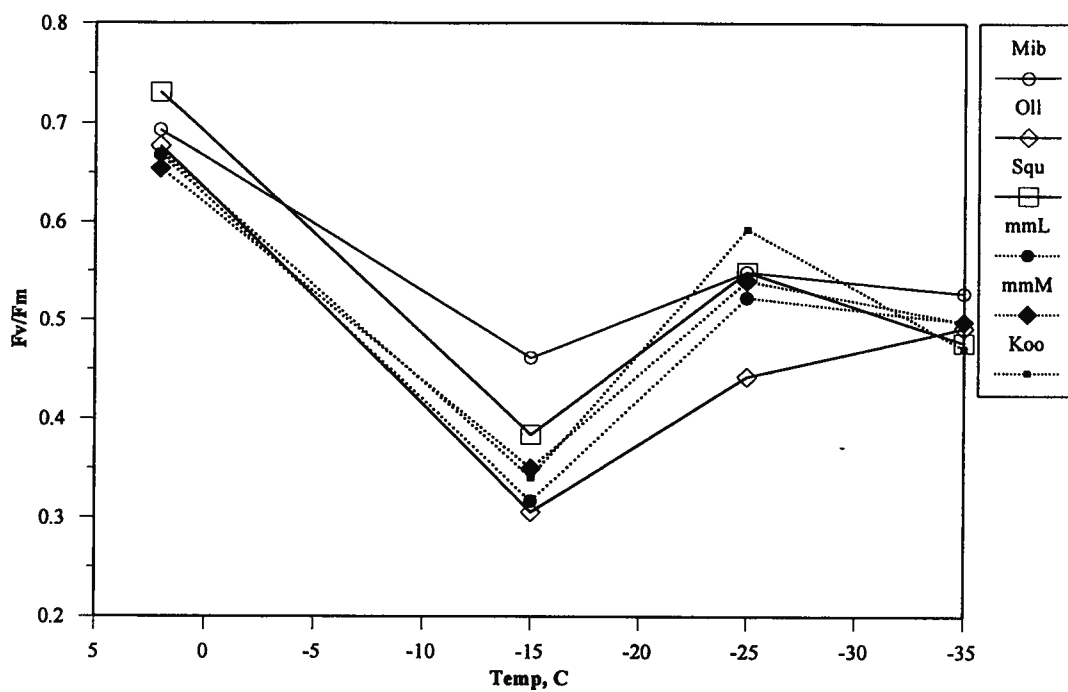
Figure 3.15. F_v/F_m (top) and F_v/F_o (bottom) per test temperature in January.
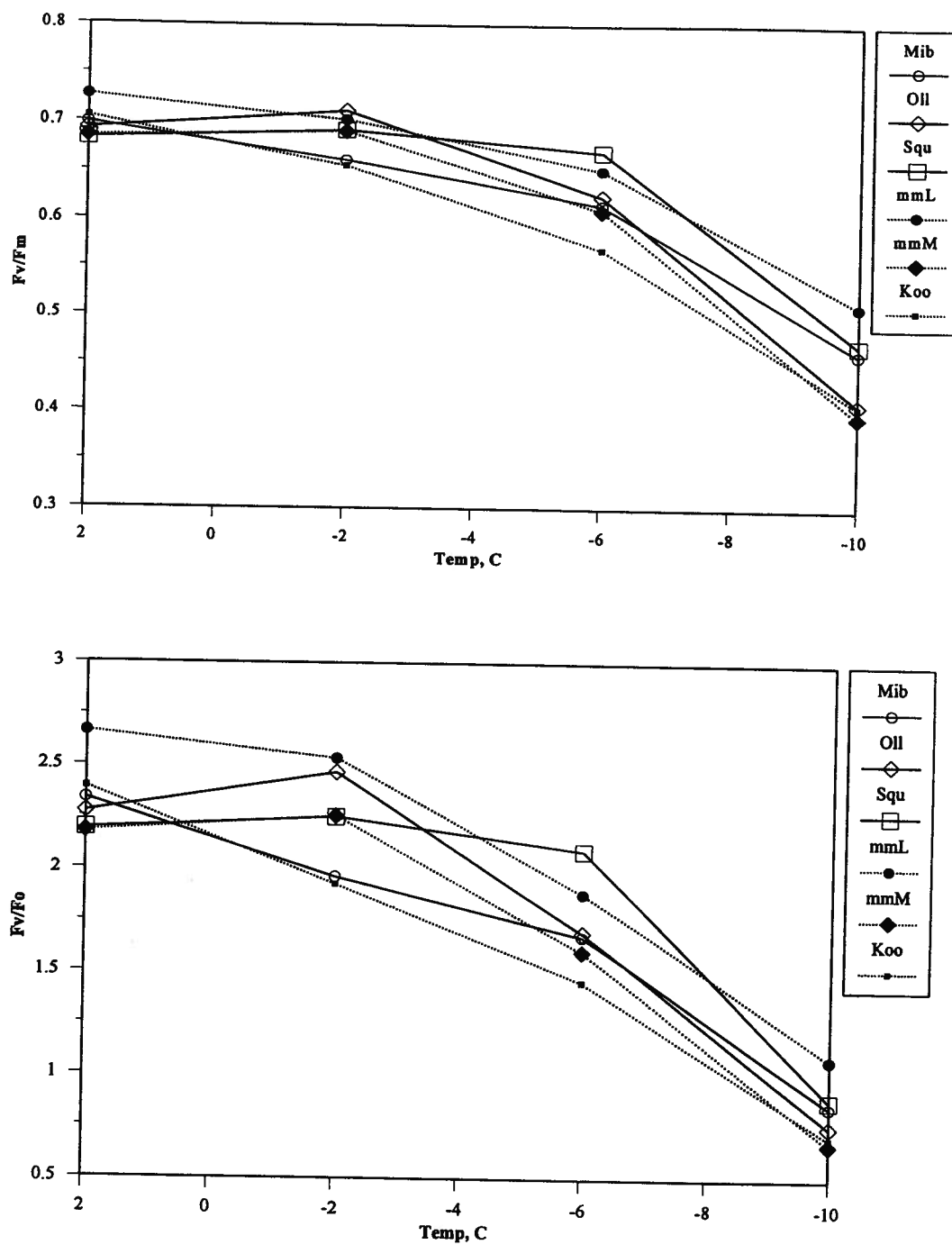
Figure 3.16. F_v/F_m (top) and F_v/F_o (bottom) per test temperature in April.

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the case for April 1. On the January 13 graphs, both ratio values at -15°C were lower than those at -25°C. The unexpected dips in these curves appear to have been due to some factor affecting only those samples tested to -15°C, but could alternatively have been due to some factor occurring with the -25°C samples. A low light was briefly turned on by accident just prior to the measuring of fluorescence of the -25°C samples; however, this short light exposure was too brief to have had any effect.

Analysis of variance of measured variables showed no zonal differences in any trait on either test date. Provenance differences were seen in the F_v/F_m and F_v/F_0 ratios on both test dates. While F_0 in January and F_v and F_m in April varied by provenance, these values are meaningless, as these absolute values will differ with the amount of tissue surface area analyzed per seedling, which could not be constant from tree to tree (R. Guy, UBC, pers. comm., 1994).

General provenance rankings based on F_v/F_m and F_v/F_0 at the temperature estimated to be closest to the expected LT_{50}'s (January 13: -35°C; April 1: -6°C) were compared to mean provenance LT_{50} rankings of these same provenances as found by electrical conductivity frost tests taken near the dates of the variable chlorophyll fluorescence tests (Table 3.8). Provenance rankings of F_v/F_m and F_v/F_0 were not similar to LT_{50} rankings.

When the average LT_{50} per provenance was compared to the fluorescence results obtained for each provenance, the ratio of F_v/F_m at the test temperature closest to that at which the LT_{50} occurred ranged from about 0.47 to 0.53 in January and about 0.57 to 0.67 in April; the F_v/F_0 ratio ranged from about 0.91 to 1.13 in January and about 1.45 to 2.09 in April. It is evident that none of the ranges overlapped. It seems that the relationship between fluorescence and electrical conductivity

Table 3.8. Comparison of provenances based on ranking by LT_{50} estimated from electrical conductivity testing and by variable chlorophyll fluorescence results from two test dates; ratios for January 13 are from samples tested to -35°C, while ratios for April 1 are from samples tested to -6°C.

Date	LT_{50}, °C		Variable chlorophyll fluorescence F_v/F_m		F_v/F_o	
Jan. 13 / 92	Mib	-30.0	Mib	0.53	Mib	1.13
	Squ	-32.0	mmL	0.50	mmM	1.02
	Koo	-33.0	mmM	0.50	mmL	1.01
	mmL	-33.0	Oll	0.49	Oll	1.00
	Oll	-37.0	Squ	0.48	Squ	0.95
	mmM	-43.0	Koo	0.47	Koo	0.91
April 1 / 92	Mib	-5.1	Squ	0.67	Squ	2.09
	Squ	-5.2	mmL	0.65	mmL	1.88
	Koo	-5.4	Oll	0.62	Oll	1.69
	mmL	-6.0	Mib	0.61	Mib	1.67
	mmM	-6.0	mmM	0.61	mmM	1.60
	Oll	-6.7	Koo	0.57	Koo	1.45

measurements of cold hardiness is thus not clearcut and hence the predictability of frost damage from the fluorescence test is not immediately obvious.

3.4. DISCUSSION

The interpretation of frost hardiness data was hampered by the low number of provenances and families per provenance which were able to be tested. For instance, comparison of provenance and family variance components of hardiness traits on different dates did not give a very cohesive story. However, it is reasonable to conclude that genetic variation does exist in the cold hardiness of western red cedar, both between and within provenances. It appears that more of the variation found when seedlings are acclimating or deacclimating occurs within rather than between provenances, and greater differences between entities occur when the trees are at their maximum stage of hardiness. Zonal differences and significant temperature interactions only occurred at the times when acclimation or deacclimation was rapid.

In observing the hardiness curves of the same genetic entries grown at the same mild coastal location over two winters, no differences in date when acclimation commenced or when deacclimation had terminated were apparent between zones, provenances, or families; nor could differences in the date of estimated maximum hardiness be found between genetic entries. Once hardening had begun, provenances from the interior range acclimated at a faster rate. However, both coastal and interior provenances growing at UBC all dehardened at the same rate. It appears that acclimation began about a week earlier in 1991 than in 1990, although deacclimation appeared to have been completed at about the same time for both test seasons. Timing of the onset of hardiness responses thus seemed to be plastic in nature, following the pattern of a generalist, and hence it can be inferred that timing of acclimation, and probably deacclimation, is not under direct genetically variable (although plasticity itself may vary genetically).

With an everchanging state of hardiness occurring between autumn and spring, it is not surprising to find one test out of ten carried out per year where no within-population differences could be found. The two dates when this occurred for index of injury, in early winter of the first test year and in midwinter of the second year of testing, coincided with a period just prior to the time when maximum hardiness was reached.

By comparing cold hardiness of trees grown at UBC vs trees grown at Salmon Arm during 1991 / 92, a comparison of the same genetic entries of the same age during the same year was being made between seedlings grown at a mild coastal site and those grown at a relatively harsh interior location. Trees at Skimikin began hardening sooner in the fall and dehardened later in the spring, although the degree of maximum hardiness reached in those trees was not deeper than trees from UBC.

