

POPULATION DIFFERENCES IN WATER-USE EFFICIENCY FOR *PINUS CONTORTA*
DOUGL. AS INDICATED BY STABLE CARBON ISOTOPIC COMPOSITION

by

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Abstract

Stable carbon isotopic composition ($\delta^{13}\text{C}$), an index of water-use efficiency (WUE), was used to test genetic and environmental responses of *Pinus contorta* Dougl. populations to moisture gradients. To gain insight into patterns of natural selection for high or low WUE, trends in $\delta^{13}\text{C}$ were measured in genotypes from different habitats. Correlations between $\delta^{13}\text{C}$ and productivity were tested. Sixteen-year-old *P. contorta* provenance trials at three climatically different sites in British Columbia were sampled. To determine whether early selection for WUE was possible in *P. contorta*, greenhouse grown seedling shoots from the same seedlots as the saplings were analyzed. Correlations among seedling $\delta^{13}\text{C}$, growth variables and yield were tested as well.

A sampling technique to accurately reflect field $\delta^{13}\text{C}$ was determined by assessing $\delta^{13}\text{C}$ variation in foliage and wood for five open-grown *P. contorta* saplings at one site (Juliet Creek, latitude 121° 00' N, longitude 49° 43' W, elevation 1010-1067 m). Variation in $\delta^{13}\text{C}$ within and among trees was smaller in wood than needles. Paired north and south aspects (wood) appeared to accurately track $\delta^{13}\text{C}$ year to year variation. Therefore, field $\delta^{13}\text{C}$ was determined for whole wood stem cores spanning ten years growth from the north and south sides of trees. The cores were taken at stump level to avoid missing years. Depending on the site, ten or eleven populations representing a wide range of habitats (from 49° 26' to 59° 59' N latitude and 114° 25' to 132° 58' W longitude) were sampled.

There were genetic differences in $\delta^{13}\text{C}$ among populations. Differences were related to provenance (habitat of origin), temperature and precipitation. A particular population from the wet, maritime Pacific coast (*P. contorta* var. *contorta*) stood out from the others, which were all from the continental interior (*P. contorta* var. *latifolia*). There were no genetic differences in plasticity among populations. Indicated relative WUE increased progressively from the wettest to the driest trial site. The magnitude and direction of this increase was similar in all populations.

The relationship between $\delta^{13}\text{C}$ and biomass increment in controlled environments as well as in nature, was not clear. High relative WUE was related to low or high yield, depending on the population, its growing conditions, and its physiological and morphological attributes. However, correlating mean population yield at each trial site with mean $\delta^{13}\text{C}$ showed a positive correlation between high productivity and high WUE. To understand differences within and among populations, it was apparent that the physiological and morphological bases for high productivity must be measured.

Of the seedling provenances, only the coastal one had a different relative WUE. Favorable growth conditions may have nullified expression of genetic differences. In *P. contorta*, it appeared that early selection for WUE may be possible. Mean isotopic compositions of saplings and greenhouse grown first year seedling shoots from the same seedlots were positively correlated.

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List of Abbreviations

A/E	photosynthetic carbon assimilation rate/transpiration rate (<i>i.e.</i> , instantaneous water-use efficiency)
A/g	photosynthetic carbon assimilation rate/stomatal conductance (<i>i.e.</i> , intrinsic water-use efficiency)
C ₃	plant species for which the first product of photosynthesis is a 3-carbon molecule
D _c	diffusivity of CO ₂
D _e	diffusivity of water vapor
e _i	water vapor pressure inside the leaf
e _a	ambient water vapor pressure outside the leaf
g _c	stomatal conductance to CO ₂
g _w	stomatal conductance to water vapor
kPa	kilopascal
LSD	least significant difference
LSM	least squares mean
map	mean annual precipitation
mat	mean annual temperature
mpdm	mean precipitation of the driest month
msp	mean summer precipitation
mtwm	mean temperature of the warmest month
MPa	megapascal
PAR	photosynthetically active radiation
PDB	fossil Pee Dee belemnite
p _i /p _a	intercellular partial pressure of CO ₂ /ambient partial pressure of CO ₂
PPFD	photosynthetic photon flux density
R	molar abundance ratio ¹³ C/ ¹² C
r _a	boundary layer resistance to diffusion

r_m	mesophyll resistance to diffusion
r_s	stomatal resistance to diffusion
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
WUE	water-use efficiency
Δ	isotope discrimination
Δc	leaf-to-air concentration difference for CO ₂
Δe	leaf-to-air concentration difference for water vapor
$\delta^{13}\text{C}$	stable carbon isotope abundance variable
ϕ_c	portion of carbon fixed that is lost to respiration
ϕ_w	non-productive water loss due to incomplete stomatal closure, cuticular openings and evaporation from the soil
v	water vapor pressure difference between the inside and outside of the leaf
‰	per mil (parts per thousand)

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1.0 Introduction

The term, water-use efficiency (WUE), relates plant production to water consumption. An indicator of plant performance, WUE is used to study genetic differences in mechanisms that maximize performance in arid environments.

The concept of water use economy is not new. Drought limits vegetation growth, distribution and yield more than any other environmental factor (Kramer 1983). Plant breeders, concerned with improved harvests under arid conditions, focus on breeding for drought resistance rather than increased WUE, *i.e.*, gaining higher yields from given amounts of water (Tesar 1984). In agriculture, WUE is desired because it is perhaps the foremost yield component (Martin and Thorstenson 1988). Although short-term yield usually declines with increased WUE, total yield per given land tract will increase if water supply limits growth, *i.e.*, growth is maintained for a longer time (Fisher and Turner 1978). In cases where water is non-limiting, however, high WUE may restrict yield (Meinzer *et al.* 1990, Condon *et al.* 1990).

Compared with agricultural crops, we know little about genetic variation in WUE or its physiological basis in woody plants. We do not know the circumstances under which efficient water use confers competitive advantage or promotes acceptable growth rates in trees. We have yet to examine the effect of drought and the magnitude of genetic, and genotype x environment interactions as sources of variation in WUE.

Water-use efficiency varies intra-specifically in *Populus* L. (Blake *et al.* 1984), *Pinus ponderosa* Laws. (Monson and Grant 1989) and *Pseudotsuga menziesii* (Mirb.) Franco (Smit and van den Driessche 1992). *Populus* studies (Dickmann *et al.* 1992) show that the relationship between dry-weight increment and high WUE is not clear. Monson and Grant (1989) and Eickmeier *et al.* (1975) have used WUE in *Pinus ponderosa* and *Tsuga canadensis* (L.) Carr, respectively, as measures of differential adaptation to moisture-limited habitats. DeLucia *et al.* (1988, 1989) show that high WUE in *Pinus ponderosa* and *Pinus*

jeffreyi Grev. & Balf. may be a competitive disadvantage in water-limited environments. In desert soils, *Pinus* seedlings cannot effectively compete with the shrub species. The shrubs' relatively high water-use rates deplete the soil water, thus restricting the pines to areas with greater water availability.

In agriculture, stable carbon isotope analysis effectively screens crops for variation in WUE (Farquhar *et al.* 1989b). Recently the application of this technique to shrubs and trees has burgeoned (Read and Farquhar 1991; Fu *et al.* 1992a, b; Jackson *et al.* 1992; Leavitt and Danzer 1992; Livingston 1992; Newberry 1992; Zhang *et al.* 1993). The isotopic composition of a plant is typically expressed as the abundance ratio $^{13}\text{C}/^{12}\text{C}$ relative to a standard and is called $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ of a plant changes due to discrimination against $^{13}\text{CO}_2$ over $^{12}\text{CO}_2$ during photosynthetic carboxylation and gaseous diffusion. Isotopic composition or $\delta^{13}\text{C}$ of plant tissue is positively related to WUE. The reasons for this are well understood. Stomatal closure causes increased instantaneous WUE since the photosynthetic rate is reduced proportionally less than the transpiration rate. At the same time, stomatal closure decreases the partial pressure of CO_2 in the intercellular air spaces (p_i). Discrimination against $^{13}\text{CO}_2$ decreases with low p_i , thereby increasing $\delta^{13}\text{C}$. Thus both seasonal WUE and $\delta^{13}\text{C}$ increase as a result of stomatal closure in response to drought. The relationship between $\delta^{13}\text{C}$ and WUE would be similar also if CO_2 fixation rate and p_i were affected by photosynthetic disturbances of the mesophyll rather than by stomatal behavior.

This study uses $\delta^{13}\text{C}$ (an integrated index of WUE) to determine ecotypic variation in WUE for *Pinus contorta* Dougl. The species has broad edaphic and climatic tolerances, and grows in a wide range of moisture-limited habitats. Based on Rehfeldt's research (1986, 1987) supporting "specialization" as the species' mode for survival, I expect *P. contorta* populations to be specialized in WUE along moisture gradients. Specialization will be evident in "ecotypes" arising in response to local conditions. To accept that such ecotypes exist, differences in WUE must be demonstrated as heritable by appearing when plants from across the species' range are grown in "common gardens" (Clausen *et al.* 1948).

Research objectives:

1. To develop sampling methods for field trials in this project, as well as for future projects;
2. To use $\delta^{13}\text{C}$ to test for genetic variation in WUE among seedling and sapling *P. contorta* provenances;
3. To correlate $\delta^{13}\text{C}$ of seedlings with shoot, root and total weight, as well as shoot/root ratio; and to correlate $\delta^{13}\text{C}$ of saplings with diameter, height and volume;
4. To correlate $\delta^{13}\text{C}$ of seedlings with that of saplings grown from the same seed source;
5. To correlate $\delta^{13}\text{C}$ of saplings with provenance climate variables: mean temperature of the warmest month, mean annual temperature, mean summer precipitation, mean precipitation of the driest month, mean annual precipitation; and
6. To assess the plasticity of populations by examining their ranked $\delta^{13}\text{C}$ values among field sites distinct in available moisture.

2.0 Literature Review

2.1 Definition of Water-Use Efficiency

In agriculture or ecosystem science, WUE is defined as the ratio of yield or net primary production to evapotranspiration (Fisher and Turner 1978; Gardner *et al.* 1985). Yield is expressed as economic yield (*e.g.*, grain or seed, forage in grasses) or dry weight of the whole plant, including roots. The plant parts used depend on the experimenter's objectives. If roots or other vegetative organs are not included, the WUE will be lower than if the entire plant is the basis for measurement. Use of evapotranspiration gives increased variation in WUE, since evaporation is influenced by leaf cover and frequency of soil wetting, independently of transpiration (Tanner 1981). The definition which follows is the most useful in agronomy (Kramer 1983):

$$\text{WUE} = \frac{\text{Dry matter or Crop Yield (g)}}{\text{Water used in evapotranspiration (kg)}} \quad (1)$$

Physiologists often discuss WUE in terms of gas exchange, expressing it as net CO₂ uptake per unit of transpired water (Fisher and Turner 1978). Simply defined, at the level of a single leaf:

$$\text{WUE} = \frac{\text{Net carbon dioxide uptake (mmol)}}{\text{Water used in transpiration (mol)}} \quad (2)$$

More recently, Hubick *et al.* (1988) have used the term "transpiration efficiency" as well as water-use efficiency to discuss dry matter production and water use. They distinguish the terms according to type of water loss. In the single plant, transpiration efficiency is the ratio of moles of carbon in the plant to moles of water used to accumulate that carbon. Water-use efficiency, assigned to determinations in the field, is the ratio of dry matter to total water use including losses such as soil evaporation and runoff.

Water-use efficiency is determined by biophysical processes such as CO₂ and water vapor gradients between leaf and air, stomatal opening, boundary layer resistance and mesophyll resistance. These effects are accounted for in different ways by Fisher and Turner (1978), Nobel (1980), Osmond *et al.* (1980) and Farquhar *et al.* (1989b). Water loss accompanies CO₂ uptake since water vapor diffuses out of a leaf by the same route that CO₂ diffuses in. For a given stomatal resistance (r_s), more water will be lost than CO₂ acquired. The concentration gradient of water vapor is usually greater than that of CO₂; water vapor has a higher diffusion coefficient than CO₂; and CO₂ diffusion is hindered by an added resistance (r_m or mesophyll resistance) not encountered by water vapor (Nobel 1983). The two gas-exchange processes are:

$$\text{Assimilation} = \frac{(\text{Conc. CO}_{2\text{ext.}} - \text{Conc. CO}_{2\text{int.}}) \times \text{diffusivity}}{r_a + r_s + r_m} \quad (3)$$

$$\text{Transpiration} = \frac{(\text{Conc. H}_2\text{O}_{\text{int.}} - \text{Conc. H}_2\text{O}_{\text{ext.}}) \times \text{diffusivity}}{r_a + r_s} \quad (4)$$

The equations can be combined to give (Fisher and Turner 1978):

$$\text{WUE} = \frac{\Delta c \times D_c (r_a + r_s)}{\Delta e \times D_e (r_a + r_s + r_m)} \quad (5)$$

where Δc and Δe are the leaf-to-air concentration differences for CO₂ and water vapor, respectively; D_c and D_e are the diffusivities of CO₂ and water vapor, respectively; and r_a , r_s and r_m are the boundary layer, stomatal and internal resistances to diffusion, respectively. Gas exchange through the cuticle is ignored.

Researchers interpret mesophyll resistance differently. For many (Nobel 1983), mesophyll resistance represents the diffusion pathway from the substomatal cavity to the chloroplast, and from inside the chloroplast to the site of fixation at ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). For others (Jones 1976; Farquhar *et al.*

1982b), mesophyll resistance comprises several components, of which the diffusion pathway is only minor since the physical distance between the cell surface and a chloroplast is very small. Carboxylation resistance is more important. Kramer (1983) states that in practice, r_m includes biochemical limitations on CO_2 fixation as well as physical limitations on diffusion since r_m for CO_2 is calculated as the difference between $(r_a + r_s)$ and the total resistance to CO_2 uptake.