Seedlings at both UBC and Skimikin which were tested during the winter of 1991 / 92 became hardier than the same genetic entries tested the previous year at UBC. While seedling age may have been a factor, it seems more likely that the estimated maximum LT_{50}'s obtained during 1991 / 92 from the two locations represent the maximum hardiness capability of western red cedar, as no other study to date reports hardiness levels lower than the levels found in this study for western red cedar. The maximum degree of hardiness thus appears to be a plastic response in this species.

The graphical representations of daily maximum and minimum temperatures at the two locations where seedlings were growing may offer some explanation into seedling responses. During the first test season, the minimum temperature had not dipped below +11°C until the very end of of September 1990, when the temperature

fell to +7.3°C. The temperature did not go below +5°C until November 1, and freezing temperatures were not encountered until December 19. Interior provenances were acclimating fairly rapidly from late October onwards, while coastal provenances did not acclimate at a faster rate until about November 20, about three weeks after temperatures below +5°C were experienced. Acclimation rates slowed in all provenances from about mid-December.

By contrast, in the fall of 1991, temperatures fell below +10°C on September 14, reached down to +7°C on September 21, and went below +5°C on October 18. There was an increase in the number of hours when the temperature was below +5°C during early November compared to the same time period in the previous year. The first below-freezing temperature occurred on October 28. Interior provenances acclimated during the fall of 1991 at a steady rate until January 1992. Coastal provenances increased in rate of acclimation around November 25, about the same time as in the previous year, but did not slow until January. All provenances were hardier than they had been a year earlier by about December 16.

At Salmon Arm, temperatures below +10°C occurred intermittently throughout July and August of 1991, below +7°C on August 26, and below +5°C by September 3. The first frost occurred on September 22. By the first week in November, the average LT_{50} of seedlings growing at Skimikin, about -28.8°C, was on average -15.4°C lower than that of trees at UBC. Trees at Salmon Arm only decreased in hardiness a further -5.8°C for the winter. Thus under extreme weather conditions, seedlings of all provenances acclimated much sooner and reached maximum hardiness much earlier, although seedlings were maintained at maximal hardiness at least until the end of January, when maximal hardiness was believed to

take place in UBC trees.

The occurrence and timing of low temperature thus appear to be the factors most strongly responsible for triggering hardening in this species. If temperatures below +5°C are critical for inducing acclimation, then the results of this study would indicate that changes in the rate of acclimation can be observed roughly three to four weeks after such temperatures are encountered, at least in trees growing in mild locations. It is suspected that a more sudden response may be found in harsher environments. These conclusions seem to be supported by those of Silim (1991), who reported a lag phase of metabolic adjustment of western red cedar in response to a low temperature stimulus of about three weeks.

Below-freezing temperatures undoubtedly affect the depth of hardiness reached in any one year. However, as much lower winter temperatures were experienced during midwinter 1990 / 91 (to -13.4°C on December 29) than in 1991 / 92 (to -2.9 on January 7) at UBC, but trees did not become as hardy in the first test year as in the second, timing of low temperatures seems to be the critical element.

Shoots of at least some seedlings were still elongating by December 1, 1990, prior to any frost. As stated earlier, western red cedar is an opportunist and will grow as long as conditions are favourable. Thus acclimation commences before shoot growth has ceased, although deep hardening probably cannot occur until growth has stopped.

At UBC, minimum temperatures began rising by the first of February in 1991 (however, late frosts happened in early March). In 1992, the last frost was on January 19. During both years, deacclimation was underway by mid-February, and was

complete by about mid-April. By contrast, temperatures substantially below freezing were occurring at least until the end of April 1992 at Salmon Arm. By March 25, seedlings at Skimikin were still hardy to about -20.8°C.

It is obvious that a period of above-freezing temperatures is necessary for the onset of deacclimation to take place. These results concur with those of Silim (1991) and Krasowski and Owens (1991) for one western red cedar seed source each. Silim (1991) also noted a lag of only five days after exposure to warm temperatures before dehardening commenced in this species. There appears to be genetic uniformity in the response to the environmental cues affecting dehardening.

While the response of western red cedar to the external stimuli responsible for initiating acclimation and deacclimation and the winter maximum hardiness level reached appear to fit Rehfeldt's (1984) definition of a generalist exhibiting phenotypic plasticity, specialist behaviour was also observed in the form of within-population variation in hardiness per test date. If western red cedar is an outcrosser with good gene flow, within-population differences would be expected to be higher than between-population differences.

However, selective pressures are obviously present in the form of winter environmental conditions. These selective pressures could be low temperature extremes, factors contributing to winter desiccation, and possibly some unknown factors affecting the relationship between a tree's genotype and its ability to survive the winter with minimum damage. It seems that the severity of winter conditions is the most influential factor shaping the patterns of frost hardiness variation in western red cedar.

Between-provenance differences were evident in January 1992 when seedlings grown at UBC were at their hardiest and were also evident on all dates on trees grown at Skimikin. Thus when conditions are most severe, between-population differences seem to overshadow within-population differences, and adaptive variation is at the provenance level. While these results could be compared to survival at Skimikin after a severe winter, another indicator of adaptive variation in which provenance differences were apparent, it must be kept in mind that frost analysis was carried out on a small number of provenances having family structure. Thus the determination of whether variability in degree of maximal hardiness obtained in any one year is mainly allocated between or within populations cannot be definitively made at this time.

Scrutiny of individual provenance performance showed that Kooskia in Idaho, the only provenance tested which can safely be assumed to have remained unglaciated during the last ice age, was one of the least hardy of the interior provenances, along with Mt. Mara Low elevation. Mt. Mara Mid elevation was consistently the most hardy of all provenances from about November to March. From the coastal provenances, the high latitude Oliver Lake near Prince Rupert, B.C. was the hardiest throughout the two winters. These observations were corroborated by cluster analysis of the mean midwinter provenance LT_{50}'s.

Although it seems that elevational clines do exist in hardiness traits, too few provenances were sampled to get an accurate picture of the steepness of such clines. It is quite possible that latitudinal clines also exist, especially in the interior where temperature is not moderated by the ocean.

The exploratory investigations into using alternative methods for assessing cold hardiness were not promising. While variable chlorophyll fluorescence may give

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indications of relative frost hardiness on any one date, this method appeared to have a poor predictive ability compared to the electrical conductivity method. No obvious relationships between the ratios F_v/F_m and F_v/F_o and the LT_{50} as found by electrical conductivity were observed.

Calorimetry was also unpromising. The two exotherms that occurred per sample tree were not related to hardiness levels or to differences between genetic entries. Reasons for the absence of a low temperature exotherm indicating the point of serious injury are unknown. Although an exotherm may have occurred but was too small for detection, it is probable that western red cedar does not supercool its cellular water, so no water is available at very low temperatures for freezing and hence producing an exotherm.

Although provenance variability occurred in the nutrient levels of trees tested for frost hardiness, as was noted in the growth study, no relationships between nutrient status and frost hardiness were observed. None of the macronutrients were at critically low or excessively high levels. If nutrients were at limiting or toxic levels, it is possible that hardiness may be affected.

The positive correlations between frost hardiness parameters and seedling survival and the inverse relationship between frost hardiness and seedling height have practical implications for selection within this species, as all of these traits would be desirable. Silvicultural techniques such as planting seedlings under an overstory might be considered as tools to assist in survival and minimize the effects of cold temperatures on trees selected for other traits such as growth. Alternately, correlation-breakers could be sought, selection indices developed, or tandem selection be practiced.

In summation, the degree of adversity of winter environmental conditions seems to be the most important factor shaping the variability of cold hardiness in western red cedar. During the times when unfavourable environmental conditions were most extreme, between-population differences were higher than within-population differences, although the opposite was true during the most rapid periods of acclimation and deacclimation. Maximum differences between populations occurred when the trees were at their annual maximum hardiness levels.

Low temperatures experienced in the autumn appeared to initiate the induction of the acclimation process, with the timing of these cold temperatures seeming to determine the depth of tree hardening reached over a winter. The severity of winter conditions at the test locations was a factor in the timing of hardening and dehardening (which were plastic in nature), and in the absolute hardiness levels reached (down to a lower limit). Of the geographic aspects associated with each provenance that were studied, elevation was the most influential.

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4. SELF-FERTILIZATION VS POLYCROSSING

4.1. INTRODUCTION

Most temperate zone coniferous forest tree species studied to date have been found to have high levels of genetic diversity. Although partial self-fertilization does occur in most species (Muona, 1990), conifers are predominantly wind-pollinated outcrossers, with the average proportion of outcrossed progeny for a species usually close to or greater than 0.90, and average individual population estimates usually greater than 0.80 (Hamrick *et al.*, 1979; Muona, 1990; Adams and Birkes, 1991). For such species, inbreeding depression is common, and may be manifested in low filled seed production, low germination rates, poor growth, higher mortality rates, and differential physiological response to stress (Franklin, 1970; Park and Fowler, 1982; Woods and Heaman, 1989; Yazdani and Lindgren, 1991; Blake and Yeatman, 1989), with the most commonly reported effects being the first two.

Members of the genus *Thuja* that have been studied have been found to have lower outcrossing rates than those of many other conifers. Mean multilocus outcrossing rates of 0.75 for *Thuja orientalis* (Xie *et al.*, 1991) and 0.635 for *Thuja occidentalis* (Perry and Knowles, 1990) were observed over seven and four loci respectively. Perry *et al.* (1990) found a mean heterozygosity of 0.094 over for *T. occidentalis*, three times that found by Yeh (1988) for one Vancouver Island and seven southern interior B.C. populations of *Thuja plicata*. El-Kassaby *et al.* (1994) obtained an outcrossing rate for two loci of 0.32 in a western red cedar seed orchard population consisting of 28 trees, and suggested that western red cedar has a high

level of natural inbreeding, with possibly as high as a 50 % selfing rate. As mentioned earlier, other studies have found little variation in isozymes (Copes, 1981) and leaf oil terpenes (von Rudloff and Lapp, 1979; von Rudloff *et al.*, 1988) of weestern red cedar.

Owens *et al.* (1990) found that selfed seed from two clones of western red cedar had only a slightly higher embryo abortion rate than outcrossed seed, and selfing had no apparent effect on amount of filled seed produced. A further small nonreplicated study (Colangeli, unpubl. manuscript) also found similarities in number of full-sized seeds, percentage filled seed, and in germination rates between selfed and outcrossed seed.

It is unknown whether the similarities between selfed and outcrossed seed will hold true in western red cedar for a larger sample population. It is also not known whether any effects of inbreeding depression resulting from self-pollination will start to become apparent in a nursery environment and later in a field plantation environment. The purpose of the current preliminary study was to investigate these issues in a small number of families.

Self-pollinated and polycrossed maternal half-sib seedlings formed the sample population. Cone and seed attributes were monitored to determine whether they were related to subsequent growth. Seedling growth was represented by height, root collar diameter (RCD), and dry weight measurements. Seedling frost hardiness was investigated, and served as an example of an adaptive trait.

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4.2. METHODS

4.2.1. Seed Sources

During the spring of 1990, 23 potted six-year old western red cedar grafted clones growing at the B.C. Ministry of Forests CLRS (elevation: 200 m; latitude: 48°49' N; longitude: 124°10' W; mean annual frost-free period: 173 days; mean annual precipitation: 214.7 cm (Envir. Canada, pers. comm., 1994); biogeoclimatic variant: CWHxm2) were selected as maternal parents. Sixteen of these were from the relatively dry CDFmm, CWHxm, and CWHdm subzones (Table 4.1), with the remaining seven from the very wet CWHvh and CWHvm subzones (see Meidinger and Pojar, 1991 for details on the biogeoclimatic ecosystem classification of B.C.).

Each female was selfed by maintaining male and female strobili inside one pollination bag and outcrossed by injecting, with a hypodermic needle, a 10-clone polycross pollen mixture of unrelated genotypes into a second pollination bag on another branch in which all male strobili had been removed. A separate tester mix (Tester 1), where contributing pollen parents were from the same three subzones as the female parents, was used with females from the dry subzones than that used with the trees from the wet subzones (Tester 2). The latter tester was made up of pollen from the CWHvh, CWHvm, and CWHwh subzones (Table 4.2).

Seed was collected and sown into plug styroblock 415 containers on April 11, 1991 in a randomized complete block design. Seedlings were grown in a heated fiberglass greenhouse under a normal growing regime for this species. A subset of 100 seeds per female parent (herein referred to as family) and treatment (self vs polycross) were X-rayed; unfilled seeds were then separated out and the remaining

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Table 4.1. Maternal parents used in the selfing / outcrossing trials, classified by tester used in the polycross.

	Clone	BGC Subzone / Variant	Seed / Height	Dry Weight	Frost Hardiness
Tester 1 (dry)	181	CDFmm	x		
	398	CDFmm	x	x	x
	400	CDFmm	x		
	435	CDFmm	x		
	355	CWHxm1	x		
	432	CWHxm1	x	x	x
	438	CWHxm1	x		
	439	CWHxm1	x	x	x
	421	CWHxm2	x		x
	445	CWHxm2	x		
	518	CWHxm2	x		
	519	CWHxm2	x		
	198	CWHdm	x	x	x
	206	CWHdm	x		
	408	CWHdm	x		
	411	CWHdm	x		
Tester 2 (wet)	307	CWHvh1	x		
	312	CWHvh1	x		
	330	CWHvm1	x		
	341	CWHvm1	x		
	344	CWHvm1	x	x	x
	367	CWHvm1	x		
	486	CWHvm1	x		

Table 4.2. Pollen contributions of the two testers used.

	Clone	BGC Subzone / Variant	%
Tester 1 (dry)	441	CWHxm2	20.2
	200	CWHdm	15.1
	182	CDFmm	15.1
	431	CDFmm	15.1
	437	CDFmm	7.6
	514	CWHxm2	7.6
	180	CWHxm1	7.6
	520	CWHxm1	6.0
	436	CDFmm	3.8
	506	CWHxm2	1.9
	Total	CDFmm	41.6
		CWHxm2	29.7
		CWHxm1	13.6
		CWHdm	15.1
Tester 2 (wet)	271	CWHwh1	20.0
	166	CWHvm1	13.3
	293	CWHvm1	13.3
	413	CWHvm1	13.3
	174	CWHvm1	6.7
	337	CWHvm1	6.7
	453	CWHvm1	6.7
	487	CWHvm2	6.7
	466	CWHvh1	6.7
	493	CWHvh1	6.7
	Total	CWHvm1	60.0
		CWHvm2	6.7
		CWHvh1	13.3
		CWHwh1	20.0

filled seeds weighed.

4.2.2. Seedling Measurements

Seedling heights were taken repeatedly throughout the growing season on four seedlings per family / treatment combination in each of six blocks for the purposes of examining growth curves. Measurements were taken every two weeks, starting five weeks after sowing, until October 31, 1991, at which time growth had slowed considerably but not completely. Analysis was only carried out on the final height measurements taken near the end of the growing season, as it was deemed unnecessary to analyze heights on each test date, but growth curves were generated from the biweekly data. Root collar diameters were also taken at the end of the measurement period.

The choice of families to be used in tests involving subsets of families were limited by inconsistencies in the amount of available seed per family; more of the families from Tester 1 had surplus germinants for such studies. Dry weight measurements were taken in January 1992 on selfed and outcrossed offspring of a subset of five families (of which all but one were from Tester 1). Twenty-four seedlings were measured from each family / treatment combination. Samples were cut at the root collar, bagged, and dried in a convection oven at 100°C for 24 hours and then immediately weighed upon removal from the oven.

During the winter of 1991 / 92, cold hardiness testing was carried out on three separate dates on six families, all but one of which were from Tester 1. Four separate, nonbulked trees per family / treatment combination were tested at three temperatures

per test date. The electrical conductivity method (Glerum, 1985; also see Section 3.2.4.2 in Chapter 3) was used to assess relative cold hardiness. Freezing was done using a programmable freezing unit. Test dates were November 13, 1991, February 5, 1992, and March 17, 1992.

A second winter of frost testing involving eight selfed and polycrossed families was carried out during 1992 / 93. Seedlings had been transplanted to a site close to Jordan River on Vancouver Island, very close to the ocean at about 300 m elevation. Frost testing was done six times over the winter. Test dates were somewhat limited by an inability to access the site throughout the winter due to snowfall on unplowed secondary logging roads, making them impassable.

4.2.3. Data Analysis

Correlations were estimated between cone and seed data, between dry weight parameters, and between mean seed, final height, and dry weight data to determine to what extent certain traits were influenced by others, e.g. whether maternal effects of seed affected early growth, and whether biomass allocation shifted with changes in total weight. Due to nonrepliction of samples, only separate ANOVA's on cone and seed data could be done, where either treatment was used as the error term to test family effects, or where family was used as the error term to test treatment effects.

Analyses of variance were performed on tree height measurements and frost test data collected on all test dates and on dry weight measurements, using SAS® PROC GLM. In all tests, treatment effects were assumed to be fixed; family effects were treated as random where all families were tested (cone, seed, and growth) and

were treated as fixed where a subset of the families were tested (dry weight and frost), as members of these subsets were chosen for a particular reason and were not assumed to represent the total population. Tester was not included in the models for dry weight and frost injury, as only one family from each of these small data sets was from Tester 2. Tester and its interactions were not significant for height and RCD, so these terms were subsequently removed from the model. Replication (rep) interactions were lumped into the error term of the height and root collar diameter models, as they too were not significant. General forms of the linear models were:

$$S_{fmt} = \mu + M_m + F(M)_{f(m)} + \varepsilon_{(fm)t}$$

$$S_{tmf} = \mu + M_m + T(M)_{t(m)} + \varepsilon_{(tm)f}$$

$$Ht_{rtfn} = \mu + R_r + T_t + F_f + T{*}F_{tf} + \varepsilon_{(rtf)n}$$

$$Dw_{tfn} = \mu + T_t + F_f + T{*}F_{tf} + \varepsilon_{(tf)n}$$

$$It_{ctfn} = \mu + C_c + T_t + C{*}T_{ct} + F_f + C{*}F_{cf} + T{*}F_{tf} + C{*}T{*}F_{ctf} + \varepsilon_{(ctf)n}$$

where: S = cone and seed traits; Ht = seedling first year height (cm) and root collar diameter (mm); Dw = dry weight parameters; I_t = frost index of injury; R = replication; M = male tester; F = family; T = treatment; and C = temperature (°C)

Expected mean square equations for height and root collar diameter are given in Appendix 1.11. For frost testing, all factors were considered to be fixed, so the mean square error term was the approriate denominator for testing all factors.

4.3. RESULTS

4.3.1. Cone and Seed Traits

Table 4.3 lists the overall means and standard errors of measured seed, growth, and dry weight traits by treatment, and gives family ranges of traits. Maternal parents from the CWHvh and CWHvm subzones (Tester 2) appeared to have higher average values for all cone and seed traits, although this could not be tested due to nonreplication of samples. Families seemed to have large ranges for all of these traits except for percentage of female strobili (herein referred to as "flowers") maturing into cones, as most flowers did develop into a cone. Differences between treatments did not seem great for any of these parameters.

All cone and seed parameters were greater in Tester 2 than in Tester 1; however, these differences were found to be statistically significant only in percentage seedfill and in number of female flowers counted. Significant family differences were apparent in all cone and seed traits except percentage of female flowers maturing into cones (Table 4.4). No treatment differences occurred in any cone or seed trait. Seed weight and percentage filled seed were not correlated with each other; nor were they found to be correlated to the percentage of female flowers which developed into cones, or to the mean seedling height at the end of one year.

4.3.2. Seedling Growth: Height, Root Collar Diameter, and Dry Weight

Significant differences between families in final height and root collar diameter (P < 0.001) occurred (Table 4.4). However, there was no evidence of height

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Table 4.3. Treatment (self-pollinated vs polycrossed) means ± standard errors and
ranges of family means of studied first-year growth traits.

Trait	# families	Poly (± s.e.)	Self (± s.e.)	Family Range
#Female flowers	23	139.9 (14.01)	146.0 (15.01)	49 ... 272
% flowers/cones[1]	23	76.6 (5.59)	57.9 (6.53)	31.5 ... 100
Seeds/cone	23	· 10.8 (0.94)	10.0 (0.94)	4.9 ... 20.6
Seed fill %	23	33.5 (4.44)	33.7 (4.04)	7.0 ... 73.9
Seed weight (g)[2]	23	0.15 (0.007)	0.15 (0.009)	0.10 ... 0.20
Height (cm)	23	28.8 (0.24)	28.5 (0.24)	23.2 ... 33.2
RCD (mm)	23	3.65 (0.03)	3.60 (0.03)	3.19 ... 4.25
Shoot dry wt (g)	5	2.69 (0.063)	2.49 (0.059)	2.49 ... 2.72
Root dry wt (g)	5	1.33 (0.036)	1.19 (0.033)	1.15 ... 1.33
Total dry wt (g)	5	4.02 (0.093)	3.68 (0.088)	3.68 ... 4.05
Shoot/Root	5	2.11 (0.046)	2.17 (0.043)	2.14 ... 2.28

[1] % female flowers developing into fullsized cones was assumed to be an indication of cone
abortion rate, and seed fill % an indication of seed viability; # female flowers and
seed weight are maternal in nature, and are included here as an indication of initial
test conditions

[2] Seed weight is based on 100 seeds

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Table 4.4. Summary of significance levels found through analyses of variance, where *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Trait	# Families	Family	Treatment (S vs P)	Fam*Treat
Female flower	23	***	-	-
Female flower	23	-	ns	-
% flowers/cones	23	ns	-	-
% flowers/cones	23	-	ns	-
Seeds/cone	23	***	-	-
Seeds/cone	23	-	ns	-
Seed fill	23	*	-	-
Seed fill	23	-	ns	-
Seed weight	23	*	-	-
Seed weight	23	-	ns	-
Height, 1st year	23	***	ns	***
RCD, 1st year	23	***	ns	**
Shoot dry weight	5	ns	*	ns
Root dry weight	5	ns	**	*
Total dry weight	5	ns	**	ns
Shoot/Root ratio	5	ns	ns	***

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differences between selfed and cross-pollinated half-sib offspring from the same family. Growth curves of overall mean height per treatment were almost identical, although mean heights per treatment per family diverged slightly. Interactions involving family*treatment were significant in ANOVA's performed on all measurement dates, as selfs were larger than outcrosses in some families.

Family variance components for polycrosses were about 2/3 those of the selfs (Table 4.5). A Variance Ratio F test (Zar, 1984) showed that the ratio of the family variance components of selfs to polycrosses was significantly less than the expected 4:1 ratio for both height and root collar diameter. Assuming no dominance or inbreeding in the parental generation or in the polycrosses, it would be expected that the family variance components of the selfed progeny should be four times greater than those of the half-sib outcrosses (Namkoong, 1966; Wilcox, 1983). The same test showed that the family variance components for height and root collar diameter did not significantly differ between the two levels of inbreeding.