Farquhar *et al.* (1989b) use the terms "conductance" and "partial pressure" to define WUE. [Conductance, or the reciprocal of resistance, refers to ease of flow (m s^{-1}) of gaseous species through a medium or material]. They begin with an approximate expression for the ratio of instantaneous carbon assimilation rate (A) and transpiration rate (E):

$$A/E = g_c(p_a - p_i) / g_w(e_i - e_a) \quad (6)$$

$$= (p_a - p_i) / 1.6v \quad (7)$$

$$= p_a(1 - p_i/p_a) / 1.6v \quad (8)$$

where g_c and g_w are conductances to CO_2 and water vapor, respectively; p_a and p_i are partial pressures of CO_2 in the atmosphere and inside the leaf, respectively; and e_i and e_a are the water vapor pressures inside and outside the leaf, v being the difference between them. The ratio of the diffusivities of water vapor and CO_2 in air equals 1.6.

Using the ratio, moles of carbon in the plant to moles of water transpired during the growth period, water-use efficiency is defined as:

$$\text{WUE} = (1 - \phi_c) p_a(1 - p_i/p_a) / 1.6v \quad (9)$$

where ϕ_c is the portion of carbon fixed during the day that is lost to respiration by the leaf at night and by other plant parts over the whole period.

Equations (5) and (9) summarize the important environmental and genotypic influences on WUE. Through its effect on v , vapor pressure deficit (air) alters WUE. In single leaves WUE decreases with increasing v , although the opposite may occur in whole plants (Rawson *et al.* 1977). Vapor pressure deficit changes daily, seasonally and regionally,

depending on temperature and absolute humidity. Air temperature acts on WUE via its effect on v . Since v is usually closely coupled to air temperature in the field (Schulze *et al.* 1973), increased air temperature reduces WUE unless leaf temperature is suboptimal for photosynthesis (Miller and Mooney 1974).

Incident irradiance is important, due to the non-linear relationship between photosynthesis and transpiration. Transpiration is always positive, showing an upward linear or curvilinear response with increasing light due to rising leaf temperature. Stomatal conductance may or may not increase. Net photosynthesis plateaus with increased light (reflecting saturation kinetics). Accordingly, there is an optimum irradiance for maximum WUE (Bierhuizen and Slatyer 1965; Downes 1970; Jones 1976).

The leaf traits – boundary layer, stomatal conductance and biochemical capacity for fixation of carbon dioxide – influence WUE (see Fisher's and Turner's 1978 review). Comparisons between and within genotypes must take place under the same ambient conditions, and genotype x environment interactions must be considered.

2.2 Physiology of *Pinus contorta*

Pinus contorta is one of the most researched conifers in the Pacific northwest, yet relatively little is known of its basic physiology (Bassman 1985). Few studies concern its tolerances to crucial, limiting environmental factors such as air and soil temperature, soil moisture, relative humidity and nutrients.

On the other hand, the species has been a frequent subject for ecophysiological studies trying to explain distributional patterns of overstory conifers along elevational and geographic gradients in montane forests. Its physiological characteristics have been compared with those of other conifers. Having reviewed earlier work, Lopushinsky (1975) and Smith (1985) characterize the water relations for the species: *P. contorta* shows moderate to relatively high transpiration rates, largely decreasing with soil drought; stomatal closure occurs at a relatively high leaf water potential; the sapwood layer is relatively thick,

conferring large stem water storage capacity; drought resistance is moderate – less than that for *P. ponderosa*; the species shows high tolerance to saturated soils.

Photosynthetic data are hard to interpret, since they are measured under various circumstances and expressed in different units. Brix (1979) compared photosynthesis in *Pseudotsuga menziesii*, *Picea glauca* (Moench) Voss, *Tsuga heterophylla* (Rafn.) Sarg. and *Pinus contorta*. He measured gas exchange on 4-month-old seedlings (greenhouse grown), following 2 months acclimation in a growth room. *Pinus contorta* had relatively high photosynthesis per unit leaf area but the lowest rate per unit leaf dry weight. As well, photosynthesis in *P. contorta* declined most steeply with drying soil and at a higher threshold soil water potential than the other species. Dykstra (1974) observed that 2-year-old *P. contorta* seedlings (acclimated for 2 months in a greenhouse) attained maximum net photosynthetic values of about $6.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Light saturation occurred at close to 400 W m^{-2} of PAR. The temperature optimum for photosynthesis was near 20°C , with a four-fold reduction occurring at about 2°C . Photosynthesis decreased almost linearly from maximum values to zero when xylem pressure potential decreased from -0.3 MPa to -1.5 MPa . Mesophyll resistance to CO_2 uptake was greater than r_s under all conditions, especially drought. Dykstra included carboxylation resistance in r_m ; by implication, WUE would have decreased with drought. Photosynthetic rate increased proportionally with decreases in r_m and r_s .

Sweet and Wareing (1968) reported intraspecific variation in net photosynthesis for 12 and 19-week-old *P. contorta*. Photosynthetic rates for the 12-week-old seedlings were in the order: California-Oregon coast (var. *contorta*) > Rocky Mountains, Alberta (var. *latifolia*) > Cascade Mountains, Oregon (var. *murrayana/latifolia*) > Washington coast (var. *contorta*). Ranking changed in the 19-week-old seedlings with different leaf morphology. The authors cautioned that large differences in photosynthetic rates might not reflect field performance, since seedling photosynthetic rate, provenance and growth conditions often interacted. Variation among provenance photosynthetic rates could not be related to climate or location

of the seed source (a finding common to studies using non-stressed growing conditions). Correlations with climate/geography have been shown under extreme conditions (Bourdeau 1963).

Changes in leaf-to-air water vapor pressure difference (v) account for much of the stomatal regulation in *P. contorta* (Kaufmann 1982); as well, stomatal response is controlled by light intensity. Sandford and Jarvis (1986) observed large variation in stomatal response to v in current year needles of 3-year-old seedlings of *Picea sitchensis* (Bong.) Carr., *Larix x eurolepis* Mill., *Pinus sylvestris* L. and *Pinus contorta*. All species' stomates partly closed as v was increased from 0.4 to 2.0 kPa; *P. contorta* ranked second in degree of stomatal closure. Only the stomates of *P. contorta* and *L. x eurolepis* behaved optimally (Cowan 1986), maximizing the exchange rate of CO₂ for water during transpiration as v increased, *i.e.*, change in assimilation/change in transpiration remained constant as v increased. Murdiyaroy *et al.* (1985) found no significant differences in transpiration and stomatal conductance among seven provenances of non-stressed 2-year-old *P. contorta*. After drought stressing newly grown seedlings from three of the provenances, they found significant provenance x treatment interactions in stomatal response.

On exposed early-successional sites, *Pinus contorta* saplings had photosynthetic gains similar to *Abies lasiocarpa* (Hook.) Nutt. and *Picea engelmannii* Parry, but with more efficient water use (Carter and Smith 1988). The pines' transpiration decreased greatly with progression from shaded to open habitats. The authors noted that all three species had remarkable stomatal and photosynthetic acclimation responses to habitat exposure.

2.3 Intraspecific Variation in WUE for Forest Tree Species

Although considerable work has been done on genetically based, intraspecific variation in photosynthetic traits and water loss rate, little has been done on variation in WUE. Blake *et al.* (1984) found genetic variation in WUE for *Populus* genotypes from various countries. Some clones showed high dry matter production combined with high

WUE. Differences in WUE were related to stomatal resistance (accounting for 40% of the variation), foliar adaptations such as cuticular ledges or hairs above pore openings, time of stomatal opening in the morning, and stomatal size and frequency. There was no evidence of a particular physiological or morphological variable which would consistently explain improved WUE in every case.

Large population differences may occur where dissimilar environments exert intense selection pressure (Turesson 1922a, 1923). Eickmeier *et al.* (1975) suggested that *Tsuga canadensis* has at least two physiological races correlated with environment. Carbon dioxide and water vapor exchange, stomatal resistance, WUE and morphology varied in ecologically meaningful ways in first year seedlings from two Wisconsin seed sources. Water-use efficiency was measured as carbon dioxide to water vapor flux ratio.

Monson and Grant (1989) found definitive heritable differences in stomatal conductance, maximum photosynthetic rate and intrinsic WUE (measured as p_i/p_a) between *Pinus ponderosa* progeny from two genetic lines. The higher WUEs in families derived from a coastal x interior cross were manifested in lower p_i/p_a values and lower stomatal conductances for any given photosynthetic rate. Results suggested that *P. ponderosa* had achieved improved WUE and lower transpiration rates in drier habitats, at the expense of reduced maximum photosynthetic rates.

Smit and van den Driessche (1992) compared WUE in *Pseudotsuga menziesii* and *Pinus contorta* seedlings. Four provenances were tested – one from a wet and one from a dry site for each species. One-year-old seedlings were planted in wet and dry soil treatments and grown outside for 20 weeks. *Pseudotsuga menziesii* had higher WUE than *Pinus contorta*. *Pseudotsuga menziesii* from the dry site was more water-use efficient than *P. menziesii* from the wet, but there was little difference between *Pinus contorta* provenances. Reasons given for *P. contorta*'s lack of response were that origin sites were not sufficiently different or the dry soil treatment was not dry enough. Root exploitation of soil water, not WUE, determined productivity differences between the species. *Pinus contorta*, with lower

WUE, produced more dry matter than *Pseudotsuga menziesii*. *Pinus contorta* seedlings used water in their root environment more completely than the *Pseudotsuga menziesii*.

2.4 Intraspecific Variation and Ecotypic Differentiation

The concept of ecotypic differentiation developed after Turesson's classic studies (1922a, b) on *Atriplex* L. species. In some cases, diverse forms among groups of individuals in different environments were directly induced by environment, *i.e.*, there was phenotypic plasticity. But in most cases, they were the result of genetic variation among populations. The inherited characteristics were related to distinct native habitats. Believing that "habitat types" arose from genotypic responses to particular environments, Turesson called them "ecotypes". The underlying evolutionary process became known as "ecotypic differentiation".

Subsequently, investigators have established that ecotypic differentiation must be mainly responsible for the ability of some species to occupy a wide range of habitats. Ecotypic differences exist with changes in altitude, latitude and precipitation and as a result of soil type, nutrient availability and metal toxicity. As major habitat factors differ spatially in a random or non-random manner, so can ecotypic variation.

2.5 The Relationship of WUE to the Ecology of *Pinus contorta*

Pinus contorta's features make it an ideal species for studying "adaptedness" – the extent to which individuals are physiologically attuned to their environment (Rehfeldt 1987). It is the most widely distributed conifer in western North America, growing in a variety of sites and in all but the most adverse conditions. Its range, centered in British Columbia, spans 33 degrees latitude, 35 degrees longitude and more than 3900 m elevation (Wheeler and Critchfield 1985).

Three distinct, interfertile, geographic races have evolved, each characterized by morphological, physiological and biochemical traits different from the others. These

geographic races and affiliated regions are:

1. *P. contorta* var. *contorta*: Pacific coast, from Alaska to northern California;
2. *P. contorta* var. *murrayana*: southern Cascades, Sierra Nevada range and the mountains of southern Baja California;
3. *P. contorta* var. *latifolia*: Rocky Mountain and Intermountain regions, northern Cascades, Washington; eastern outliers from northern Alberta to South Dakota.

The basis for the species' large distribution is the existence of specialized populations for small parts of the species' range. In seedling studies, Rehfeldt (1983, 1986, 1987, 1989) demonstrated that through natural selection, traits have evolved to overcome ecological problems such as changeable frost-free periods, insect infestations and disease epidemics. Research has established within- and among-population variance in drought resistance; however genetic variation in WUE has hardly been tested.

2.6 Stable Carbon Isotope Discrimination and WUE

Introduction

Plant adaptation and acclimation responses to water shortage have received increasing attention in recent years. During the last decade, agricultural research on WUE has increased greatly with the development of stable carbon isotope analysis as an effective screening tool for variation in WUE (Hubick *et al.* 1988). The goal of this research has been to select for improved growth in water-limited environments.

History

The fundamental terms, processes, effects and theory of carbon isotope fractionation or discrimination by plants are discussed by Hayes (1982), Farquhar *et al.* (1982b, 1984, 1989a, b), O'Leary (1981, 1988) and O'Leary *et al.* (1992). Stable carbon isotope composition was initially used to indicate photosynthetic pathways in plants (Bender 1968).

Isotopic surveys have provided information on the distribution of photosynthetic pathways among various phylogenetic groups and ecological zones (O'Leary 1981, 1988). Early studies have shown substantial variation in isotopic composition at interspecific and intraspecific levels, as well as variation associated with different environmental growth conditions and dry-matter composition (reviewed by Farquhar *et al.* 1989a).

Units of Measurement

There are four isotopes of carbon – ^{14}C , ^{13}C , ^{12}C and ^{11}C . The stable isotopes are ^{13}C and ^{12}C ; ^{14}C and ^{11}C are unstable, *i.e.*, "radioactive". Terrestrial carbon is 98.9% ^{12}C and 1.1% ^{13}C . Green plants contain relatively less ^{13}C than does air, showing their preferential use of $^{12}\text{CO}_2$ over $^{13}\text{CO}_2$. Variation in the $^{13}\text{C}/^{12}\text{C}$ ratio (R) of plants is due to "isotope effects" or fractionations, expressed during the formation and destruction of carbon bonds or as a result of processes affected by mass, such as gaseous diffusion. Isotope effects are not observable but isotopic compositions are altered by them, and from this we infer they exist. Isotopic compositions ($\delta^{13}\text{C}$), expressed as "del" values (δ), are conveyed in per mil (‰), *i.e.*, parts per thousand. Carbon isotopic composition is calculated relative to the historic standard, fossil Pee Dee belemnite (PDB):

$$\delta^{13}\text{C} = \frac{[\text{R}(\text{Sample}) - \text{R}(\text{Standard})]}{[\text{R}(\text{Standard})]} \times 10^3 \quad (10)$$

Isotope effects, expressed as isotope discrimination (Δ), are calculated from the difference in the $\delta^{13}\text{C}$ value between source and product:

$$\Delta = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}}{1 + \delta^{13}\text{C}_{\text{plant}}/1000} \quad (11)$$

The advantage of reporting Δ rather than δ is that it directly expresses the consequences of biological processes. It is important where isotopic composition of the

source air changes due to anthropogenic factors or when interpreting results from canopies where an isotopic gradient exists with height (Vogel 1978).