The heights of trees sampled for dry weight parameters were again found by ANOVA to significantly vary between families but not between self / polycross treatments. Root collar diameters of trees sampled for dry weights did not differ between either families or treatments. Treatment effects were not found in the shoot/root ratio, but were apparent in shoot, root, and total shoot + root dry weights (Figure 4.1). No family differences were noted in any dry weight parameter. Family*treatment interactions were significant in root dry weight and in the shoot/root ratio; in one out of five families for shoot, root and total dry weights (two out of five families for the shoot/root ratio), the average weight was greater in selfs than outcrosses, causing the interactions.

Table 4.5. Family variance components ± standard error, and % σ^2Family / σ^2Total for first year height and root collar diameters of selfed and polycrossed seedlings.

Trait	Treatment	σ^2F (± s.e.)	% σ^2F / σ^2T
Height	Polycross	4.478 (1.619)	14.56
	Self	6.590 (2.211)	20.86
Root collar diameter	Polycross	0.037 (0.017)	6.25
	Self	0.053 (0.021)	9.20
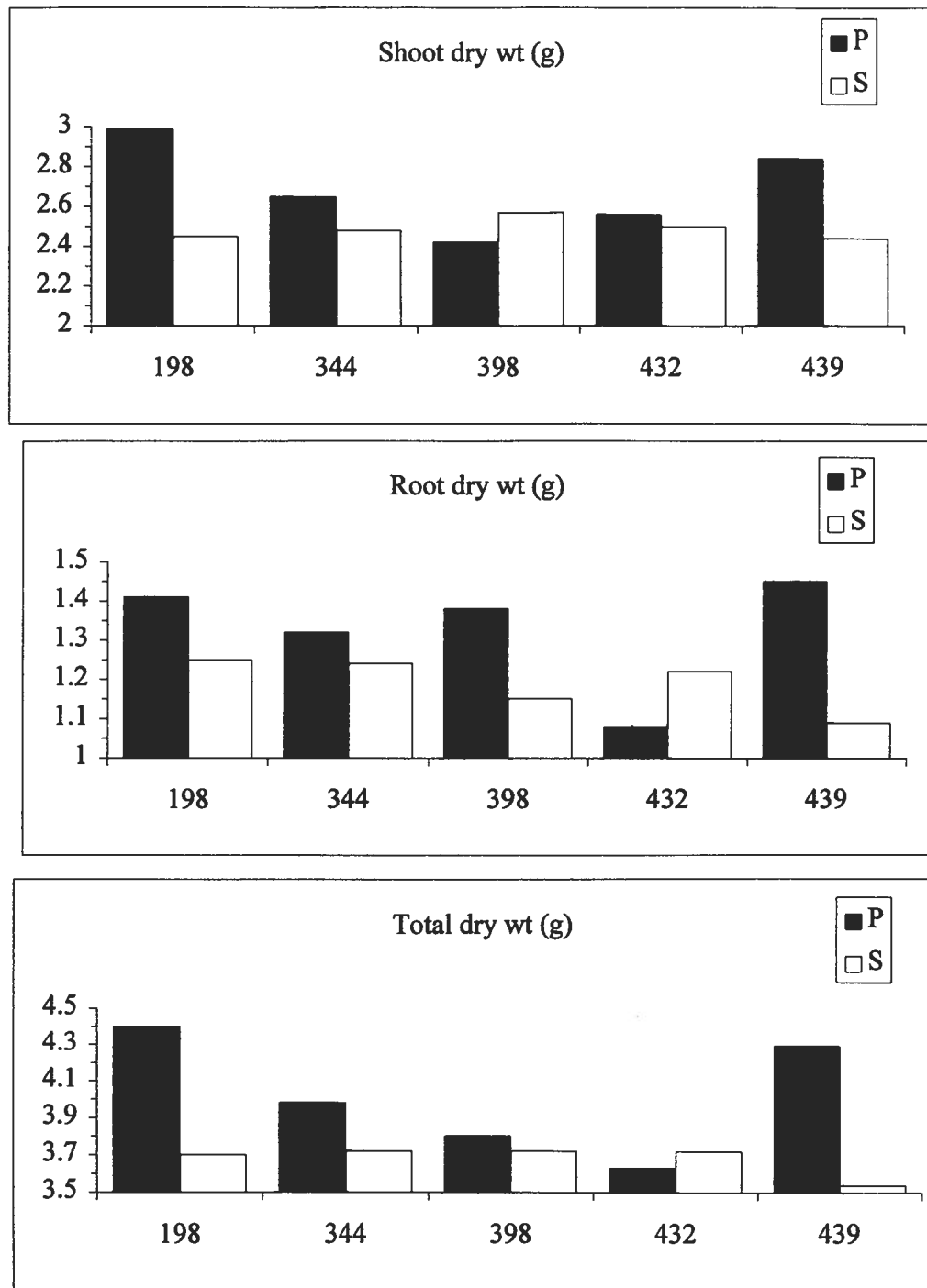
Figure 4.1. Mean shoot, root, and total dry weights per family for selfed vs polycrossed one-year old progeny.

All dry weight parameters were correlated to each other, and to the root collar diameter of trees sampled for dry weight, except for shoot dry weight with the shoot/root ratio. Seedling height of trees sampled for dry weight was correlated to the root collar diameter and to all dry weight measurements except for root dry weight. Correlations involving the shoot/root ratio were negative except with height. Table 4.6 shows results from analyzing each family individually for dry weight traits.

4.3.3. Frost Hardiness

A significant difference in the frost testing index of injury was found between families on all three test dates (Table 4.7). Treatment differences were not significant on November 13, 1991, but were significant on February 5, 1992 and March 17, 1992. It appeared that selfed trees were less hardy than the polycrosses from at least the time of maximum hardiness until trees had fully dehardened in the spring (Figure 4.2). Some families appeared to harden at different rates. Family by treatment interactions were significant in all tests; on the last two dates, selfs were more hardy than polycrosses in one family out of the six tested.

A second winter of frost testing involving eight selfed and polycrossed families showed even greater differences between the two treatments at maximum hardiness in the second season of testing, with polycrosses being significantly hardier than the selfs during the periods of maximum hardiness and dehardening (Figure 4.3).

Table 4.6. Significance levels of treatment (S vs P) found when separate ANOVA's of dry weight parameters were performed per family, where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Family	Shoot dw	Root dw	Shoot/Root	Total dw
198	*	ns	ns	*
344	ns	ns	ns	ns
398	ns	ns	**	ns
432	ns	ns	*	ns
439	ns	**	**	*

Table 4.7. ANOVA results of frost testing selfed and outcrossed seedlings growing at Cowichan Lake during 1991 / 92 (six families), and at Jordan River during the winter of 1992 / 93 (eight families), where: *** = P < 0.001; ** = 0.001 < P < 0.01; * = 0.01 < P < 0.05; ns = 0.05 < P.

Date	Fam	Treat	F*T	Temp (C)	F*C	T*C	F*T*C
Nov. 13/91	***	ns	*	***	**	ns	ns
Feb. 5/92	***	*	**	***	ns	*	ns
Mar. 17/92	***	***	*	***	ns	*	ns
Sept. 25/92	***	*	***	***	**	**	***
Oct. 2/92	ns	***	ns	***	ns	**	ns
Oct. 30/92	***	ns	***	***	ns	ns	*
Nov. 28/92	***	***	***	***	*	ns	ns
Mar. 3/93	***	***	***	***	***	***	ns
Apr. 7/93	***	*	*	***	*	**	ns
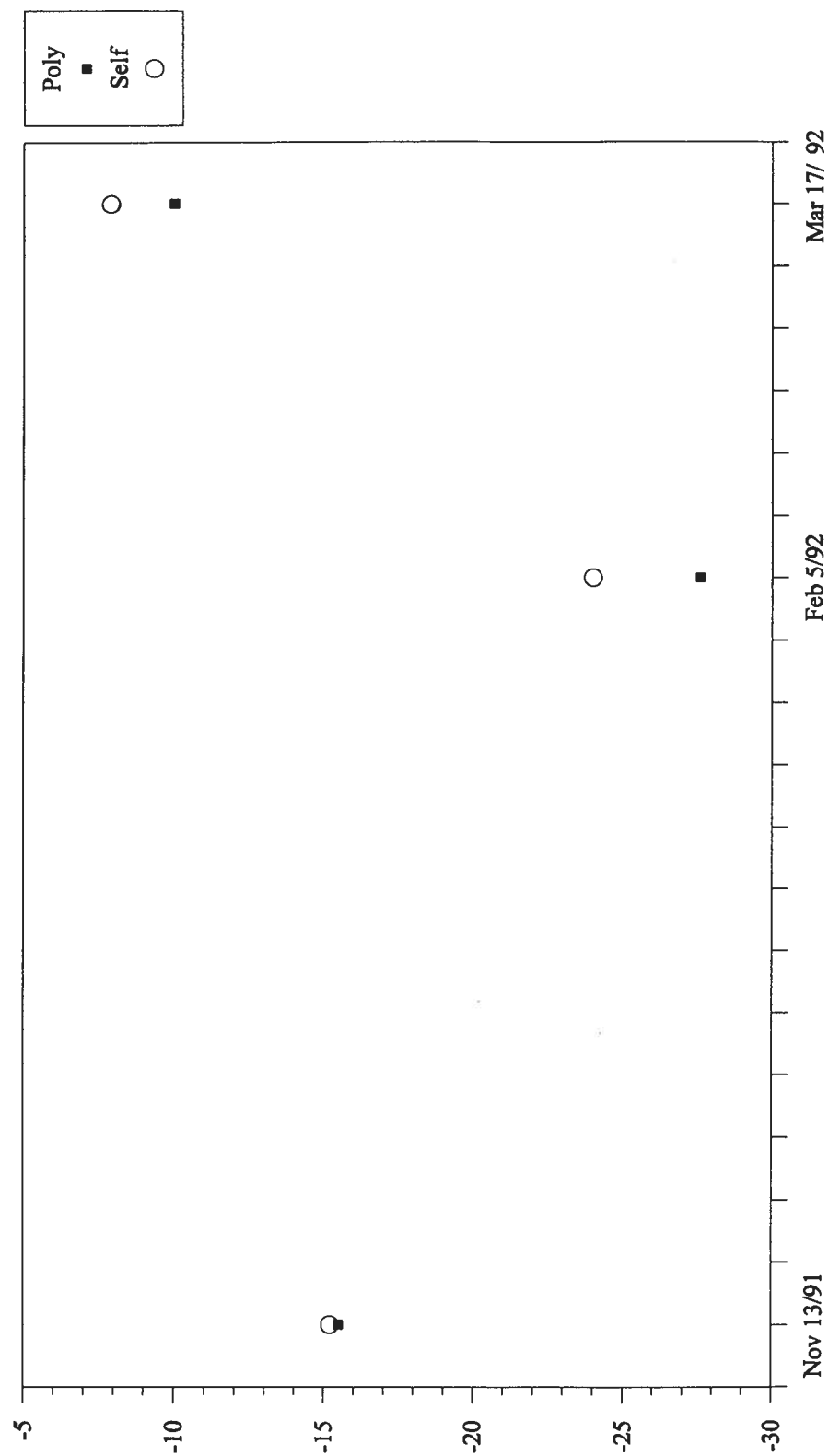
Figure 4.2.   Mean frost test LT_{50} for selfed vs polycrossed progeny during 1991 / 92.

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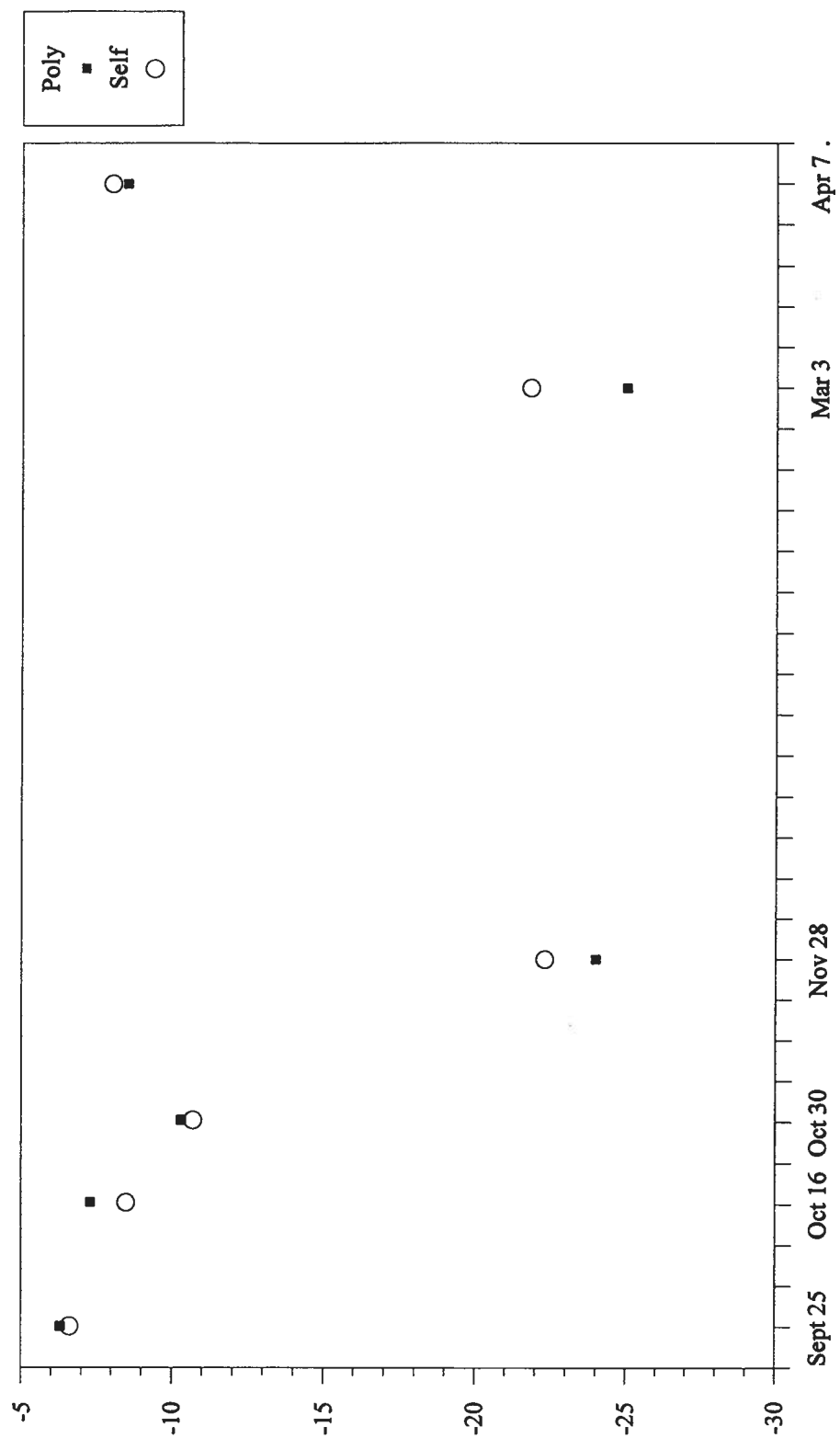
Figure 4.3. Mean frost test LT_{50} for selfed vs polycrossed progeny during 1992 / 93.

4.4. DISCUSSION

Family differences occurred in most of the traits studied: seed traits, seedling height, root collar diameter, and frost hardiness index of injury. Although provenance structure was not adhered to in the selfing study, these results concur (excepting seed traits) with the results of Chapter 2 and 3, involving a completely different set of families, in which height, RCD, and frost hardiness index of injury on most test dates varied at the family level. In both sets of families, dry weights were not found to differ at the family level. In traits where all selfed vs outcrossed families were measured, it appears that families from the wetter subzones (Tester 2) were superior; these environments are presumed to be the better sites for this species.

Greater family variance in polycrosses than expected was found when compared to selfs. The polycross family variance component should be an estimate of $1/4$ of the additive variance, while the selfed family variance component should be equal to all of the additive variance (plus $1/4$ of the dominance variance if dominance occurs). The discrepancy may partly be due to the inequality of contributions of the males in the two testers: in Tester 1, four of the ten males contributed about 65 % of the pollen, while in Tester 2, four out of ten males contributed about 60 % of the pollen. Thus the relationship between the cross-pollinated progeny of a female would be somewhat greater than that of half-sibs, possibly approaching that of full-sibs.

The significant difference between treatments in dry weight parameters compared to the nonsignificance of treatments in height and RCD might imply that wood specific gravity, bark thickness, or amount of foliage or branches differ between treatments, though these hypotheses would need to be tested.

For seed and early seedling growth traits, self-pollinated western red cedar offspring seemed to exhibit few signs of inbreeding depression when compared to cross-pollinated half-sib offspring of the same family, lending credence to the results of Owens *et al.* (1990). However, differences in frost hardiness which occurred after the first and second growing seasons, with polycrosses being hardier to lower temperatures, could be an indication that self-pollinated progeny may prove to have lower survival rates over time. It would also not be unanticipated if differences in seedling growth began to appear, with the outcrosses expected to begin outperforming the selfs; evidence for this was shown by the higher dry weight measurements of outcrossed seedlings compared to those of selfs.

It appears that western red cedar is a species which is self-fertile, but inherent detrimental effects due to inbreeding begin to be exhibited sometime later in the life cycle. This would parallel the findings of Perry and Knowles (1990), who found a lower than normal effect of inbreeding depression on embryo survival ($\sim 70\%$) of open-pollinated *Thuja occidentalis* seeds, while the proportion of heterozygotes in the parent stands did not deviate from Hardy-Weinberg expectations, leading the authors to speculate that much of the genetic load in this species may be expressed after germination and during development to a mature tree.

Other studies (e.g. Libby *et al.*, 1981; Kuittinen *et al.*, 1991; also see citations in Perry and Knowles, 1990 and Muona, 1990) have also suggested that the manifestation of inbreeding depression may be delayed until later in the life cycle of various coniferous species. One study (Sorensen and Miles, 1982) observed no detrimental effects on survival in three tree species while seedlings were growing under very favourable conditions in a nursery, but upon outplanting, severe

environmental conditions eliminated inbred seedlings prior to maturing. These theories need to be corroborated for western red cedar through more intensive studies of mature and young stands of this species, and also by obtaining results of the current study over future growing seasons.

Until the severity of latent inbreeding depression, if present as suspected, can be determined, along with the time frames when such depression may be manifested, it is suggested that the ability of western red cedar to produce viable self-pollinated seed not be interpreted to mean that inbreeding will not have any negative consequences in offspring. As with other species, care for now should be taken in seed orchards and breeding populations to avoid close inbreeding.

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5. CONCLUSIONS

It is evident that enough genetic variation in measured western red cedar seedling traits has been identified to negate the claim that little to no variability exists in this species. Heritabilities, while not absolute, are certainly within the realm of estimates made for other coniferous forest species. Typical narrow-sense individual heritabilities for growth traits of other coniferous species range from 0.1 to 0.4 (e.g. see Zobel and Talbert, 1984) and are most commonly between 0.2 to 0.3 (J.S. Brouard, pers. comm., 1994).

This study has disproven earlier inferences that western red cedar is almost or completely genetically depauperate in all traits (von Rudloff and Lapp, 1979; von Rudloff *et al.*, 1988; Copes, 1981; Yeh, 1988; Bower and Dunsworth, 1987). Much more heterogeneity has been found in growth and survival characteristics than that reported by Rehfeldt (1994), and elevational clines are much stronger for traits measured in this study than those found by Rehfeldt.

Seedling growth, as measured by height and root collar diameter, and seedling acclimation and deacclimation with respect to low temperatures predominantly exhibited within-population variation; also, about a third of sampled foliar nutrients exhibited variability at the family level. Variation between populations was evident in seedling dry weights, foliar nutrient levels, tree and foliage survival at an interior site, and in cold hardiness attributes when seedlings were experiencing the harshest conditions, the latter three traits which could be considered adaptive in nature and under the influence of selective pressure. Differences between the coastal and interior

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zones were observed in first year container plug heights, certain dry weight traits, cold acclimation and deacclimation, and in the final heights and amount of desiccation damage to foliage at the interior site after a severe winter.

From these studies, provenance differences appear to be most strongly influenced by elevation; later investigations may also find latitude to be of some importance as well, at least for some traits. Location differences were significant. Genotype by environment interactions were found at the zonal level in root collar diameter and seedling survival, at the provenance level for seedling survival and midwinter and spring hardiness, and at the family level for height after the 1991 growing season.

The provenances with the largest amounts of family variation were those of Vancouver Island. Although a small and unbalanced sample was used, when looking at all provenances found in a broad geographical region, it appears that the most between-population variation could be found in the B.C. interior, while the least was found in coastal northern B.C.

It may be speculated that more generations after the last glaciation have occurred in the southern interior than the northern coast, affecting inherent levels of variation through long term genetic mechanisms. However, a very generalized emerging pattern seems to be that where selective pressures are the most severe, between-population differences predominate, and within-population differences emerge where selective pressures are less critical.

As so few generations of western red cedar have occurred since the last glaciation, refugial populations probably retained residual levels of variation, because

it is unlikely that enough time has passed for populations to build up to the current levels of variability found within and between populations if the species was left nearly depauperate during the last ice age. It can be hypothesized that this species is probably in a state of constant change.

Early juvenile traits showed slight evidence of inbreeding depression due to selfing, albeit there are indications that inbreeding depression may be progressively expressed later in the life cycle of western red cedar. If delayed inbreeding depression is manifested, this would indicate the presence of dominance variance in such traits. However, this study has concentrated solely on the effects of additive gene action and no estimate of nonadditive genetic variation can be made. Should latent inbreeding depression be exhibited later in the life of this species, as is suspected, then an excess of homozygotes should not be found in the adult population, and the proportion of heterozygotes in mature stands should not deviate from Hardy-Weinberg expectations, assuming of course that all assumptions of Hardy-Weinberg equilibrium have been met.

The populations investigated in this study displayed phenotypic plasticity with respect to timing of growth, timing of acclimation and deacclimation, and maximum degree of hardiness attained per year (down to a limit, which appears to be genetically controlled). This opportunistic plasticity in the responses to timing of climatic events is to some degree a result of the indeterminate nature of this species, as energy is not redirected into forming an annual bud and bud flushing, and growth can continue as long as conditions are favourable.