Since Δ values are usually positive while those for δ are negative (when PDB is the reference), confusion can result. Therefore some authors use molar abundance ratios (R) and compositional deviations (δ) as intermediates in determining final isotope effects.

Carbon isotopic composition (δ), rather than Δ is reported throughout the thesis. The $\delta^{13}\text{C}$ values are the primary data which can be converted to Δ , knowing the $\delta^{13}\text{C}$ of source air. I did not measure $\delta^{13}\text{C}_{\text{air}}$ for the field sites tested or for the greenhouse where the seedlings were grown. Measuring $\delta^{13}\text{C}_{\text{air}}$ introduces its own sampling error into the calculation of Δ . Substituting $\delta^{13}\text{C}_{\text{air}}$ reported in the literature (-8.0 ‰) for $\delta^{13}\text{C}_{\text{air}}$ in my study would have given inaccurate measures of Δ for seedlings grown in the greenhouse.

Theory

Plants discriminate against ^{13}C during photosynthesis. In 1982(b), Farquhar *et al.* predicted this discrimination would be least in plants (C_3) which fixed the most carbon per unit of water transpired, *i.e.*, in those that maintained the greatest WUE. This theory was confirmed, in part, from $\delta^{13}\text{C}$ values and short term gas exchange measurements of leaves from contrasting species (Farquhar *et al.* 1982a). In 1984, Farquhar and Richards confirmed the theory fully by isotope analysis of *Triticum aestivum* L. genotypes, for which dry matter accumulation and water use had been measured over the long term. Plants with high WUE had large ratios of ^{13}C to ^{12}C , meaning that isotope effects were smaller or that the plants discriminated less against the heavier isotope, ^{13}C . These results suggested that carbon isotope analysis could be a tool for seeking differences in WUE and for genotype selection in breeding programs for increased yield under drought.

Current theory states that variation in WUE occurs because WUE is partly dependent on the ratio of CO_2 partial pressure inside the leaf to CO_2 partial pressure in the atmosphere (p_i/p_a). This ratio varies and the variation can be seen in the isotopic composition of a plant.

Since the p_i/p_a ratio varies, we expect a large degree of genetic variation in the physiological component of whole plant WUE. The nature of inheritance in p_i/p_a is largely unknown, except that it is not under simple genetic control (Farquhar *et al.* 1989a). Isotopic discrimination is likely a polygenic trait as well (Farquhar *et al.* 1989a). The genetic control of Δ appears strong in *Arachis hypogaea* (Hubick *et al.* 1988) and in *Triticum aestivum* (Condon *et al.* 1987).

To increase WUE, a leaf must decrease p_i/p_a through increased photosynthetic capacity or decreased stomatal conductance. By rewriting equation (7) to give equation (8), Farquhar *et al.* (1989a) emphasize that a smaller value of p_i/p_a leads to an increase in A/E (transpiration efficiency). A simple model for discrimination in leaves of C_3 plants is:

$$\Delta = a + (b - a)p_i/p_a - d, \quad (12)$$

where "a" is the fractionation of carbon isotopes due to diffusion in air (4.4 ‰) and "b" the net fractionation due to carboxylation (27.0 ‰). The "d" represents minor isotopic effects caused by dissolution of CO_2 , liquid phase diffusion, respiration and other processes not yet quantified (Masle and Farquhar 1988). Since "a", "b" and "d" are largely constant, Δ may be a surrogate measure for p_i/p_a . In experiments relating gas exchange properties to short and long term Δ (with v kept constant), p_i/p_a is positively correlated with Δ (Evans *et al.* 1986).

During whole plant growth, carbon and water losses must be accounted for, requiring another equation. A portion (ϕ_c) of the carbon fixed via the stomates during the day is lost in respiration. Some water is lost from the plant independently of CO_2 uptake, due to incomplete stomatal closure and cuticular openings. Unavoidable evaporative soil water loss occurs as well. If this "nonproductive" water loss is a portion, ϕ_w , of "productive" water loss, the A/E equation can be changed to describe the molar ratio of carbon gain by a plant, to water lost:

$$WUE = \frac{p_a (1 - p_i/p_a) (1 - \phi_c)}{1.6v (1 + \phi_w)} \quad (13)$$

This equation, combined with equation (12), predicts a negative linear dependence of WUE on Δ :

$$\text{WUE} = \frac{p_a \frac{(b - d - \Delta)}{(b - a)} [(1 - \phi_c)]}{1.6v (1 + \phi_w)} \quad (14)$$

Results from pot experiments, using various watering treatments and genotypes, fit the theory well for a number of species – *Triticum aestivum* (Farquhar and Richards 1984; Masle and Farquhar 1988), *Arachis hypogaea* L. (Hubick *et al.* 1986, 1988; Wright *et al.* 1988), *Lycopersicon esculentum* Mill. (Martin and Thorstenson 1988), *Coffea arabica* L. (Meinzer *et al.* 1990) and *Vigna unguiculata* (L.) Walp. (Hall *et al.* 1990).

2.7 Carbon Isotope Discrimination and Plant Growth Characteristics

Although Hubick *et al.* (1988) observed a negative relationship between dry matter production and Δ of field grown *Arachis hypogaea* cultivars, Condon *et al.* (1987) found a positive relationship in *Triticum aestivum* cultivars receiving greater than usual rainfall. Clearly, associations between Δ and carbon partitioning are important. If strains with larger p_i/p_a partition more carbon to the shoot, then selecting for large Δ may result in increased yield when water is not limiting. Masle and Farquhar (1988) examined relations between individual growth components and Δ in *T. aestivum*. The grain was grown in a soil resistant to root penetration. Resistance was increased by augmenting soil density or by lowering soil water content. Stomatal conductance and shoot growth responses were similar regardless of which method was used. Masle and Farquhar (1988) found a negative relationship between "partitioning ratio" (total plant carbon/leaf area) as well as root/shoot ratio and Δ . Although the relationship was not stable across environments, the authors suggested that it might have contributed to the positive relationship between plant mass and Δ reported by Condon *et al.* (1987). Relative growth rate and Δ correlated positively with decreasing soil resistance (as Δ increased, there was a relatively greater decrease in partitioning ratio than in CO_2

assimilation rate). Assimilate partitioning, as well as leaf gas-exchange properties must be considered when interpreting the relationship between plant growth and Δ .

2.8 Environmental Effects on Carbon Isotope Discrimination

Light

Field studies have reported an increase in $\delta^{13}\text{C}_{\text{plant}}$ with increased irradiance (Farquhar *et al.* 1989a). It is debatable whether, in the field, carbon isotopic composition of leaves in different light levels can be interpreted. It is hard to separate effects of light on Δ from correlated effects of source air ($\delta^{13}\text{C}_{\text{air}}$), both of which affect leaf carbon isotopic composition (Farquhar *et al.* 1989a).

Vogel (1978) found a pattern of isotopic variation in canopy leaves, where light varied substantially. Foliar $\delta^{13}\text{C}$ decreased by 3.0 ‰ between the top (19 m) and bottom (1 m) of the canopy. The $\delta^{13}\text{C}$ of soil CO_2 was approximately -19.0 ‰ while $\delta^{13}\text{C}_{\text{air}}$ above the canopy was only -7.0 ‰. [The CO_2 released by decomposing plant litter in soil is more depleted in ^{13}C (*ca.* -27.0 ‰) than that of atmospheric CO_2 (*ca.* -7.0 ‰), since plants discriminate against $^{13}\text{CO}_2$ in air]. Without measuring isotopic composition of CO_2 within the canopy and assuming constant physiological discrimination throughout, Vogel attributed the decrease in $\delta^{13}\text{C}$ of leaves at lower layers to the use of recycled soil CO_2 .

Medina and Minchin (1980) reported $\delta^{13}\text{C}_{\text{plant}}$ gradients of 4.7 and 5.6 ‰ between upper and lower canopy leaves for two tropical forest types. Results were also attributed to the photosynthetic assimilation of recycled soil CO_2 in the lower leaves.

In a moist tropical forest (Panama), Jackson *et al.* (1992) compared shade tolerant and light demanding species in understory and gap environments. They concluded that different $\delta^{13}\text{C}$ values between plants of the same species growing in gaps and understory were physiologically based and not solely due to differences in $\delta^{13}\text{C}$ of source air (CO_2).

Francey *et al.* (1985) examined $\delta^{13}\text{C}_{\text{air}}$ as well as $\delta^{13}\text{C}_{\text{plant}}$ in temperate coniferous forest canopies. They noted a decrease in $\delta^{13}\text{C}_{\text{plant}}$ with canopy depth but no corresponding

decrease in $\delta^{13}\text{C}_{\text{air}}$, thus indicating a physiological effect. Deeper in the canopy $\delta^{13}\text{C}_{\text{plant}}$ and p_i were negatively correlated. While soil CO_2 contributes to the decrease in leaf isotope composition, most of the change can be attributed to stomatal and photosynthetic effects (Farquhar *et al.* 1989a).

Fu *et al.* (1992a) reported on leaf $\delta^{13}\text{C}$ variation in open-grown *Quercus virginiana* Mill. canopies where fixation of respiratory CO_2 was likely minimal. Foliar Δ increased significantly from the upper to lower canopy, attributed to reduced photosynthetic photon flux density (PPFD) in the lower canopy. Cardinal directions showed significant differences in Δ values. North facing leaves experiencing lowest PPFD showed highest Δ . South facing leaves with highest PPFD showed lowest Δ . Foliar Δ values were significantly higher during the wet sampling period than the dry, suggesting greater WUE during the dry period. There were no significant interactions between canopy height, aspect or sample period (wet, dry). Differences in foliar Δ were attributed to PPFD changes and perhaps to water vapor pressure differences between leaves and air. It was suggested that leaves subject to low PPFD had higher p_i/p_a ratios, which might increase quantum yield and give higher photosynthetic carbon gain under light-limiting conditions. The effect of vapor pressure deficit on Δ would have been largely due to decreased stomatal conductance, resulting in lower p_i/p_a ratios.

Water

A common physiological response to drought is decreased photosynthesis, transpiration and leaf conductance concurrently (Farquhar and Sharkey 1982). Partial pressure of CO_2 inside the leaf will decline if leaf conductance decreases more than photosynthetic demand for CO_2 . This effect will be manifested as an increase in $\delta^{13}\text{C}_{\text{plant}}$ or a decrease in Δ . Carbon isotope analysis reveals that, over the long term, plants under water stress (induced by low soil moisture) produce leaves with lower p_i values. Over the short term, without new growth, reduction in p_i as a measure of stress can be detected in leaf soluble sugars (Guy and Wample 1984; Brugnoli *et al.* 1988; Lauteri *et al.* 1992) or by using

the "on-line" approach to measure variation in $\delta^{13}\text{C}$ of CO_2 in air as it flows by a leaf inside a stirred cuvette (Hubick *et al.* 1988). An increase in leaf-to-air vapor pressure difference will cause reduction of p_i and Δ in the short term (Brugnoli *et al.* 1988; Madhavan *et al.* 1991) and long term (Farquhar and Richards 1984).

Nutrients

Water-use efficiency in any genotype increases with more available nitrogen. The patterns that occur presumably reflect available water and nitrogen (or phosphorus) in the habitat (reviewed by Farquhar and Richards 1984).

Fu *et al.* (1992b) reported the effect of soil water and nutrients on Δ , in relation to leaf developmental stage in two *Phaseolus* species. In all treatments, the species native to warmer, drier environments had significantly lower Δ (suggesting greater long term WUE). Both species were more water-use efficient in high nitrogen.

2.9 Carbon Isotope Analysis in Woody Plants

Craig (1953) was among the first to document carbon isotope compositions of plant materials. He analyzed wood from 22 trees world-wide. Wyoming trees showed a "slight local" pattern, with samples from the northwest of the state [*Abies lasiocarpa*, -27.9 ‰; *Pinus contorta*, -27.1 ‰; *Artemesia tridentata* Nutt., -26.6 ‰] being lighter than those further south [*Abies lasiocarpa*, -23.8 ‰; *Artemesia tridentata*, -23.7 ‰; *Salix bebbiana* Sarg., -25.0 ‰]. Craig suggested that local environments produced notable $\delta^{13}\text{C}$ variation among trees of the same species. He supported this idea by referring to his early work on $\delta^{13}\text{C}$ differences in one tree, for which he gave no specific causes. He found no correlations between $\delta^{13}\text{C}$ and altitude, or collection date. In *P. contorta*, $\delta^{13}\text{C}$ values of wood and leaves were equal, a result unique from the 0.5 to 2.0 ‰ difference for the 13 other plants he compared.

In 1987, Smith *et al.* reported differences in photosynthetic CO₂ fixation and carbon isotope fractionation among subspecies of *Artemisia tridentata*. The differences were associated with varying ploidy levels, growth habit and habitat.

De Lucia *et al.* (1988) used carbon isotope analysis in their study of edaphic communities of *Pinus ponderosa* and *Pinus jeffreyi* in Great Basin sagebrush and pinyon-juniper communities. They examined the water available to, and the water use of the species as potential factors restricting distribution. Intraspecific comparisons were not made since insufficient numbers occupied each soil type. In September, samples of current year foliage, taken at several points around each of five plants per species per site, were analyzed for $\delta^{13}\text{C}$. Although lighter by 1.5 ± 0.3 ‰, whole leaf $\delta^{13}\text{C}$ correlated positively with that for cellulose. Carbon isotope ratios (cellulose) were inversely related to maximum stomatal conductances. Results suggested that *Pinus monophylla* T. & F. and *Juniperus osteosperma* (Torr.) Little, followed by *Pinus ponderosa* and *Pinus jeffreyi*, functioned at lower internal CO₂ concentrations and higher WUEs than the shrubs.