The provenances which can be identified from this study as being generally favourable across all traits for which they were measured include Mt. Mara Low

elevation, Hope, and Mt. Benson. Benton Flat was only average for height, but was superior in other traits. No outstanding families based on height and frost hardiness results were obvious due to the inverse relationship of the two traits, although one of the families from Mt. Mara Low elev. (#4) did look promising.

It is strongly suggested that different breeding programs be initiated for coastal vs interior populations. Trees destined for coastal sites of lower elevations and adequate moisture need be less concerned with hardiness levels, and gains in height and volume can be made. Interior populations on the other hand should be also selected on their ability to withstand relatively harsh conditions, with average to better than average heights desired in these trees.

Recommendations for seed transfer are preliminary at best, as only two sites were used in this study. However, elevation and possibly latitude have been identified as being important in setting transfer limits. As only a sampling of populations were tested, the steepness of clines or spatial patterns of variation cannot be predicted with certainty from this data.

As substantial variation has been found to exist in quantitative seedling traits of western red cedar, the next step would be to better define responses at more than one location per zone. It is hoped that an even more widespread sampling of provenances and families be included in such testing.

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APPENDICES

APPENDIX 1. GENERAL EXPECTED MEAN SQUARE EQUATIONS

Appendix 1.1. Expected mean squares equations for analyses of variance of provenances without family structure for the following model:

$$Y_{rzpn} = \mu + R_r + Z_z + P(Z)_{p(z)} + R*P(Z)_{rp(z)} + \varepsilon_{(rzp)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Rep $= \sigma^2E + n\sigma^2RP(Z) + zpn\sigma^2R$
Zone $= \sigma^2E + n\sigma^2RP(Z) + m\sigma^2P(Z) + rpn\sigma^2Z$
Prov(Z) $= \sigma^2E + n\sigma^2RP(Z) + m\sigma^2P(Z)$
R*P(Z) $= \sigma^2E + n\sigma^2RP(Z)$
Error $= \sigma^2E$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = Z   E = P(Z)
H = R R*Z P(Z)   E = R*P(Z)

Appendix 1.2. Expected mean squares equations for analyses of variance of provenances with family structure for the following model:

$$Y_{rzpfn} = \mu + R_r + Z_z + P(Z)_{p(z)} + F(P\ Z)_{f(pz)} + R*F(P\ Z)_{rf(pz)} + \varepsilon_{(rzpf)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Rep	=	$\sigma^2E + n\sigma^2RF(PZ) + zpfn\sigma^2R$
Zone	=	$\sigma^2E + n\sigma^2RF(PZ) + rn\sigma^2F(PZ) + rfn\sigma^2P(Z) + rpfn\sigma^2Z$
Prov(Z)	=	$\sigma^2E + n\sigma^2RF(PZ) + rn\sigma^2F(PZ) + rfn\sigma^2P(Z)$
Fam(P Z)	=	$\sigma^2E + n\sigma^2RF(PZ) + rn\sigma^2F(PZ)$
R*F(P Z)	=	$\sigma^2E + n\sigma^2RF(PZ)$
Error	=	σ^2E

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = Z	E = P(Z)
H = P(Z)	E = F(P Z)
H = F(P Z) R	E = R*F(P Z)

Appendix 1.3.   Expected mean squares equations for analyses of variance of provenances without family structure for the following model:

$$Y_{lrzpn} = \mu + L_l + R(L)_{r(l)} + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + R*P(L\ Z)_{rp(lz)} + \varepsilon_{(lrzp)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

$$
\begin{aligned}
\text{Location} &= \sigma^2E + n\sigma^2RP(LZ) + m\sigma^2LP(Z) + zpn\sigma^2R(L) + rzpn\sigma^2L \\
\text{Rep(L)} &= \sigma^2E + n\sigma^2RP(LZ) + zpn\sigma^2R(L) \\
\text{Zone} &= \sigma^2E + n\sigma^2RP(LZ) + m\sigma^2LP(Z) + lmn\sigma^2P(Z) + rpn\sigma^2LZ + lrpn\sigma^2Z \\
\text{L*Z} &= \sigma^2E + n\sigma^2RP(LZ) + m\sigma^2LP(Z) + rpn\sigma^2LZ \\
\text{Prov(Z)} &= \sigma^2E + n\sigma^2RP(LZ) + m\sigma^2LP(Z) + lmn\sigma^2P(Z) \\
\text{L*P(Z)} &= \sigma^2E + n\sigma^2RP(LZ) + m\sigma^2LP(Z) \\
\text{R*P(L\ Z)} &= \sigma^2E + n\sigma^2RP(LZ) \\
\text{Error} &= \sigma^2E
\end{aligned}
$$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

$$
\begin{aligned}
H &= L*Z\ \ P(Z) & E &= L*P(Z) \\
H &= R(L)\ \ L*P(Z) & E &= R*P(L\ Z) \\
H &= L & E &= R(L) + L*P(Z) - R*P(L\ Z) \\
H &= Z & E &= L*Z + P(Z) - L*P(Z)
\end{aligned}
$$

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Appendix 1.4. Expected mean squares equations for analyses of variance of provenances with family structure for the following model:

$$Y_{lrzpfn} = \mu + L_l + R(L)_{r(l)} + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + F(P\ Z)_{f(pz)}$$
$$+ L*F(P\ Z)_{lf(pz)} + R*F(L\ P\ Z)_{rf(lpz)} + \varepsilon_{(lrzpf)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Location $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + rf n\sigma^2 LP(Z) + zpfn\sigma^2 R(L)$
$+ rzpfn\sigma^2 L$

Rep(L) $= \sigma^2 E + n\sigma^2 RF(LPZ) + zpfn\sigma^2 R(L)$

Zone $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + lm\sigma^2 F(PZ) + rf n\sigma^2 LP(Z)$
$+ lrfn\sigma^2 P(Z) + rpfn\sigma^2 LZ + lrpfn\sigma^2 Z$

L*Z $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + rf n\sigma^2 LP(Z) + rpfn\sigma^2 LZ$

Prov(Z) $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + lm\sigma^2 F(PZ) + rf n\sigma^2 LP(Z)$
$+ lrfn\sigma^2 P(Z)$

L*P(Z) $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + rf n\sigma^2 LP(Z)$

F(P Z) $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ) + lm\sigma^2 F(PZ)$

L*F(P Z) $= \sigma^2 E + n\sigma^2 RF(LPZ) + m\sigma^2 LF(PZ)$

R*F(L P Z) $= \sigma^2 E + n\sigma^2 RF(LPZ)$

Error $= \sigma^2 E$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = L*Z   E = L*P(Z)
H = L*P(Z) F(P Z)   E = L*F(P Z)
H = R(L) L*F(P Z)   E = R*F(L P Z)
H = L   E = R(L) + L*P(Z) - R*F(L P Z)
H = Z   E = L*Z + P(Z) - L*P(Z)
H = P(Z)   E = L*P(Z) + F(P Z) - L*F(P Z)

Appendix 1.5. Expected mean squares equations for analyses of variance of provenances without family structure for the following model:

$$Y_{rzp} \quad = \quad \mu + R_r + Z_z + P(Z)_{p(z)} + \varepsilon_{rp(z)}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Rep	=	$\sigma^2 E + zp\sigma^2 R$
Zone	=	$\sigma^2 E + r\sigma^2 P(Z) + rp\sigma^2 Z$
Prov(Z)	=	$\sigma^2 E + r\sigma^2 P(Z)$
Error	=	$\sigma^2 E$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = Z   E = P(Z)

Appendix 1.6.   Expected mean squares equations for analyses of variance of provenances with family structure for the following model:

$$Y_{rzpf} = \mu + R_r + Z_z + P(Z)_{p(z)} + F(P\ Z)_{f(pz)} + \varepsilon_{rf(pz)}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

$$
\begin{aligned}
\text{Rep} &= \sigma^2 E + f\sigma^2 RP(Z) + zpf\sigma^2 R \\
\text{Zone} &= \sigma^2 E + r\sigma^2 F(PZ) + rf\sigma^2 P(Z) + rpf\sigma^2 Z \\
\text{Prov(Z)} &= \sigma^2 E + r\sigma^2 F(PZ) + rf\sigma^2 P(Z) \\
\text{Fam(P Z)} &= \sigma^2 E + r\sigma^2 F(PZ) \\
\text{Error} &= \sigma^2 E
\end{aligned}
$$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

$$
\begin{aligned}
H &= Z & E &= P(Z) \\
H &= P(Z) & E &= F(P\ Z)
\end{aligned}
$$

Appendix 1.7. Expected mean squares equations for analyses of variance of provenances without family structure for the following model:

$$Y_{lrzpn} = \mu + L_l + R(L)_{r(l)} + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + \varepsilon_{(lrzp)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Location $= \sigma^2E + m\sigma^2LP(Z) + zpn\sigma^2R(L) + rzpn\sigma^2L$

Rep(L) $= \sigma^2E + zpn\sigma^2R(L)$

Zone $= \sigma^2E + m\sigma^2LP(Z) + lm\sigma^2P(Z) + rpn\sigma^2LZ + lrpn\sigma^2Z$

L*Z $= \sigma^2E + m\sigma^2LP(Z) + rpn\sigma^2LZ$

Prov(Z) $= \sigma^2E + m\sigma^2LP(Z) + lm\sigma^2P(Z)$

L*P(Z) $= \sigma^2E + m\sigma^2LP(Z)$

Error $= \sigma^2E$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = L*Z   P(Z)   E = L*P(Z)
H = L   E = R(L) + L*P(Z) - Error
H = Z   E = L*Z + P(Z) - L*P(Z)

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Appendix 1.8.   Expected mean squares equations for analyses of variance of provenances with family structure for the following model:

$$Y_{lrzpfn} = \mu + L_l + R(L)_{r(l)} + Z_z + L*Z_{lz} + P(Z)_{p(z)} + L*P(Z)_{lp(z)} + F(P\ Z)_{f(pz)} + L*F(P\ Z)_{lf(pz)} + \varepsilon_{(lrzpf)n}$$

- Zone is a fixed effect; all other factors are considered to be random effects.
- Rep(Location) interactions were lumped together.

Location	$= \sigma^2E + rn\sigma^2LF(PZ) + rfn\sigma^2LP(Z) + zpfn\sigma^2R(L) + rzpfn\sigma^2L$
Rep(L)	$= \sigma^2E + zpfn\sigma^2R(L)$
Zone	$= \sigma^2E + rn\sigma^2LF(PZ) + lrn\sigma^2F(PZ) + rfn\sigma^2LP(Z) + lrfn\sigma^2P(Z)$ $+ rpfn\sigma^2LZ + lrpfn\sigma^2Z$
L*Z	$= \sigma^2E + rn\sigma^2LF(PZ) + rfn\sigma^2LP(Z) + rpfn\sigma^2LZ$
Prov(Z)	$= \sigma^2E + rn\sigma^2LF(PZ) + lrn\sigma^2F(PZ) + rfn\sigma^2LP(Z) + lrfn\sigma^2P(Z)$
L*P(Z)	$= \sigma^2E + rn\sigma^2LF(PZ) + rfn\sigma^2LP(Z)$
F(P Z)	$= \sigma^2E + rn\sigma^2LF(PZ) + lrn\sigma^2F(PZ)$
L*F(P Z)	$= \sigma^2E + rn\sigma^2LF(PZ)$
Error	$= \sigma^2E$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = L*Z	E = L*P(Z)
H = L*P(Z) F(P Z)	E = L*F(P Z)
H = L	E = R(L) + L*P(Z) - Error
H = Z	E = L*Z + P(Z) - L*P(Z)
H = P(Z)	E = L*P(Z) + F(P Z) - L*F(P Z)

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Appendix 1.9.   Expected mean squares equations for frost test index of injury analyses of variance of all provenances at UBC for two years and at Skimikin, plus variable chlorophyll fluorescence tests, for the following model:

$$Y_{tzpn} = \mu + T_t + Z_z + T^*Z_{tz} + P(Z)_{p(z)} + T^*P(Z)_{tp(z)} + \varepsilon_{(tzp)n}$$

- Zone and temperature are fixed effects; all other factors are considered to be random effects.