Read and Farquhar (1991) reported the first study relating genetic differences in Δ of individual species to their origin climates. They measured leaf Δ in 22 southern species of *Nothofagus*, grown in controlled environments. Variation in Δ was correlated with temperature range (highest mean monthly maximum temperature minus lowest mean monthly maximum temperature), mean annual temperature, mean annual precipitation, growing season precipitation (December to March) and latitude ($p < 0.05$). The positive correlation between latitude and Δ for some species, grown under common conditions, was not maintained in field samples.

Leavitt and Danzer (1992) examined the influence of light, soil moisture, relative humidity, temperature and CO₂ concentration on $\delta^{13}\text{C}$ of *Pinus resinosa* Ait. seedling growth rings. Treatment and control groups were grown in artificial conditions. Soil moisture and CO₂ showed the greatest isotopic effects; however, the true influence of CO₂ was masked by inability to regulate the $\delta^{13}\text{C}$ of chamber air. Surprisingly, relative humidity affected $\delta^{13}\text{C}$ to

a minor degree, perhaps due to varying humidities at values only >50%. Control plants showed seasonal isotopic changes, suggesting that growth and carbon allocation effects and/or genetic effects might contribute to tree ring $\delta^{13}\text{C}$ changes.

Newberry (1992) studied annual climatic variation effects on WUE of *Pinus edulis* Engelm. at two elevations (1500 m, 2300 m) with similar water stress. Water-use efficiency was determined by $\delta^{13}\text{C}$ values of different-aged leaf cohorts, collected from the southeast aspect of each tree at 1 to 2 m height. Lower elevation trees showed similar trends in annual WUE changes. High elevation trees had randomly fluctuating and more variable $\delta^{13}\text{C}$ values. Despite site differences in annual $\delta^{13}\text{C}$ patterns and foliage variation, average site $\delta^{13}\text{C}$ s were similar. Possible explanations for this were that competition, low light and herbivory confounded a simple increased drought – increased WUE relationship at the more heavily vegetated, higher elevation site. Influence of soil CO_2 was not questioned. At the lower elevation site with open canopy, WUE was constrained only by water availability.

Zhang *et al.* (1993) found genetic differences in Δ among 25 *Pseudotsuga menziesii* populations in a 15-year-old field trial. Carbon isotope discrimination was related to geographic location of the seed source and was positively correlated with altitude ($r=0.76$, $p=0.01$). Intrinsic water-use efficiency (A/g or assimilation/conductance) was negatively correlated with altitude ($r=-0.63$, $p=0.0007$). Fifteen year heights and diameters were not correlated with photosynthetic rate but were negatively correlated with Δ , p_i/p_a and g , and positively correlated with A/g (all at $p<0.01$). Efficient water use for photosynthesis appeared to be more important than absolute photosynthetic rate in determining growth rates.

Results from the physiological and genetic studies on photosynthetic traits and water loss rate for *P. contorta* suggest that populations will differ in relative WUE, as indicated by $\delta^{13}\text{C}$. The relationship between $\delta^{13}\text{C}$ and WUE is positive (while that between Δ and WUE is negative). It is expected that populations from dry habitats will maintain greater WUEs (higher $\delta^{13}\text{C}$) than those from wet habitats over a range of test sites. On dry test sites all

populations will likely increase in WUE or $\delta^{13}\text{C}$, while on wet test sites populations will decrease in WUE.

2.10 Variation in $\delta^{13}\text{C}$ Within and Among Trees

Researchers recognize that inherent spatial variation and varying proportions of organic constituents in trees present sampling problems for $\delta^{13}\text{C}$ analysis. Several studies have examined $\delta^{13}\text{C}$ variation within and among trees. Tans and Mook (1980) found "good" correlations between $\delta^{13}\text{C}$ values of whole wood and acid-alkali-acid treated latewood of mature *Quercus rubra* L. Treated samples from east and west aspects of wood within a bole correlated poorly, with the largest $\delta^{13}\text{C}$ difference for a single year being 4.0 ‰. North and south aspects were not discussed. Within the same radial direction, a single ring sampled over a 40 cm vertical distance showed a $\delta^{13}\text{C}$ fluctuation of up to 0.8 ‰. When sampled along the fiber direction, at an angle to the vertical, $\delta^{13}\text{C}$ was nearly constant. Latewood was judged no more reliable than earlywood in providing consistent circumferential $\delta^{13}\text{C}$ trends.

Leavitt and Long (1982) compared *Juniperus monosperma* (Engelm.) Sarg. leaf and wood $\delta^{13}\text{C}$ trends over one growing season. The sampled wood was from a south-facing trunk branching near the ground (noted in Leavitt and Long 1986). Leaves were lighter than wood for both whole tissue and cellulose by approximately 2.5 ‰ and 3.5 ‰, respectively. Leaves followed the same $\delta^{13}\text{C}$ trends as the wood. Although rings and leaves showed intra-annual isotopic changes, the changes were not significant.

Leavitt and Long (1984) studied $\delta^{13}\text{C}$ variability in tree rings at one site by examining $\delta^{13}\text{C}$ trends in one *Pinus edulis*, as well as differences among several trees at the site. Cellulose was analyzed. The circumferential range within one tree was about 1.0 to 1.5 ‰, while the difference among individuals was 2.0 to 3.0 ‰. The authors concluded that pooling four orthogonal cores from four trees accurately represented site $\delta^{13}\text{C}$ trends and absolute values. Also, two cores showed a marked improvement over single cores in correlations between $\delta^{13}\text{C}$ of radii and $\delta^{13}\text{C}$ of the full circumference (taken as the "true"

$\delta^{13}\text{C}$ value). Orthogonal cores yielded more representative samples than cores taken from one side of the tree only.

In 1986, Leavitt and Long compared $\delta^{13}\text{C}$ values of leaf, twig and wood cellulose in *P. edulis* from different locations in the southwestern USA. In most cases, *P. edulis* rings were heavier than leaves by 1.0 to 2.0 ‰. Leaves were isotopically heavier than their twigs and over all, followed the same directional $\delta^{13}\text{C}$ pattern as the twigs. The authors found no longitudinal $\delta^{13}\text{C}$ gradients in tree rings, but like previous researchers, they used only single radii at different levels. Single radii do not accurately represent the trend or absolute $\delta^{13}\text{C}$ values in a ring series representing all radii at a certain level aboveground (Leavitt and Long 1986). The range of circumferential variation for ring cellulose was 0.5 to 1.5 ‰; for leaves it was 1.0 to 2.0 ‰. The authors found a north trunk of *J. monosperma* yielding a different $\delta^{13}\text{C}$ trend over a growing season than a south trunk from the same tree. They suggested that the trend in the south trunk might reflect greater sun exposure of its leaves in the early part of the season, whereas the north trunk would not receive high exposure until mid season.

Yoder *et al.* (1992) measured $\delta^{13}\text{C}$ of whole wood and needles (taken 1.37 m above ground level) in *Pinus ponderosa* spanning different age and size classes. Early wood was sampled from the most recent growth ring. Wood and foliage appeared to be enriched with ^{13}C at the rate of 1.0 ‰ for each 20 m increase in tree height. Foliage remained between 1.0 to 2.0 ‰ more depleted than wood. There was no change statistically in $\delta^{13}\text{C}$ from the bottom to top of individual large trees (actual size of trees not given).

3.0 Materials and Methods

This study involved three experiments: 1. development of sampling methods for provenance field trials in British Columbia, as well as for future trials; 2. $\delta^{13}\text{C}$ analysis of wood samples from provenance trials in British Columbia; 3. $\delta^{13}\text{C}$ analysis of seedlings grown from the seed used to establish the provenance trials.

3.1 Determination of Sampling Methods for Field Trials

To decide sampling method, $\delta^{13}\text{C}$ variation in foliage and wood was assessed for five open-grown *Pinus contorta* saplings located at one site (Juliet Creek, lat. 121° 00' N, long. 49° 43' W, elev. 1010-1067 m). The site had gravelly soil and vegetation suggesting low moisture availability (*Achillea millefolium*, *Amelanchier alnifolia*, *Calamagrostis rubescens*, *Lonicera utahensis*, *Lupinus* spp., *Pachistima myrsinites*). The saplings were moderately infected with needle cast. It was assumed that each was infected to the same degree and that each responded similarly to the disease.

The sampling was done on June 11, 1989. Saplings were felled and discs of the bole and needles collected accordingly:

Tree No. 1 (Age 21.5 yrs., Ht. 5.3 m)

Discs: No. 1 - leader

No. 2 - at midsection of 1988-89 stem

No. 3 - at 1.2 m above ground

No. 4 - within 10 cm of ground

Needles: 1. Top of tree

a) needles growing on leader

b) 1989 needles growing on stem and on north, east, south and west-facing branches

2. Mid-tree - 1989 needles at four cardinal directions around the crown circumference at 1.2 m

3. Lower tree - 1989 needles at four cardinal directions on bottom branches

Trees No. 2, 3, 4 and 5 (Ages 15, 24.5, 31.5 and 23.5 yrs., respectively; Heights 2.42, 3.95, 5.45 and 9.4 m, respectively)

Discs: No. 1 - leader

No. 3 - at 1.2 m above ground

No. 4 - within 10 cm of ground

Needles: 1. growing on leader

2. 1989 needles on bottom N, E, S and W-facing branches

Four, 5 mm wide "cores" were cut from the air-dried discs, each core taken from the north, east, south or west aspect of the sapling. Within each core, rings representing years 1983, 1984, 1987 and 1989 were analyzed. Data from six climate stations around Juliet Creek showed that 1983 and 1984 had low growing season temperatures with high precipitation, relative to other years in the 1980s, while 1987 and 1989 had relatively high growing season temperatures and low precipitation.

Wood was hand filed for homogeneous well-mixed particles. Weighed samples (0.5 mg) were combusted in a fully automatic elemental analyzer (Howarth 1977). The samples were introduced into the combustion zone at 1050 °C to coincide with oxygen enrichment of the helium gas. This eased combustion which was completed when the combustion products passed through the combustion tube. The carbon was converted quantitatively to carbon dioxide, the hydrogen to water and the nitrogen to oxides of nitrogen. A secondary reaction tube removed excess oxygen and reduced the nitrogen oxides to nitrogen before the gases entered the chromatographic column for separation of the combustion products. For measurement of $\delta^{13}\text{C}$, the CO_2 was transferred to a VG Isotech Prism triple-collecting mass spectrometer (Middlewich, England) (Nier 1947). Internal precision of the spectrometer was better than ± 0.02 ‰. Samples were corrected for ^{17}O contribution to mass 45 abundance.

Needles: After freeze-drying and interim refrigeration, the needles were cleaned, ground in a Wiley Mill (40 mesh), pulverized in a Planetary Micro Mill "Pulverizette 7" (Fritsch GmbH) and analyzed as above.

3.2 Determination of Genetic Variation in WUE Among Sapling Provenances

I obtained permission from Dr. Cheng C. Ying (Provenance Forester, British Columbia Ministry of Forests) to sample field trials in British Columbia, set up in 1974 to assess genotype x environment effects in *Pinus contorta* (Illingworth 1978). From 60 test sites, I chose three: 70 Mile House, Holden Lake and Salmon Lake. They formed a north-south transect through the B.C. interior, representing contrasting soil moistures and climates (Table 1, Fig. 1). Of 140 provenances tested, I studied eleven (Table 2, Fig. 1).

Each test site comprised two blocks, with nine saplings per provenance per block, planted 2.5 m apart in square plots (Fig. 2). Wood was sampled from every living tree, extracted by coring full diameter (north to south) near ground level. Ground level, rather than 1.2 m above ground, was used to avoid missing years. Sapling heights and diameters (at 15 years) were obtained from Ministry growth surveys (1988). Volumes were calculated from a formula by Kovats (1977). Features of each sapling and surrounding neighbors which might influence carbon assimilation and partitioning were noted (*e.g.*, damaged leaders, physical erectness, sexual precociousness, canopy closure).

The sampling extended over two field seasons. During July 1990, Holden Lake was sampled. In July 1991, 70 Mile House and Salmon Lake were sampled.

Air-dried cores were microscopically magnified and cleaned of extraneous carbon sources (*e.g.*, black smudges from the increment borer, clinging bark particles) with a razor blade. Ten year segments (1980 - 1989) from each side were combined and ground in a Wiley Mill (20 mesh). Following 2 hours freeze-drying, the samples were pulverized for one-half hour in a Planetary Micro Mill and stored in plastic vials.

Table 1. Test site characteristics for 70 Mile House, Holden Lake and Salmon Lake.

Test Site	Lat	Long	Elev (m)	SMR ¹	SNR ²	Climate Station ³	Lat	Long	Elev (m)	MPDM ⁴ (mm)	MSP ⁵ (mm)	MAP ⁶ (mm)	MTWM ⁷ (°C)	MAT ⁸ (°C)	Years ⁹
70 Mile House	51 17	121 20	1070	3.0	D	70 Mile House	51 18	121 24	1079.9	16.6	167.9	327.0	14.0	2.6	7
Holden Lake	51 37	121 31	1160	3.5	C	100 Mile House	51 39	121 16	1059.2	15.0	196.0	386.0	14.4 ^c	3.0 ^d	10
Salmon Lake	54 51	123 55	950	5.0	B/C	Kalder Lake	54 58	124 10	969.3	23.0	280.4	697.1	12.5	0.2	7

¹Soil moisture regime (Classes: 0 very xeric; 1 xeric; 2 subxeric; 3 submesic; 4 mesic; 5 subhygric; 6 hygric; 7 subhydric)

²Soil nutrient regime (Classes: A very poor; B poor; C medium; D rich; E very rich)

³Normalized data from *Climatic Data Summaries for the Biogeoclimatic Zones of British Columbia*, Version 3, 1991, compiled by D. Meidinger and G. Reynolds (unpublished)

⁴Mean precipitation dry month

⁵Mean summer precipitation (May-Sept. inclusive)

⁶Mean annual precipitation

⁷Mean temperature warmest month

⁸Mean annual temperature

⁹Years station operating

^{c,d}Temperature adjusted downward 1°C to account for elevation difference between climate station and site

Site Descriptions (unpublished, compiled by Inselberg, MOF 1988/89)

70 Mile House

Block 51

Slope: 5%, with undulating shape Aspect: variable
 Soil Depth: 30+ cm Rooting Depth: 30+ cm
 Texture: loam with 5% coarse fragments to 20 cm depth
 loam with 40% coarse fragments from 20 to 30+ cm
 Horizons: Ae (2 cm) Humus mor (2 cm)
 Base Status: high

Water: No groundwater table, gleyed horizons or flooding.
 Indicator Species: *Arctostaphylos uva-ursi*, *Astragalus miser*, *Calamagrostis rubescens*, *Galium boreale*, *Rosa acicularis*, *Shepherdia canadensis*.
 Block 51 shows low productivity, despite its fine-textured rich soil. Soil depth is shallow and precipitation low. Considerable height variation, even within provenances, occurs.

Site descriptions (cont'd)

70 Mile House

Block 52

Slope: 12%, with some mounds Aspect: 50°
Soil Depth: 60 cm Rooting Depth: 60 cm
Texture: loam with 5% coarse fragments to 25 cm
 loam with 35% coarse fragments from 25 to 60 cm
Horizons: Ae (2 cm) Humus mor (2 cm)
Base Status: high

Water: No groundwater table, gleyed horizons or flooding.
Indicator Species: *Achillea millefolium*, *Arctostaphylos uva-ursi*,
Arnica cordifolia, *Astragalus miser*, *Calamagrostis*
rubescens, *Galium boreale*, *Rosa acicularis*, *Shepherdia*
canadensis.

Block 52 has deeper soil and a slightly cooler exposure than Block 51.
Vegetation is only slightly more productive.

Holden Lake

Block 53

Slope: 0 - 5%, with mounds Aspect: flat
Soil Depth: 50+ cm Rooting Depth: 50+ cm
Texture: silty loam with 5% coarse fragments to 50 cm
 silty loam with 35% coarse fragments to 50+ cm
Horizons: Ae (3 cm) Humus mor (3 cm)
Base Status: medium

Water: No groundwater table, gleyed horizons or flooding.
Indicator Species: *Arctostaphylos uva-ursi*, *Calamagrostis rubescens*,
Rosa acicularis, *Shepherdia canadensis*, *Vaccinium scoparium*.
Block 53 has uniform soil characteristics throughout, with most
provenances growing well. Exceptions are stunted, multiple leader
samples showing poor vigor.

Holden Lake
Block 54

Water: No groundwater table, gleyed horizons or flooding.
Indicator Species: Same as for Block 53.
Provenance performance is similar to that on Block 53.

Slope: 0%, with a straight shape Aspect: flat
Soil Depth: 30+ cm Rooting Depth: 30+ cm
Texture: loamy-sand with 15% coarse fragments to 15 cm
 sandy-loam with 15% coarse fragments from 16 to 30+ cm
Horizons: Ae (10 cm - variable) Humus mor (5 cm)
Base Status: medium

Water: No groundwater table or flooding. Gleyed horizons (light mottles) to 8 cm.

Indicator Species: *Arnica cordifolia*, *Aulacomnium palustre*, *Calamagrostis rubescens*, *Cornus canadensis*, *Galium boreale*, *Lonicera involucrata*, *Peltigera aphosa*, *Petasites palmatus*, *Polytrichum juniperinum*, *Rosa acicularis*, *Ribes lacustre*, *Rubus pubescens*, *Spirea betulifolia*, *Spirea douglasii*, *Vaccinium caespitosum*, *Veratrum viride*.

The blocks have uniform, fine-textured soils which become dense at 30 cm. Mottles at 10 cm suggest a fluctuating water table due to poor drainage. Several provenances have reddish older foliage. Some provenances are growing slowly; as well, some have broken or forked leaders suggesting minor snow damage. Most of the original forest floor was burned away by a slash fire but mineral soil was not exposed.

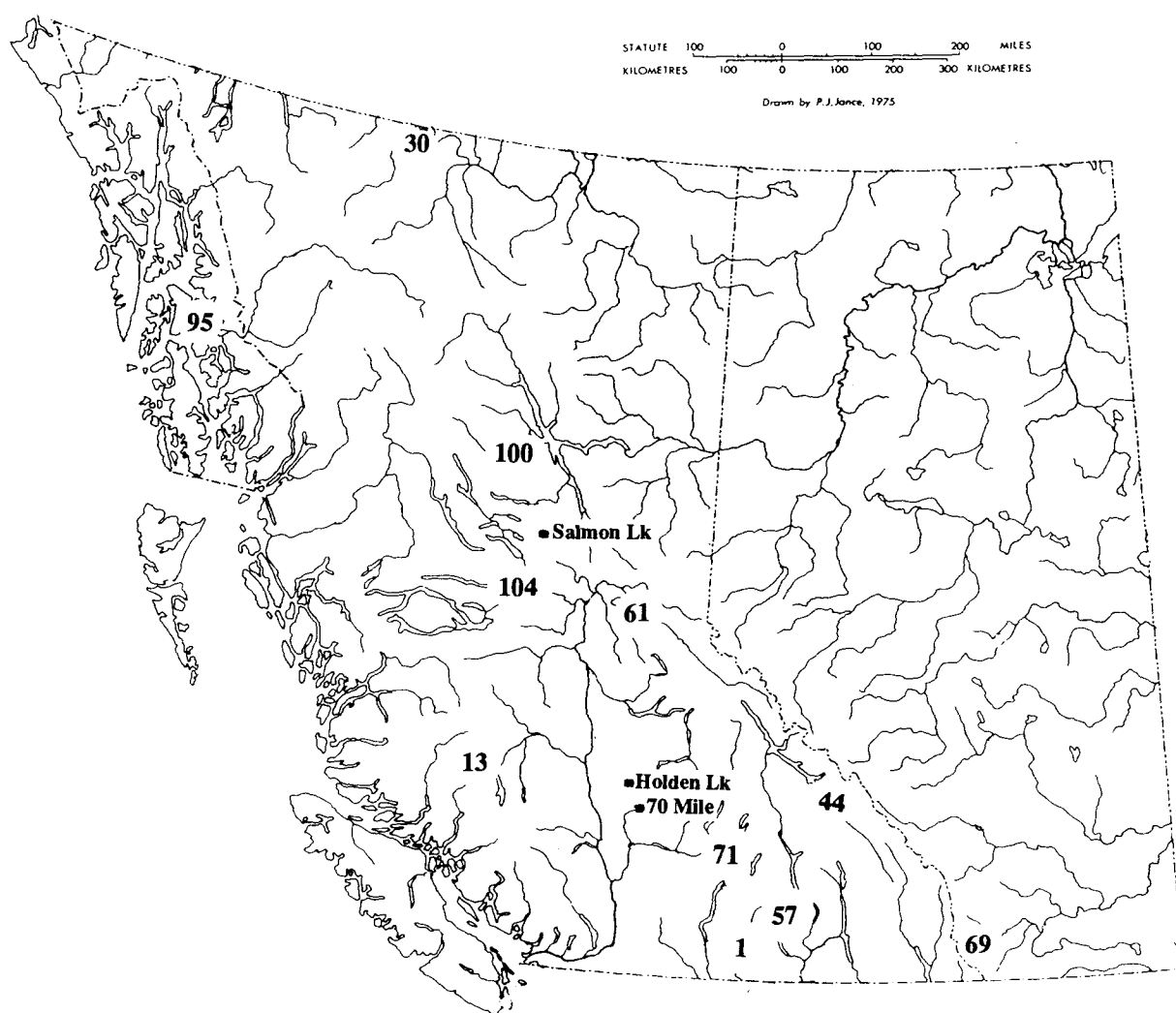


Fig. 1. Map of British Columbia and Alberta showing locations of provenances and test sites (Salmon Lake, Holden Lake, 70 Mile House). Provenance locations are represented by the provenance numbers on the map.

Table 2. Provenance characteristics and available climate data.

Prov	Location	Lat	Long	Elev (m)	Mtwm ¹ (°C)	Mat (°C)	Msp (mm)	Map (mm)	Mpdm (mm)	Climate Station ²	Lat	Long	Elev (m)	Years ³
1	Trapping Creek	49 35	119 01	1006										
13	Horne Lake	51 46	124 44	914	13.2	1.6	133.3	334.2	13.8	Kleena Kleene	51 59	124 56	899	17 ^b
30	Lower Post	59 59	128 33	640	15.5	-2.9	222.5	460.8	13.9	Lower Post	59 56	128 30	583	8 ^b
44	Marl Creek	51 31	117 11	945										
57	Inonoaklin Valley	49 54	118 12	579	17.8 ^a	6.4 ^a	254.5	621.8	34.5	Fauquier	49 52	118 04	472	30
61	Purden Lake	53 52	121 44	838										
69	Link Creek (Ab)	49 26	114 25	1379	14.3	3.0	325.1	852.0	39.0	Castle R. S.	49 24	114 20	1364	14
71	Fly Hills	50 43	119 27	1524										
95	Petersburg	56 47	132 58	23	13.2	5.2	885.8	2671.3	134.0	Petersburg	56 82	132 95	15	24
100	Nina Creek	55 48	124 49	762	13.6	0.3	223.5	493.7	23.7	Germansen Ld.	55 47	124 42	747	27
104	Nechako River	54 01	124 32	732	13.9	2.0	258.4	525.2	22.2	Fort Fraser 13S	53 53	124 35	701	5 ^b

¹Refer to Table 1 for the meaning of Mtwm, Mat, Msp, Map and Mpdm.

²Sources: Canadian Climate Normals 1951-1980; Climatic Data Summaries for the Biogeoclimatic Zones of British Columbia, Version 3, 1991, compiled by D. Meidinger and G. Reynolds (unpublished); personal communication, staff at Atmospheric Environment Service (AES), Vancouver, B.C.

³Years climate station in service.

^a Temperature adjusted downward 1 °C to account for elevation difference between climate station and provenance location.

^b Adjusted normals based on 5-19 years, including those from 1951-1980 and any other data from 1931-1950.

Schematised Test Block Layout

CHILCOTIN REGION

70 Mile House, Site 1: Block 52

Note: NOT PLANTED = ● ; DEAD = ✕

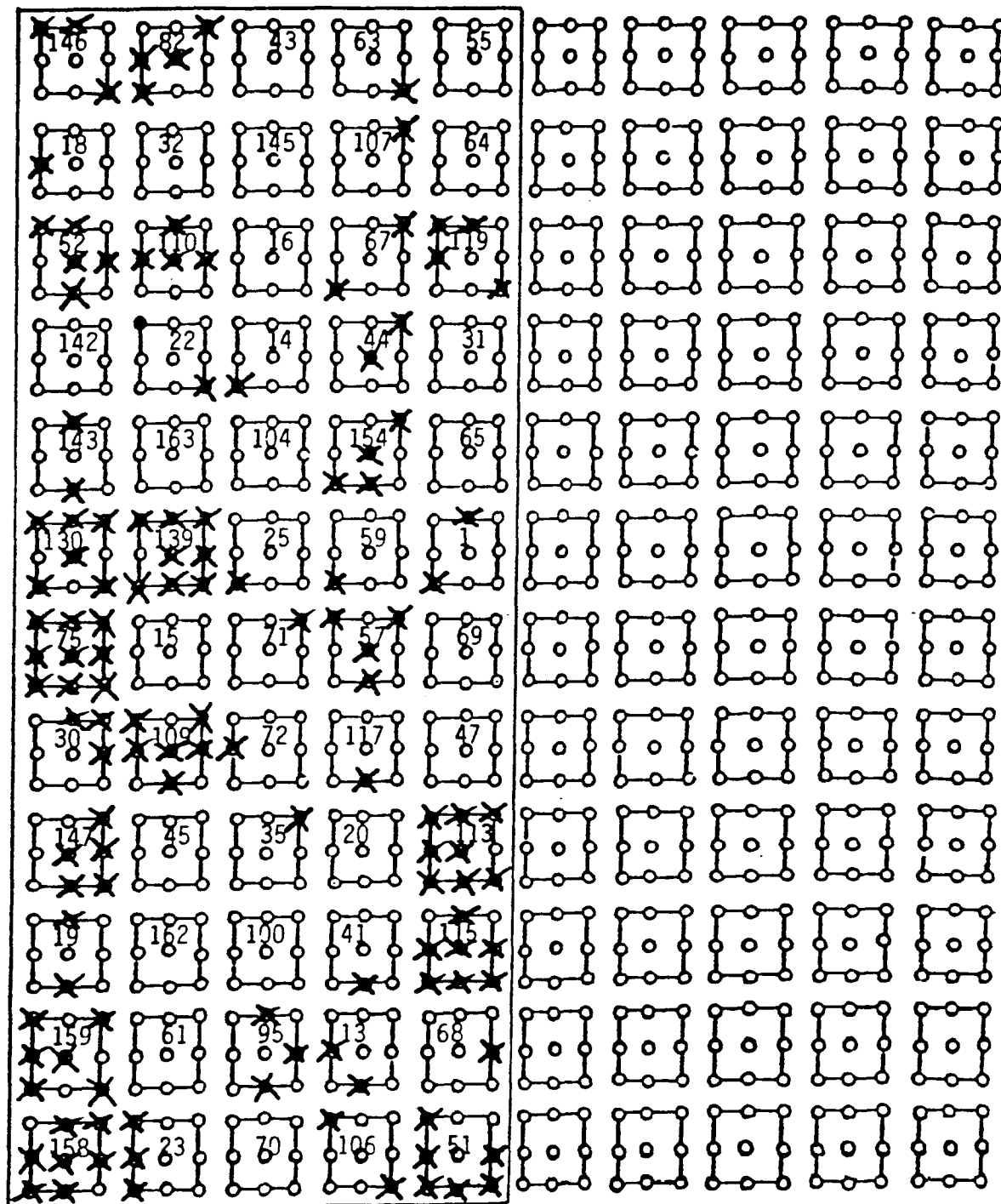


Fig. 2. Schematised test block layout for one field site. The squares represent nine-tree plots, the positioning of individuals designated by open circles. The numbers inside the squares show the provenances allocated to those plots.