Temp	=	$\sigma^2E + n\sigma^2TP(Z) + zpn\sigma^2T$
Zone	=	$\sigma^2E + tn\sigma^2P(Z) + tpn\sigma^2Z$
T*Z	=	$\sigma^2E + n\sigma^2TP(Z) + pn\sigma^2TZ$
Prov(Z)	=	$\sigma^2E + tn\sigma^2P(Z)$
T*P(Z)	=	$\sigma^2E + n\sigma^2TP(Z)$
Error	=	σ^2E

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = Z	E = P(Z)
H = T T*Z	E = T*P(Z)

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Appendix 1.10.   Expected mean squares equations for frost test index of injury analyses of variance of provenances having family structure at UBC for two years and at Skimikin for the following model:

$$Y_{tzpfn} = \mu + T_t + Z_z + T*Z_{tz} + P(Z)_{p(z)} + T*P(Z)_{tp(z)} + F(P\ Z)_{f(pz)} + T*F(P\ Z)_{tf(pz)} + \varepsilon_{(tzpf)n}$$

- Zone and temperature are fixed effects; all other factors are considered to be random effects.

$$
\begin{aligned}
\text{Temp} &= \sigma^2 E + n\sigma^2 TF(PZ) + fn\sigma^2 TP(Z) + zpfn\sigma^2 T \\
\text{Zone} &= \sigma^2 E + tn\sigma^2 F(PZ) + tfn\sigma^2 P(Z) + tpfn\sigma^2 Z \\
T*Z &= \sigma^2 E + n\sigma^2 TF(PZ) + fn\sigma^2 TP(Z) + pfn\sigma^2 TZ \\
\text{Prov}(Z) &= \sigma^2 E + tn\sigma^2 F(PZ) + tfn\sigma^2 P(Z) \\
T*P(Z) &= \sigma^2 E + n\sigma^2 TF(PZ) + fn\sigma^2 TP(Z) \\
\text{Fam}(P\ Z) &= \sigma^2 E + tn\sigma^2 F(PZ) \\
T*F(P\ Z) &= \sigma^2 E + n\sigma^2 TF(PZ) \\
\text{Error} &= \sigma^2 E
\end{aligned}
$$

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = Z	E = P(Z)
H = P(Z)	E = F(P Z)
H = T T*Z	E = T*P(Z)
H = T*P(Z)	E = T*F(P Z)

Appendix 1.11. Expected mean squares equations for analyses of variance of selfed vs polycrossed seedling height and root collar diameter for the following model:

$$Y_{rftn} = \mu + R_r + T_t + F_f + T^*F_{tf} + \varepsilon_{(tf)n}$$

- Treatment (selfed vs polycrossed) is a fixed effect; all other factors are considered to be random effects.
- Replication interactions were lumped together.

Rep	=	$\sigma^2E + tfn\sigma^2R$
Treat	=	$\sigma^2E + rn\sigma^2TF + rfn\sigma^2T$
Family	=	$\sigma^2E + rtn\sigma^2F$
T*F	=	$\sigma^2E + rn\sigma^2TF$
Error	=	σ^2E

The above expected mean squares equations were used to determine the appropriate error terms to be used in F tests and pseudo-F tests for each factor. Constructed F tests were as follows (H: hypothesis effects; E: error term):

H = T   E = T*F

Appendix 2. Mean Squares: Seed Traits

Appendix 2.1. Mean squares of seed traits for all provenances and for provenances having family structure, where zone is a fixed effect.

Trait	All provenances[1]		Provenances with family Structure[2]		
	Zone	Error	Zone	Prov(Z)	Error
Seed fill %	2,456.13**	380.65	2,658.72	709.71	347.06
Seed weight	48×10^{-8}***	4×10^{-8}	20×10^{-8}	7×10^{-8}	5×10^{-8}
Germination %	482.80	550.36	27.84	1,919.12***	352.08

[1] $Y_{zn} = \mu + Zone_z + \varepsilon_{(z)n}$

[2] $Y_{pzn} = \mu + Zone_z + Provenance(Z)_{p(z)} + \varepsilon_{(pz)n}$; where provenance was used as the error term to test zone effects

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Appendix 2.2. Mean squares by seed collection of seed traits for all provenances and for provenances having family structure, where collection is a fixed effect.

Trait	All provenances[1]		Provenances with family Structure[2]		
	Collection	Error	Collection	Prov(Coll)	Error
Seed fill %	1,440.23*	379.76	1,335.04	825.52*	347.06
Seed weight	30×10^{-8}**	4×10^{-8}	19×10^{-8}	6×10^{-8}	5×10^{-8}
Germination %	3,680.38***	421.22	2,245.57	1,335.99**	352.08

[1] $Y_{cn} = \mu + Collection_c + \varepsilon_{(c)n}$

[2] $Y_{pcn} = \mu + Collection_c + Provenance(C)_{p(c)} + \varepsilon_{(pc)n}$; where provenance was used as the error term to test collection effects.

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Appendix 2.3. Mean squares by region of seed traits for all provenances and for provenances having family structure, where region is a fixed effect.

Trait	All provenances[1]		Provenances with family Structure[2]		
	Region	Error	Region	Prov(Reg)	Error
Seed fill %	1,214.50**	353.84	1,657.57	530.10	347.06
Seed weight	19×10^{-8}***	4×10^{-8}	17×10^{-8}	5×10^{-8}	5×10^{-8}
Germination %	2,458.47***	386.53	2,451.89	1,030.28*	352.08

[1] $Y_{gn} = \mu + Region_g + \varepsilon_{(g)n}$

[2] $Y_{pgn} = \mu + Region_g + Provenance(Reg)_{p(g)} + \varepsilon_{(pg)n}$; where provenance was used as the error term to test region effects.

APPENDIX 3. MEAN SQUARES: HEIGHT AND ROOT COLLAR DIAMETER

Appendix 3.1.   Height and root collar diameter mean squares of provenances without family structure.

Trait	Age	Rep	Zone	Prov(Z)	R*P(Z)	Error
Height '90, plugs	1,014.01***	14.18	295.42*	52.14***	7.68***	4.66
Height '91, UBC	1,456.44***	668.94***	54.75	366.18***	57.44**	38.37
Height '92, UBC	4,281.91***	4,649.16***	1,402.44	1,142.19***	139.51	112.26
Height '91, Skim	1,304.53***	855.89***	2.71	175.06***	48.97	40.70
Height '92, Skim	717.39**	430.87***	773.98	184.62***	59.37	84.51
RCD, '92 UBC	77.03***	104.84***	45.48	11.76***	3.18*	2.28
RCD '92, Skim	39.85***	17.61***	10.36	6.72**	2.86	3.60

$Y_{rpzn} = \mu + \beta(Age) + Replication_r + Zone_z + Provenance(Z)_{p(z)} + R*P(Z)_{rp(z)} + \varepsilon_{(rpz)n}$

- Expected mean square equations are given in Appendix 1.1.

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Appendix 3.2. Height and root collar diameter mean squares of provenances having family structure.

Trait	Age	Rep	Zone	Prov(Z)	Fam(P Z)	R*F(P Z)	Error
Height '90, plugs	2,420.91***	34.26***	1,054.80*	205.09***	46.03***	8.21***	4.23
Height '91, UBC	2,677.61***	751.63***	920.18	1,001.18***	294.58***	85.06***	39.85
Height '92, UBC	3,829.07***	10,049.92***	2,586.46	3,013.20*	1,011.70***	295.75***	119.85
Height '91, Skim	3,447.28***	1,175.40***	15.12	392.84*	162.75***	52.87**	40.39
Height '92, Skim	762.44**	540.40***	3,014.03**	243.15	131.55*	86.46	83.62
RCD, '92 UBC	162.99***	231.50***	64.48	15.53	10.38***	4.40***	2.45
RCD '92, Skim	50.20***	9.58	42.81	16.44	7.78*	4.54***	3.05

$Y_{rfpzn} = \mu + \beta(Age) + Replication_r + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{ft(pz)} + R*F(P\ Z)_{rft(pz)} + \varepsilon_{(rfpz)n}$

- Expected mean square equations are given in Appendix 1.2.

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Appendix 3.3. Mean squares of seedling height at the end of the 1991 growing season and root collar diameter at the end of the 1992 growing season at two locations on provenances without family structure.

	Age	Location	Rep(L)	Zone	L*Z	Prov(Z)	L*P(Z)	R*P(LZ)	Error
Height '91	2,759.49***	27,123.66***	762.64***	34.32	16.97	465.39***	66.60	53.17**	39.49
RCD '92	116.87***	31.94	61.48***	1.27	36.23**	14.58***	3.17	3.03	2.77

$Y_{lrzpn} = \mu + \beta(Age) + Location_l + Replication(L)_{r(l)} + Zone_z + L*Z_{lz} + Provenance(Z)_{p(z)} + L*P(Z)_{lp(z)} + R*P(L\,Z)_{rp(lz)} + \varepsilon_{(lrzp)n}$

- Expected mean square equations are given in Appendix 1.3.

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Appendix 3.4. Mean squares of seedling height at the end of the 1991 growing season and root collar diameter at the end of the 1992 growing season at two locations on provenances having family structure.

Age	Location	Rep(L)	Zone	L*Z	Prov(Z)	L*P(Z)	Fam(P Z)	L*F(P Z)	R*F(L P Z)	Error
Height '91										
6,092.47***	49,115.89***	965.72***	564.92	269.55	1,191.54*	154.77	338.53***	111.17*	68.94***	40.11
RCD '92										
213.17***	93.60	120.86***	9.82	160.30**	19.88	11.44	8.92	6.48	4.47***	2.61

$Y_{lrzpfn} = \mu + \beta(Age) + Location_l + Replication(L)_{r(l)} + Zone_z + L*Z_{lz} + Provenance(Z)_{p(z)} + L*P(Z)_{lp(z)} + Family(P Z)_{f(pz)}$
$+ L*F(P Z)_{lf(pz)} + R*F(L P Z)_{rf(lpz)} + \varepsilon_{(lrzpf)n}$

- Expected mean square equations are given in Appendix 1.4.

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Appendix 3.5.   Mean squares of seedling height at the end of the 1992 growing season at UBC on provenances analyzed individually.

Provenance	Rep	Family	R*F	Error
Quinsam	823.96	1,166.88*	351.54***	105.23
Tofino	1,849.72***	1,232.82**	219.75***	94.22
Mill Bay	859.64*	4,437.61***	270.46**	128.26
Cheakamus	1,217.09*	822.42	304.44**	145.19
Hope	1,809.86**	390.65	327.28**	140.14
Mt. Mara Low	1,875.95	391.35	690.71***	135.35
Mt. Mara Mid	1,955.40***	575.24	219.85	133.78
Mt. Mara High	121.79	93.96	67.62	124.47
Benton Flat	1,341.66**	357.43	198.62*	99.90

$Y_{rfn} = \mu + Replication_r + Family_f + R*F_{rf} + \varepsilon_{(lrzp)n}$; where R*F was used as the error term to test Rep and Family

Appendix 3.6. Mean squares of seedling root collar diameter at the end of the 1992 growing season at UBC on provenances analyzed individually.