Whole wood was analyzed, with reproducible analyses to 0.05 ‰ (8 replicates). The decision to use whole wood rather than alpha cellulose was based on previous studies and an experiment on 10 cores representing a range of whole wood $\delta^{13}\text{C}$ values from -23.358 to -27.312 ‰. Alpha cellulose was isolated from the wood in Dr. N. Livingston's laboratory, University of Victoria, British Columbia. Alpha cellulose $\delta^{13}\text{C}$ values were regressed on $\delta^{13}\text{C}$ values of the wood from which it was isolated. The regression r^2 of 0.981 was highly significant ($p < 0.0001$) (Fig. 3).

3.3 Seedling Experiment

One gram of seed per provenance, cold-stored approximately 20 years, was procured. Germination trials showed 60 to 95% viability. In September 1990, the seed was sown in 164 ml super cell cone-tainers (Stuewe and Sons, Inc.) containing peat and vermiculite 2:1. To each 113 litre-bag of peat was added 662.25 g Dolomite (WesGrow) and 112.5 g trace elements (WesGrow). Soil pH at planting was 4.7. Seeds were covered with No. 2 Granite Grit (Imasco). The seedlings were grown in a greenhouse. After emergence they were watered to field capacity as necessary and after 4 weeks, fertilized every 4 days with 100 ppm 20-8-20 (Plant Prod). The 18 hour photoperiod was supplemented by high intensity discharge sodium lamps. Irradiance ranged from 94 to 513 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ PAR, depending on seedling location and cloud cover. Therefore, seedling positions were rotated weekly. In full sunlight (which seldom occurred) seedlings were exposed to 1400 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ PAR. Relative humidity varied from 40 to 45%, while air temperature above seedlings was $20 \pm 2^\circ\text{C}$ on average (Vaisala RH/temperature meter, Cole-Parmer). Air above the seedlings was well mixed by fans. Near the end of December 1990, sulfur deficiency was corrected by supplementing seedling nutrition with K_2SO_4 (10 ppm) and a commercial (STEM) micronutrient mix (1 ppm).

On February 18, 1991, thirty randomly selected seedlings per provenance were harvested. Most had set bud and had mature as well as primary needles. Shoots were frozen

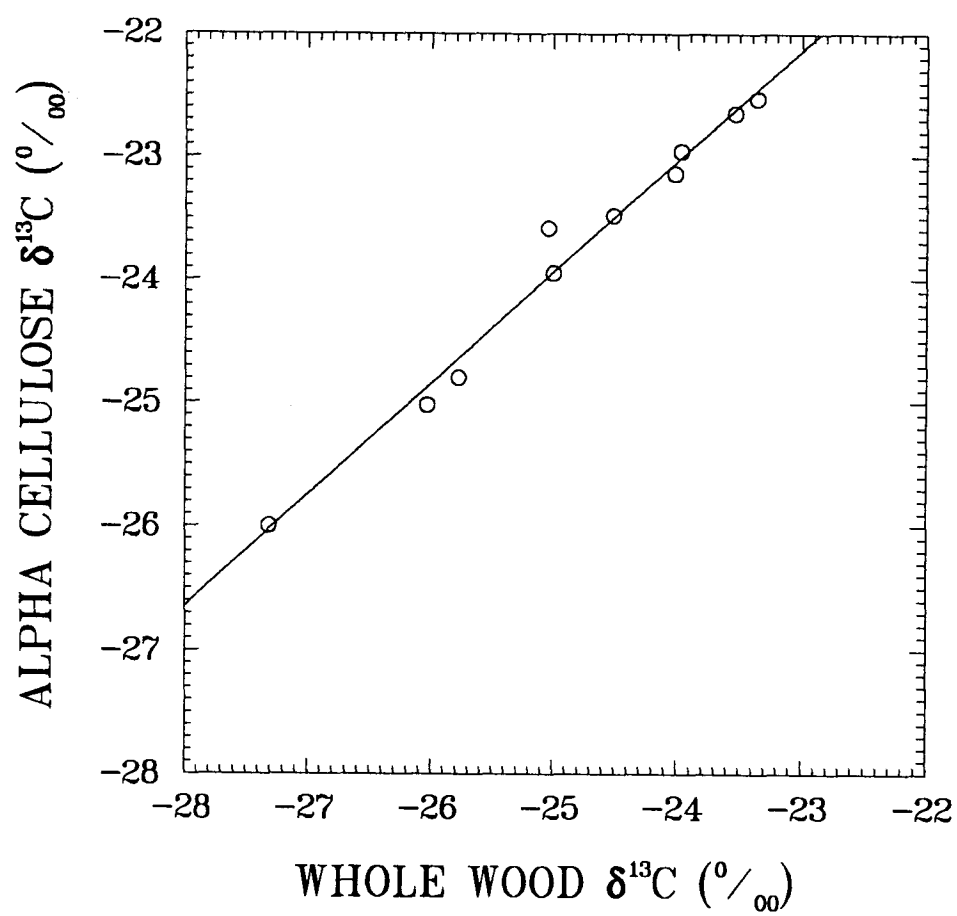


Fig. 3. Regression of alpha cellulose $\delta^{13}\text{C}$ on whole wood $\delta^{13}\text{C}$: $\delta^{13}\text{C}$ (cellulose) = $-1.357 + 0.903 \delta^{13}\text{C}$ (wood). Regression $r^2=0.981$ ($p<0.0001$).

in liquid nitrogen, freeze-dried and weighed. Roots were washed, frozen, freeze-dried and weighed. An additional five seedlings were prepared for $\delta^{13}\text{C}$ analysis of primary needles, mature needles and stem parts. Since ANOVA showed no significant differences among parts, total shoots were analyzed for $\delta^{13}\text{C}$ following grinding in a Wiley Mill (40 mesh) and pulverization. Analyses were reproducible to 0.1 ‰ (5 replicates).

3.4 Data Analysis

Determination of Sampling Methods for Field Trials

Three ANOVAs were used to test effects. Analyses were done with SAS/STAT software, 6.03 Edition (SAS Institute 1988). The first ANOVA was a three-way analysis of $\delta^{13}\text{C}$ values in wood for year, aspect and tree effects as well as interactions in *Pinus contorta* saplings. The second ANOVA was a three-way analysis, specifically for material effects (needle vs. wood) and material x aspect interactions. The third ANOVA was a three-way analysis on one sapling, specifically for needle x position effects. The models were mixed models, with aspect fixed and the other factors random. It could be argued that year samples were repeated measures and therefore dependent. They were analyzed as if they were independent, however, since repeated measures analysis produced similar results to factorial ANOVA. Hicks (1982) shows that a repeated measures design is but a special case of factorial and nested-factorial experiments. Numerical results are the same.

Determination of Genetic Variation in WUE Among Sapling Provenances

Three-way ANOVA (using SAS/STAT software) was applied to the replicated randomized complete block design of the field trials. All effects were random. The data set had missing cases (or cells) as well as missing observations. Provenance 57 was not tested at Salmon Lake. Provenance 95 at 70 Mile House was deleted from statistical analyses since only four saplings survived from a possible 18. As well, saplings which were dead, too small to core or girdled from top to bottom were removed from the data set. Other outliers with no

basis for removal were treated as very unusual measurements and not deleted. To simplify results and test all provenances, balanced data sets were analyzed in three ANOVAs. The data sets were extracted from the following site-combinations: 1. 70 Mile House, Holden Lake and Salmon Lake (provenances 57 and 95 excluded); 2. 70 Mile House and Holden Lake (provenance 95 excluded); and 3. Holden Lake and Salmon Lake (provenance 57 excluded).

The design posed a pseudoreplication problem, since sites were not replicated. Pseudoreplication was avoided by not interpreting significant differences among sites as site treatment effects (Hurlbert 1984). Inferences were made on provenances within sites.

Correlation and regression analyses for $\delta^{13}\text{C}$ and climate variables were done with SYSTAT software, Version 5.01 (Wilkinson 1990). Multiple regression modelled site mean $\delta^{13}\text{C}$ values as a function of provenance mean temperature of the warmest month (mtwm), mean annual temperature (mat), mean summer precipitation (msp), mean precipitation of the driest month (mpdm) and mean annual precipitation (map). Only provenances 13, 30, 57, 69, 95, 100 and 104 were used, since nearby climate stations did not exist for the others.

Seedling Experiment

Statistical analyses were done with SYSTAT software. The seedling design was completely randomized. One-way ANOVAs compared provenance means for $\delta^{13}\text{C}$ values, shoot weights, root weights, total weights and shoot/root ratios. Correlations were determined among growth variables and $\delta^{13}\text{C}$.

4.0 Results

4.1 Determination of Sampling Methods for Field Trials

Table 3 shows the effects of tree, aspect and year on $\delta^{13}\text{C}$ values of stump wood in *Pinus contorta* saplings. Analysis of variance on the mixed model (trees and years random, aspects fixed) showed significant tree, year and tree x aspect effects ($\alpha=0.05$). The aspect, aspect x year and tree x year effects were not significant, although the tree x year effect was nearly so ($p=0.057$). The tree x aspect x year effect (error variance) could not be tested and was assumed non-significant. Multiple range tests (Ryan-Einot-Gabriel-Welsch, Tukey) defined two homogeneous tree subsets (trees 1 and 5; trees 2, 3 and 4). As well, two homogeneous subsets for years were 1987; and 1983, 1984 and 1989.

Figures 4 and 5 depict the lack of interaction between tree x year and aspect x year effects. Fig. 4 shows that ranking in $\delta^{13}\text{C}$ changed little among saplings for the years tested. Fig. 5 shows that north, south and west aspects maintained ranking over the years tested. During 1987 (a dry year), $\delta^{13}\text{C}$ was greater in all directions.

Sampling allowed two cores per sapling in the provenance field trials. For the saplings at Juliet Creek, it appeared that certain paired aspects would accurately track $\delta^{13}\text{C}$ year to year variation among the years tested. The $\delta^{13}\text{C}$ values in stump wood portrayed by averaging north and south, and east and west aspect $\delta^{13}\text{C}$ values are shown in Figs. 6 and 7. Ranking in $\delta^{13}\text{C}$ among saplings appears to be more stable over the years tested when north and south aspects are used as combined samples rather than east and west aspects.

Table 4 shows the effects of tree, aspect and sampling material (needles vs. wood) on $\delta^{13}\text{C}$ values near ground level in 1989. Analysis of variance on the mixed model (trees and materials random, aspects fixed) showed non-significant tree and aspect effects, as well as tree x aspect and tree x material interactions. (Tree 3 was missing an easterly $\delta^{13}\text{C}$ value).

Table 3. ANOVA for five *Pinus contorta* saplings at Juliet Creek (discs at stump level).

Source	df	SS	MS	EMS	F Value	P > F
Tree	4	11.7332	2.9333	$\sigma^2 + 4\sigma^2_{TY} + 16\sigma^2_T$	22.250	0.0001
Aspect	3	1.1313	0.3771	$\sigma^2 + 5\sigma^2_{AY} + 4\sigma^2_{TA} + \theta_A$	2.192	0.1539
Year	3	3.8856	1.2952	$\sigma^2 + 4\sigma^2_{TY} + 20\sigma^2_Y$	9.820	0.0015
T*A	12	2.0315	0.1693	$\sigma^2 + 4\sigma^2_{TA}$	2.530	0.0155
T*Y	12	1.5819	0.1318	$\sigma^2 + 4\sigma^2_{TY}$	1.970	0.0573
A*Y	9	0.6262	0.0696	$\sigma^2 + 5\sigma^2_{AY}$	1.040	0.4273
T*A*Y	36	2.4042	0.0668	σ^2		

Satterthwaite Approximation to Test Aspect:

Source: Aspect

Error: $MS(T*A) + MS(A*Y) - MS(Error)$

DF	MS	Denominator DF	Denominator MS	F Value	P>F
3	0.3771	9.71	0.1721	2.192	0.1539

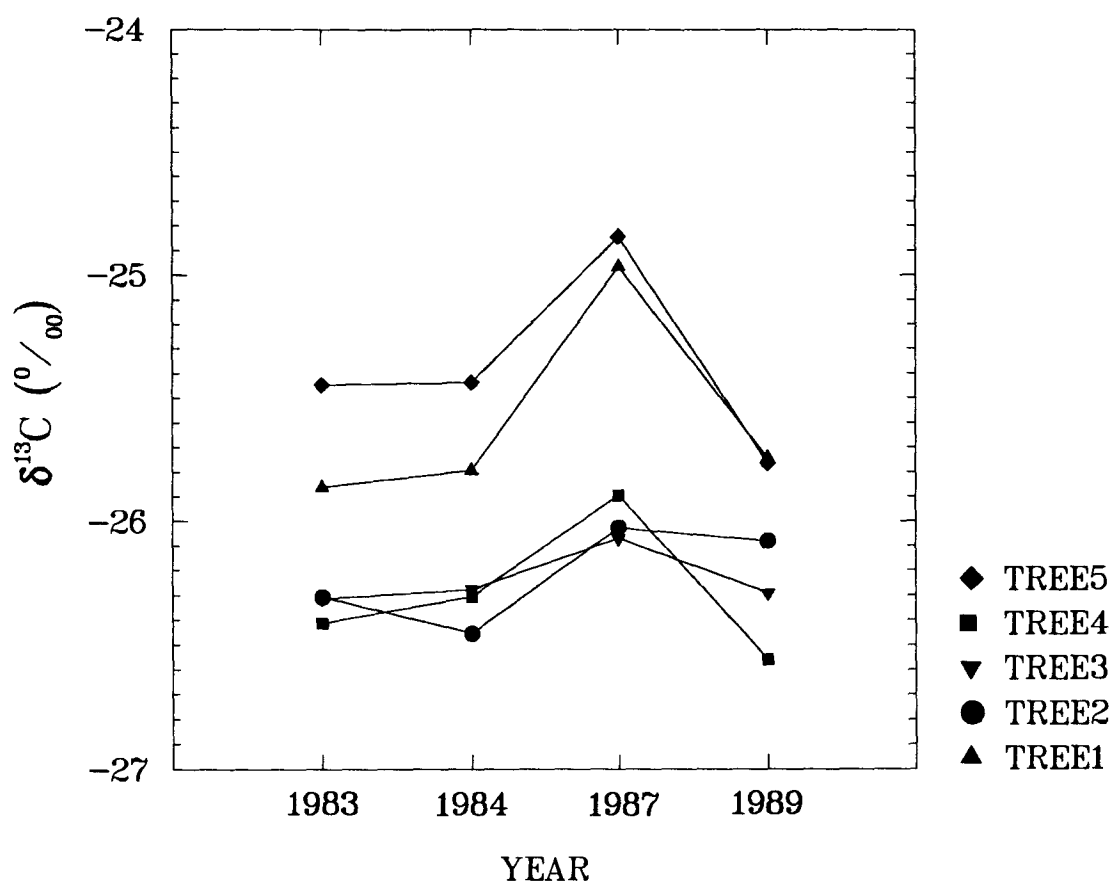


Fig. 4. Stump wood $\delta^{13}\text{C}$ values for years 1983, 1984, 1987 and 1989 in five *Pinus contorta* saplings. Isotopic compositions are the means of four observations taken circumferentially per year tested per tree, *e.g.* sum of north, east, south and west aspect $\delta^{13}\text{C}$ values for 1983/4. Ranking in $\delta^{13}\text{C}$ among saplings is relatively stable over the years tested when the average circumferential $\delta^{13}\text{C}$ per tree is the measure for comparison.

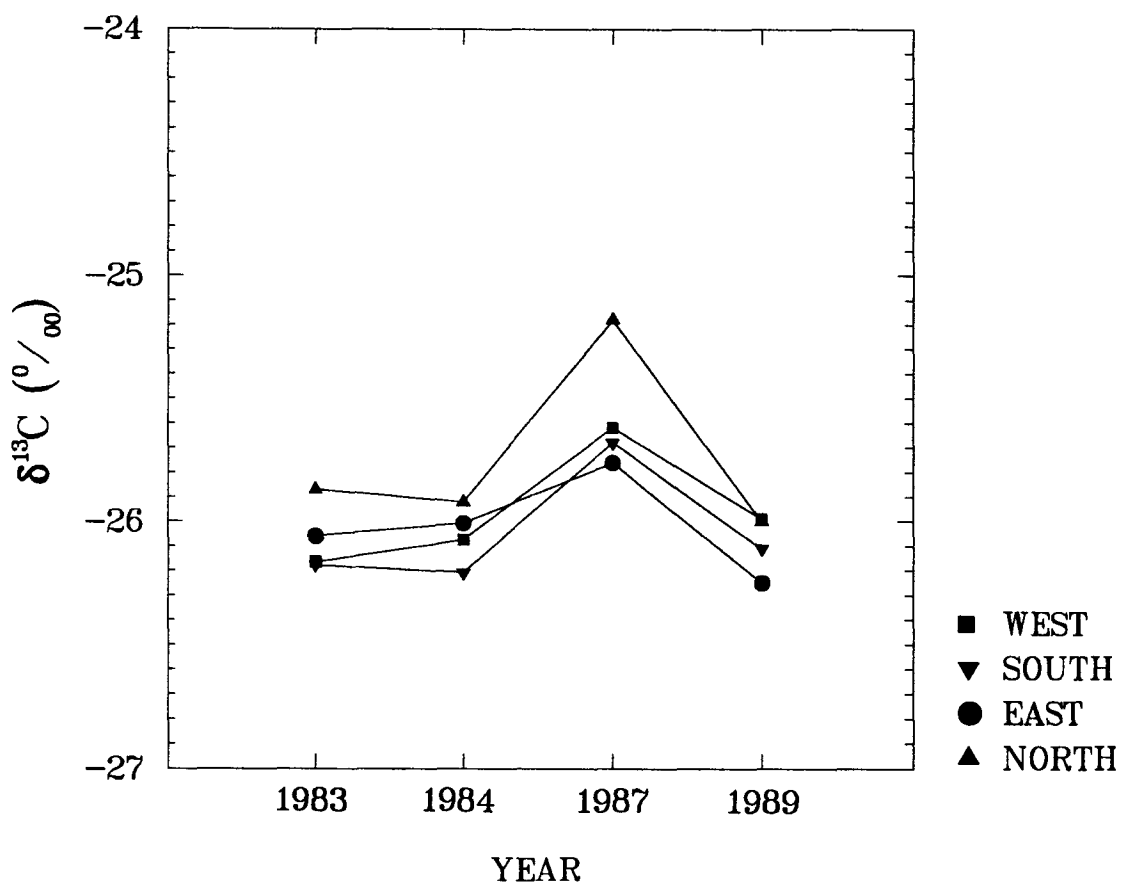


Fig. 5. Aspect $\delta^{13}\text{C}$ values (stump wood) for years 1983, 1984, 1987 and 1989. Isotopic compositions are means of five saplings, each sapling contributing one $\delta^{13}\text{C}$ value per year per aspect. Ranking in $\delta^{13}\text{C}$ among aspects remains relatively stable over the years tested, when aspect $\delta^{13}\text{C}$ is a measure of several saplings.

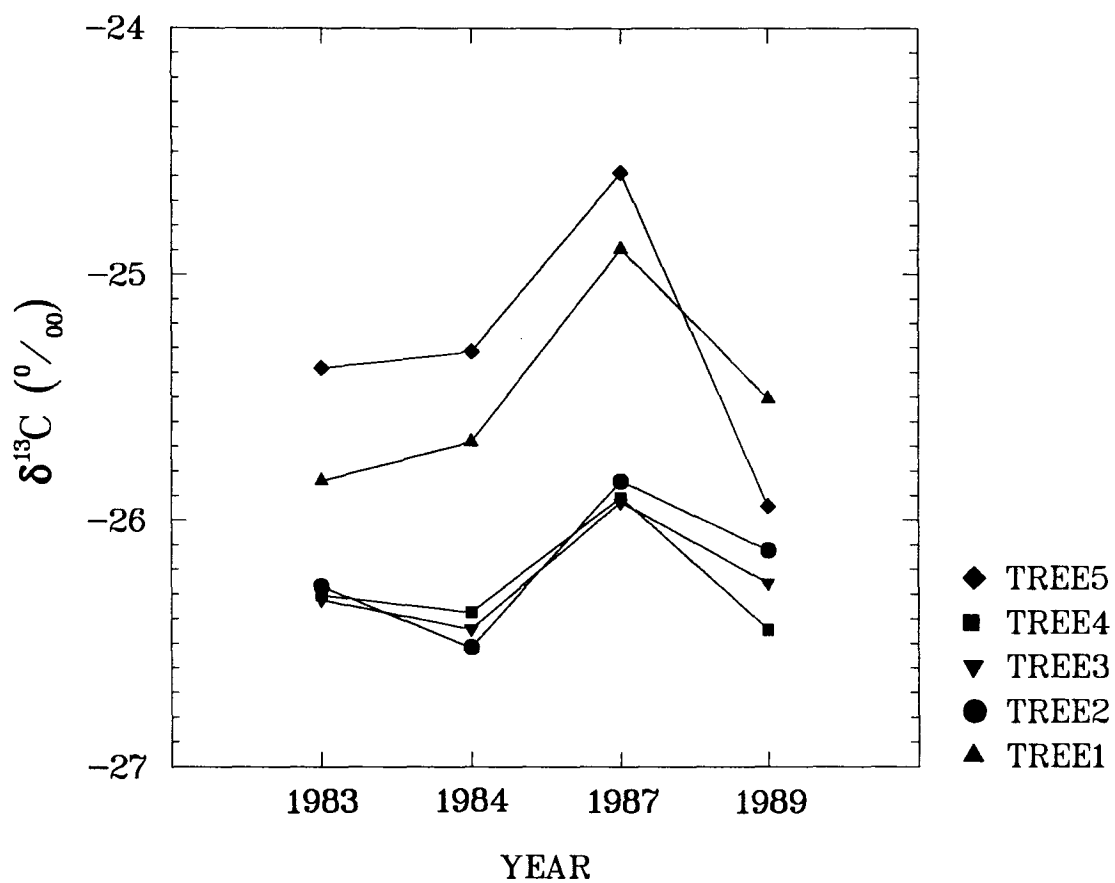


Fig. 6. Stump wood $\delta^{13}\text{C}$ values for years 1983, 1984, 1987 and 1989 as portrayed by averaging north and south aspects in five *Pinus contorta* saplings. Isotopic compositions are means of two samples per tree. Ranking in $\delta^{13}\text{C}$ among trees remains quite stable over the years tested when north and south aspects are used as combined samples.

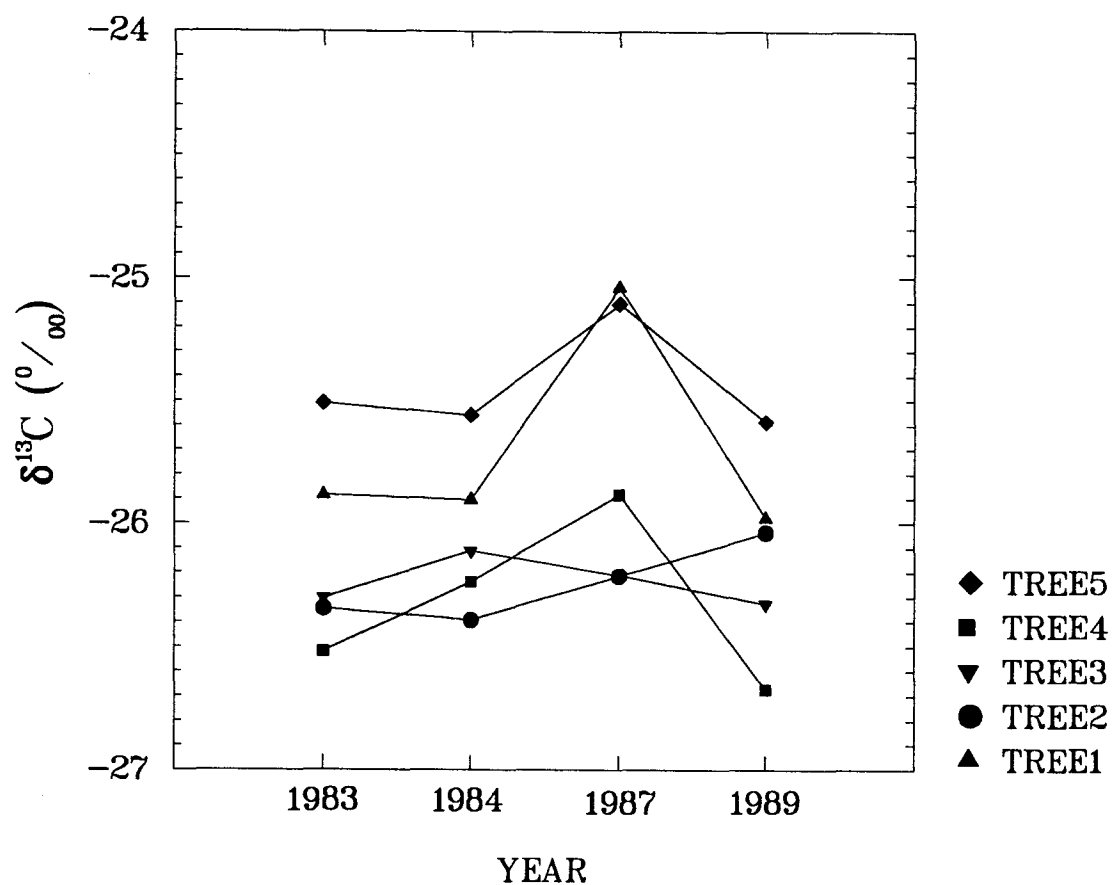


Fig. 7. Stump wood $\delta^{13}\text{C}$ values for years 1983, 1984, 1987 and 1989 as portrayed by averaging east and west aspects in five *Pinus contorta* saplings. Isotopic compositions are means of two samples per tree. Ranking in $\delta^{13}\text{C}$ among trees is not as stable over the years tested when east and west aspects are used as combined samples rather than north and south aspects.

Table 4. ANOVA for $\delta^{13}\text{C}$ comparisons of wood and needles at stump level in five *Pinus contorta* saplings at Juliet Creek.

Source	df	SS	MS	EMS	F Value	P > F
Tree	4	4.4530	1.1132	$\sigma^2 + 3.75\sigma_{\text{TM}}^2 + 1.75\sigma_{\text{T}}^2$	2.540	0.1943
Aspect	3	1.8463	0.6154	$\sigma^2 + 4.6667\sigma_{\text{AM}}^2 + 1.8667\sigma_{\text{TA}}^2 + \theta_{\text{A}}$	0.592	0.6624
Material	1	18.8254	18.8254	$\sigma^2 + 3.75\sigma_{\text{TM}}^2 + 18.462\sigma_{\text{M}}^2$	42.960	0.0028
T*A	12	2.2291	0.1858	$\sigma^2 + 1.9167\sigma_{\text{TA}}^2$	0.940	0.5419
T*M	4	1.7528	0.4382	$\sigma^2 + 3.75\sigma_{\text{TM}}^2$	2.230	0.1327
A*M	3	3.1490	1.0497	$\sigma^2 + 4.6667\sigma_{\text{AM}}^2$	5.330	0.0164
T*A*M	11	2.1659	0.1969	σ^2		

Satterthwaite Approximation to Test Aspect:

Source: Aspect

Error: $0.9739 \cdot \text{MS}(\text{T} \cdot \text{A}) + \text{MS}(\text{A} \cdot \text{M}) - 0.9739 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P>F
3	0.6154	2.89	1.0388	0.592	0.6624

Material and aspect x material effects were significant. The influence of aspect on $\delta^{13}\text{C}$ for needles and wood is shown in Fig. 8. Wood at 1.2 m height is included for interest; it could not be compared to wood near ground level in this ANOVA. It appeared that the influence of aspect was prominent for needles but not wood. Needles had more negative $\delta^{13}\text{C}$ values than wood. The aspect x position effect in wood appeared non-significant (standard error bars overlap).