Provenance	Rep	Family	R*F	Error
Quinsam	27.96**	30.23**	4.22*	2.41
Tofino	37.10***	2.06	4.25*	2.31
Mill Bay	27.25**	12.75	5.05*	2.54
Cheakamus	39.19***	21.01**	4.64	2.90
Hope	34.60***	1.58	4.30	3.06
Mt. Mara Low	39.94**	3.04	7.54***	2.63
Mt. Mara Mid	24.07***	9.11*	2.70	2.33
Mt. Mara High	1.96	0.34	1.35	3.58
Benton Flat	25.44**	6.39	3.70*	2.10

$Y_{rfn} = \mu + Replication_r + Family_f + R*F_{rf} + \varepsilon_{(frzp)n}$; where R*F was used as the error term to test Rep and Family

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APPENDIX 4. MEAN SQUARES: DRY WEIGHTS

Appendix 4.1. Dry weight trait mean squares of provenances without family structure.

Trait	Rep	Zone	Prov(Z)	R*P(Z)	Error
Stem wt	109.72***	86.34	47.53***	8.12	7.80
Foliar wt	502.83***	2,674.84**	134.58**	34.00	36.49
Shoot wt	1,079.18***	3,722.35***	210.44**	62.84	73.22
Root wt	17.92	229.61**	14.42	8.22	5.63
Total wt	1,332.13***	5,800.96**	308.18**	102.93	113.08
Shoot/Root	9.39***	3.64	2.12*	0.97**	0.50
Shoot/Total	0.04***	0.01	0.008*	0.004***	0.002
Stem/Total	0.002	0.15	0.03***	0.004***	0.001
# Lateral branches	129.92***	105.22*	15.39	13.07*	8.30

$Y_{rpzn} = \mu + Replication_r + Zone_z + Provenance(Z)_{p(z)} + R*P(Z)_{rp(z)} + \varepsilon_{(rpz)n}$

- Expected mean square equations are given in Appendix 1.1.

Appendix 4.2.   Dry weight trait mean squares of provenances having family structure.

Trait	Rep	Zone	Prov(Z)	Fam(P Z)	R*F(P Z)	Error
Stem wt	126.60***	143.34	67.24***	14.46	12.42***	7.42
Foliar wt	476.23***	1,454.00	352.96***	66.64	45.46*	35.08
Shoot wt	1,079.96***	2,510.40	585.70***	129.68	102.01*	69.78
Root wt	8.05	151.06	36.61**	11.60	12.28***	7.06
Total wt	1,168.61***	3,893.06	828.88**	194.04	162.83**	110.88
Shoot/Root	10.02***	5.36	4.26**	0.99	0.67**	0.42
Shoot/Total	0.04***	0.01	0.02****	0.003	0.003***	0.002
Stem/Total	0.008**	0.02	0.02***	0.003*	0.002*	0.001
# Lateral branches	72.13***	25.92	21.17	12.88	11.12**	6.81

$Y_{rfpzn} = \mu + Replication_r + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{f(pz)} + R*F(P\ Z)_{rf(pz)} + \varepsilon_{(rfpz)n}$

- Expected mean square equations are given in Appendix 1.2.

Appendix 4.3. Branch angle mean squares of provenances without family structure and those having family structure, where zone is a fixed effect.

Provenances without family structure[1]				Provenances with family structure[2]			
Zone	Prov(Z)	Error		Zone	Prov(Z)	Fam(P Z)	Error
45.36	83.69	43.18		35.31	44.74	138.25	80.51

[1] $Y_{pzn} = \mu + Zone_z + Provenance(Z)_{p(z)} + \varepsilon_{(pz)n}$; where provenance was used as the error term to test zone effects

[2] $Y_{fpzn} = \mu + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{f(pz)} + \varepsilon_{(fpz)n}$; where provenance was used as the error term to test zone effects, and family was used as the error term to test provenance effects

APPENDIX 5. MEAN SQUARES: GROUPING BY GEOGRAPHIC REGION

Appendix 5.1.   Mean squares of UBC seedling traits of all provenances grouped by region.

Trait	Rep	Reg	Prov(Reg)	R*P(Reg)	Error
Height '92	6,319.58***	4,560.33	2,398.91***	235.48**	157.07
RCD '92	132.35***	67.14*	18.87***	4.16**	2.87
Stem dry wt	237.46***	201.01**	42.43***	11.65*	8.83
Foliar dry wt	1,044.64***	1,316.82**	251.55***	43.88	40.24
Shoot dry wt	2,264.03***	2,240.93**	418.20***	90.76	80.87
Root dry wt	14.39	161.93**	29.68**	10.77*	7.65
Total dry wt	2,490.64***	3,583.18***	616.32***	145.38	126.54
Shoot / Root	23.93***	3.33	3.12***	0.81**	0.54
Shoot / Total	0.11***	0.009	0.01***	0.003***	0.002
Stem / Total	0.009**	0.08**	0.01***	0.002***	0.001

$Y_{rpgn} = \mu + \text{Replication}_r + \text{Region}_g + \text{Provenance(Reg)}_{p(g)} + \text{R*P(Reg)}_{rp(g)} + \varepsilon_{(rpg)n}$

- Expected mean square equations are given in Appendix 1.1, substituting Region for Zone.

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APPENDIX 6. MEAN SQUARES: FOLIAR NUTRIENT ANALYSIS

Appendix 6.1. Mean squares of foliar nutrient content for all provenances and for provenances having family structure, where zone is a fixed effect.

Nutrient	All provenances[1]			Provenances with family structure[2]			
	Zone	Prov(Z)	Error	Zone	Prov(Z)	Fam(P Z)	Error
%N	0.003	0.041*	0.015	0.021	0.068	0.037**	0.011
%P	0.001	0.003***	0.0002	0.003	0.002**	0.0002	0.0003
%K	0.0007	0.130***	0.017	0.008	0.248*	0.047**	0.014
%Ca	0.008	0.058**	0.012	0.004	0.088*	0.020	0.009
%Mg	0.008	0.016****	0.032	0.0002	0.001	0.0006	0.0004
%Mn	11.57	2,201.41**	418.09	289.00	439.61	704.75	361.03
ppm Fe	262.24	653.91	383.61	46.69	524.25	914.64*	318.92
ppm Cu	9.71	16.60*	6.05	1.48	24.85	6.61	5.49
ppm Zn	208.07	151.26*	45.17	87.11	26.89	31.06	23.86
P/N	0.0005	0.003***	0.0004	0.0004	0.005*	0.0007	0.0004
K/N	0.007	0.160***	0.011	0.035	0.297***	0.011	0.013
K/Ca	0.012	0.412***	0.051	0.003	0.796*	0.119*	0.045

[1] $Y_{pzn} = \mu + Zone_z + Provenance(Z)_{p(z)} + \varepsilon_{(pz)n}$; where provenance was used as the error term to test zone effects

[2] $Y_{fpzn} = \mu + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{f(pz)} + \varepsilon_{(fpz)n}$; where provenance was used as the error term to test zone effects, and family was used as the error term to test provenance effects

APPENDIX 7. MEAN SQUARES: SURVIVAL TRAITS

Appendix 7.1. Survival trait mean squares of provenances without family structure.

Trait	Rep	Zone	Prov(Z)	Error
% Planted '90/Alive '92, UBC	9.79	403.14	343.40*	146.58
% Planted '90/Alive '92, Skimikin	1,807.39*	250.43	822.32	623.43
% Alive '91/Alive '92, Skimikin	2,275.57*	174.10	857.95	703.79
# Live '92, Skimikin	9.77**	0.02	4.67	2.49

$Y_{rpz} = \mu + \text{Replication}_r + \text{Zone}_z + \text{Provenance}(Z)_{p(z)} + \varepsilon_{rp(z)}$

- Expected mean square equations are given in Appendix 1.5.

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Appendix 7.2.   Survival trait mean squares of provenances having family structure.

Trait	Rep	Zone	Prov(Z)	Fam(P Z)	Error
% Planted '90/Alive '92, UBC	13.90	837.97**	68.92	136.57	105.76
% Planted '90/Alive '92, Skimikin	3,196.16***	23,098.78	5,181.87***	299.00	411.75
% Alive '91/Alive '92, Skimikin	3,305.92***	24,007.43	5,286.28***	330.75	409.10
# Alive '92, Skimikin	15.56***	75.05	27.60***	1.74	2.38

$Y_{rfpz} = \mu + Replication_r + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{f(pz)} + \varepsilon_{rf(pz)}$

- Expected mean square equations are given in Appendix 1.6.

Appendix 7.3. Skimikin foliar desiccation damage mean squares of provenances without family structure.

Rep	Zone	Prov(Z)	R*P(Z)	Error
2,955.21**	590.06	2,117.89**	731.84	519.99

$Y_{rpzn} = \mu + \text{Replication}_r + \text{Zone}_z + \text{Provenance(Z)}_{p(z)} + \text{R*P(Z)}_{rp(z)} + \varepsilon_{(rpz)n}$

- Expected mean square equations are given in Appendix 1.1.

Appendix 7.4. Skimikin foliar desiccation damage mean squares of provenances having family structure.

Rep	Zone	Prov(Z)	Fam(P Z)	R*F(P Z)	Error
6,333.64***	150,352.17*	14,988.88***	1,286.46**	739.27	621.79

$Y_{rfpz} = \mu + Replication_r + Zone_z + Provenance(Z)_{p(z)} + Family(P\ Z)_{f(pz)} + R*F(P\ Z)_{rf(pz)} + \varepsilon_{rf(pz)}$

- Expected mean square equations are given in Appendix 1.2.

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Appendix 7.5. Mean squares of percentage of seedlings planted in 1990 still alive after 1992 at two locations on provenances without family structure.

Location	Rep(L)	Zone	L*Z	Prov(Z)	L*P(Z)	Error
73,757.78***	908.59*	645.54	10.12	677.32	482.41	388.36

$Y_{lrzpn} = \mu + Location_l + Replication(L)_{r(l)} + Zone_z + L*Z_{lz} + Provenance(Z)_{p(z)} + L*P(Z)_{lp(z)} + \varepsilon_{(lrzp)n}$

- Expected mean square equations are given in Appendix 1.7.

Appendix 7.6. Mean squares of percentage of seedlings planted in 1990 still alive after 1992 at two locations on provenances having family structure.

Location	Rep(L)	Zone	L*Z	Prov(Z)	L*P(Z)	Fam(P Z)	L*F(P Z)	Error
242,183.18***	1,605.03***	7,650.45	16,449.34*	2,553.07	2,811.43***	272.16	163.15	258.28

$$Y_{lrzpfn} = \mu + \text{Location}_l + \text{Replication(L)}_{r(l)} + \text{Zone}_z + L*Z_{lz} + \text{Provenance}(Z)_{p(z)} + L*P(Z)_{lp(z)} + \text{Family}(P\ Z)_{f(pz)} + L*F(P\ Z)_{lf(pz)} + \varepsilon_{(lrzpfn)}$$

- Expected mean square equations are given in Appendix 1.8.