Figures 9 and 10 depict tree $\delta^{13}\text{C}$ trends with aspect, in needles and wood at stump level. The tree x aspect x material effect (error variance) could not be tested and was assumed non-significant. Overall, wood (base) $\delta^{13}\text{C}$ values were less variable from tree to tree than the needles. In 1989, isotope values among trees ranged from -25.267 to -26.840 ‰ in stump wood (a difference of 1.573 ‰) and from -25.750 to -28.989 ‰ in lower branch needles (a difference of 3.239 ‰).

Analysis of variance (Table 5) shows the effects of aspect, position (base, 1.2 m, top) and material (needles, wood) on $\delta^{13}\text{C}$ values in one sapling during 1989. Materials were significantly different; other effects were not. Figure 11 depicts the lack of response of needle $\delta^{13}\text{C}$ to aspect and position in the sapling.

4.2 Determination of Genetic Variation in WUE Among Sapling Provenances

Table 6 gives summary statistics for provenance $\delta^{13}\text{C}$ values at 70 Mile House, Holden Lake and Salmon Lake. Block designs with missing observations require unbiased estimates of treatment means, therefore least squares means (LSMs) are reported. All provenances, but 71, showed progressively decreasing $\delta^{13}\text{C}$ values from the driest to wettest site. Provenance 71 broke the $\delta^{13}\text{C}$ trend from 70 Mile House to Holden Lake by a small increase (0.069 ‰), rather than a decrease. Standard error of the LSM was 0.1 ‰ for all but two provenance-site pairs.

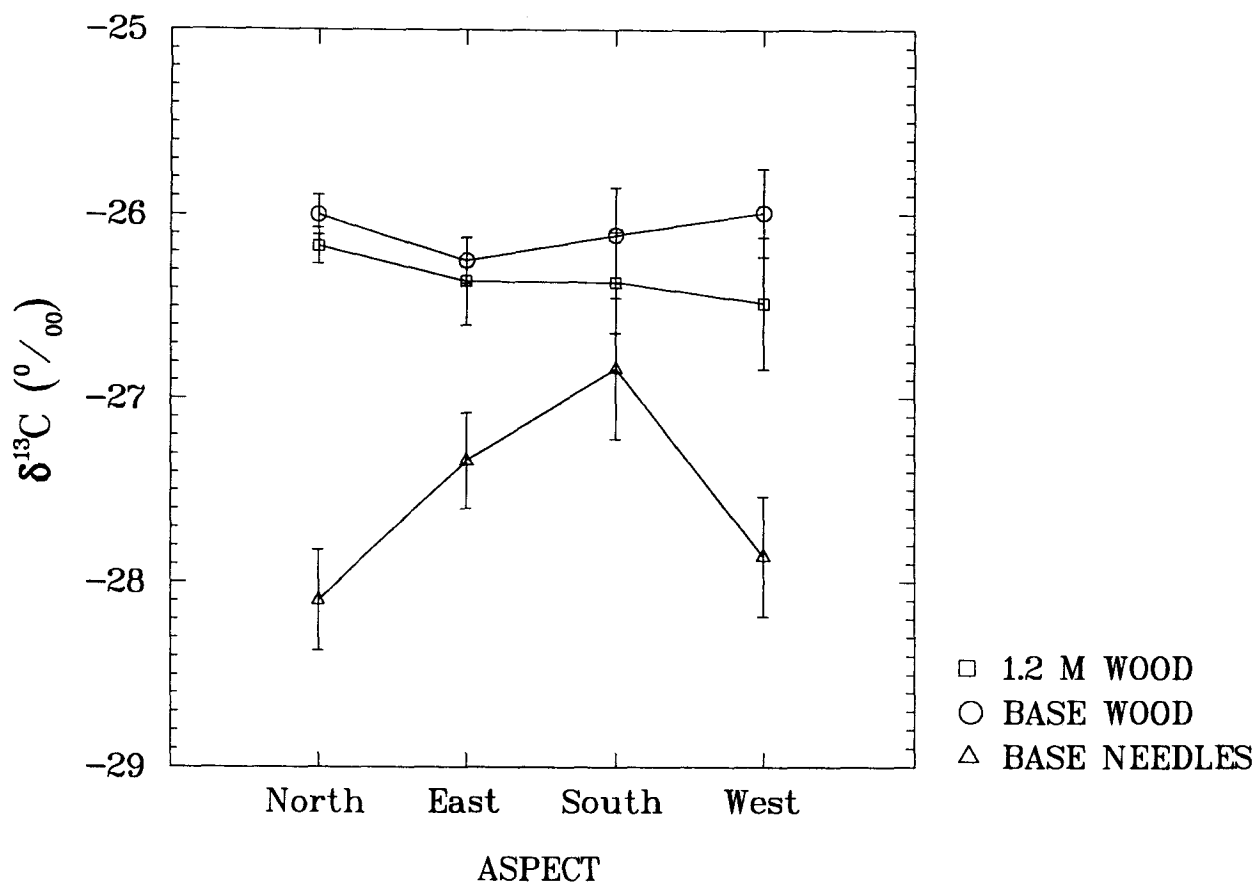


Fig. 8. Aspect x material interactions (1989) near ground level and at 1.2 m height (wood) in *Pinus contorta* saplings ($\pm\text{SE}$). Isotopic compositions are means of five trees (one sample per tree). Needle $\delta^{13}\text{C}$ is influenced by aspect whereas wood $\delta^{13}\text{C}$ is not. Needle $\delta^{13}\text{C}$ is more negative than wood $\delta^{13}\text{C}$.

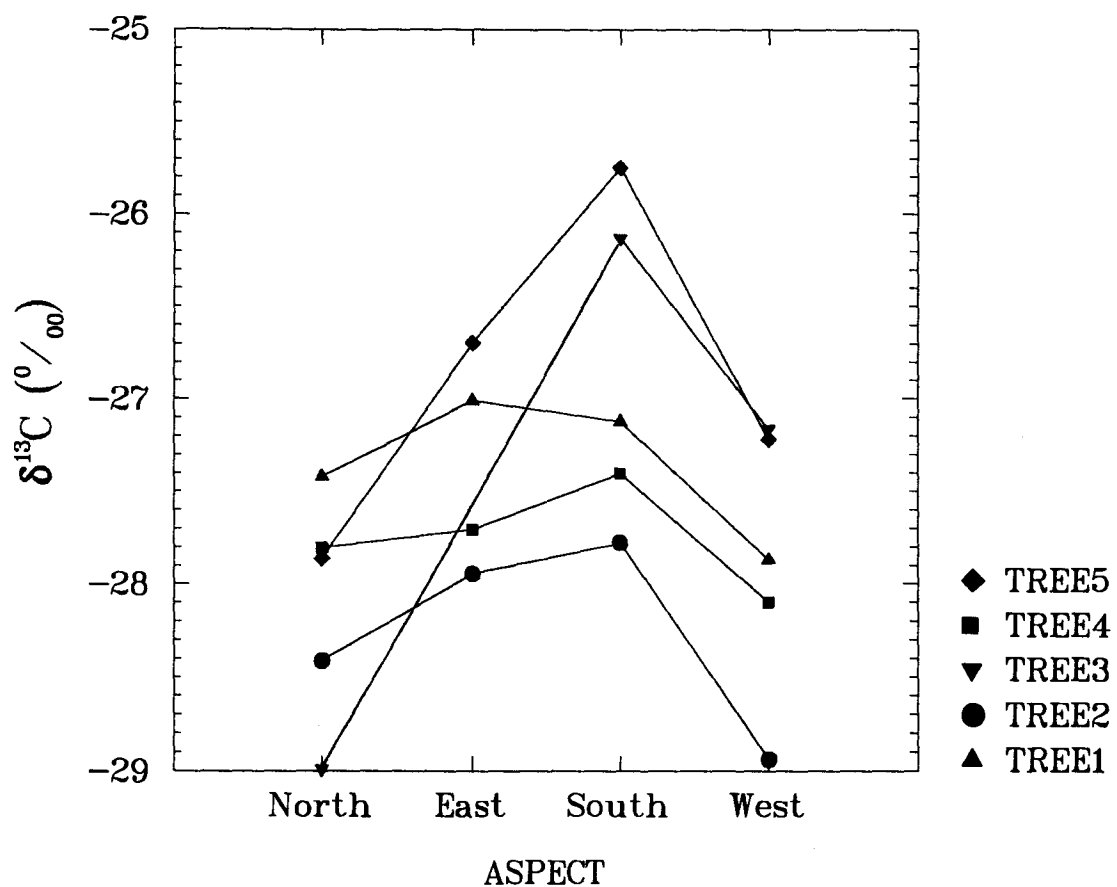


Fig. 9. Needle $\delta^{13}\text{C}$ trends with aspect (1989) in five *Pinus contorta* saplings. Needles are sampled from the lowest branches. Isotopic compositions represent one sample per tree. Sapling 3 is missing its easterly $\delta^{13}\text{C}$ value.

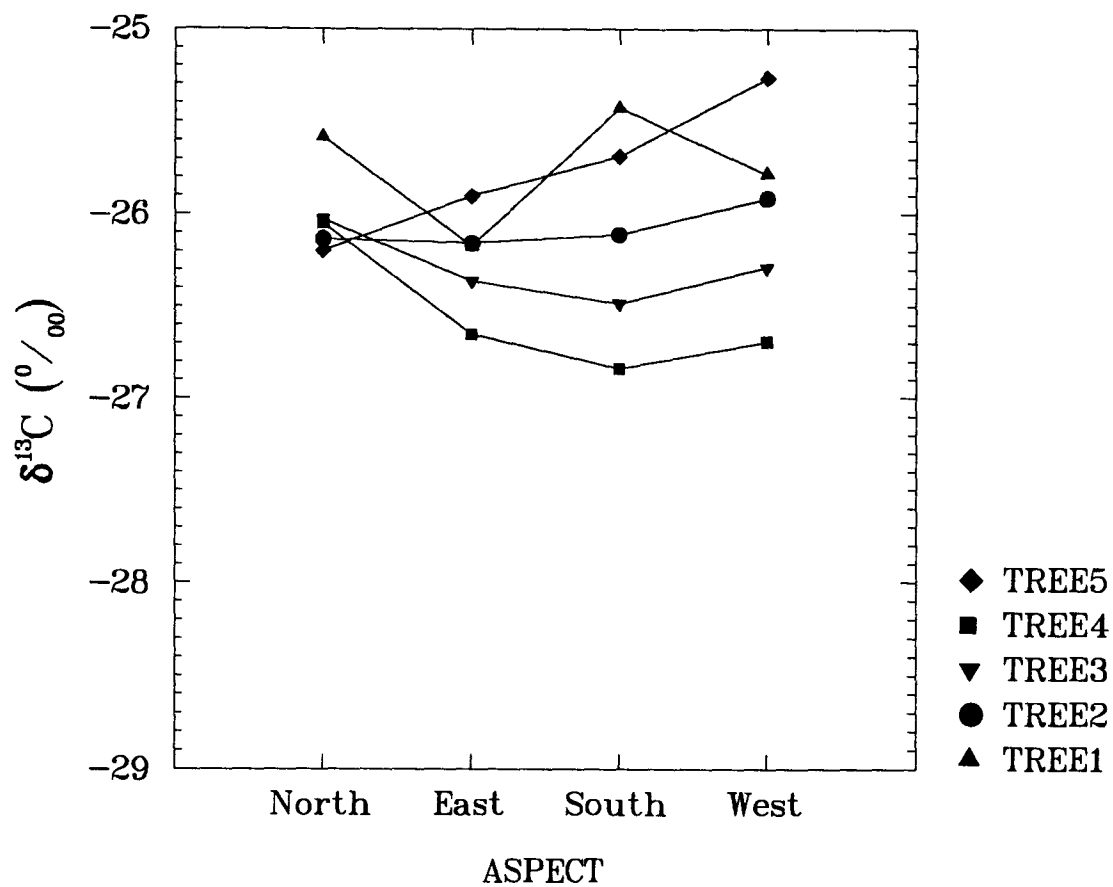


Fig. 10. Wood $\delta^{13}\text{C}$ trends with aspect (1989) in five *Pinus contorta* saplings. Wood is cored at 10 cm above ground level. Isotopic compositions represent one sample per tree.

Table 5. ANOVA for sample comparisons in one *Pinus contorta* sapling at Juliet Creek.

Source	df	SS	MS	EMS	F Value	P > F
Aspect	3	0.2092	0.0697	$\sigma^2 + 3\sigma_{AM}^2 + 2\sigma_{AP}^2 + \theta_A$	3.496	0.9097
Position	2	0.4006	0.2003	$\sigma^2 + 4\sigma_{PM}^2 + 8\sigma_P^2$	0.510	0.6624
Material	1	7.4783	7.4783	$\sigma^2 + 4\sigma_{PM}^2 + 12\sigma_M^2$	19.030	0.0487
A*P	6	0.5233	0.0872	$\sigma^2 + 2\sigma_{AP}^2$	0.491	0.7958
A*M	3	0.3307	0.1102	$\sigma^2 + 3\sigma_{AM}^2$	0.621	0.6267
P*M	2	0.7859	0.3930	$\sigma^2 + 4\sigma_{PM}^2$	2.214	0.1905
A*P*M	6	1.0651	0.1775	σ^2		

Satterthwaite Approximation to Test Aspect:

Source: Aspect

Error: MS (A*P) + MS(A*M) - MS(Error)

DF	MS	Denominator DF	Denominator MS	F Value	P>F
3	0.0697	0.04	0.0199	3.496	0.9097

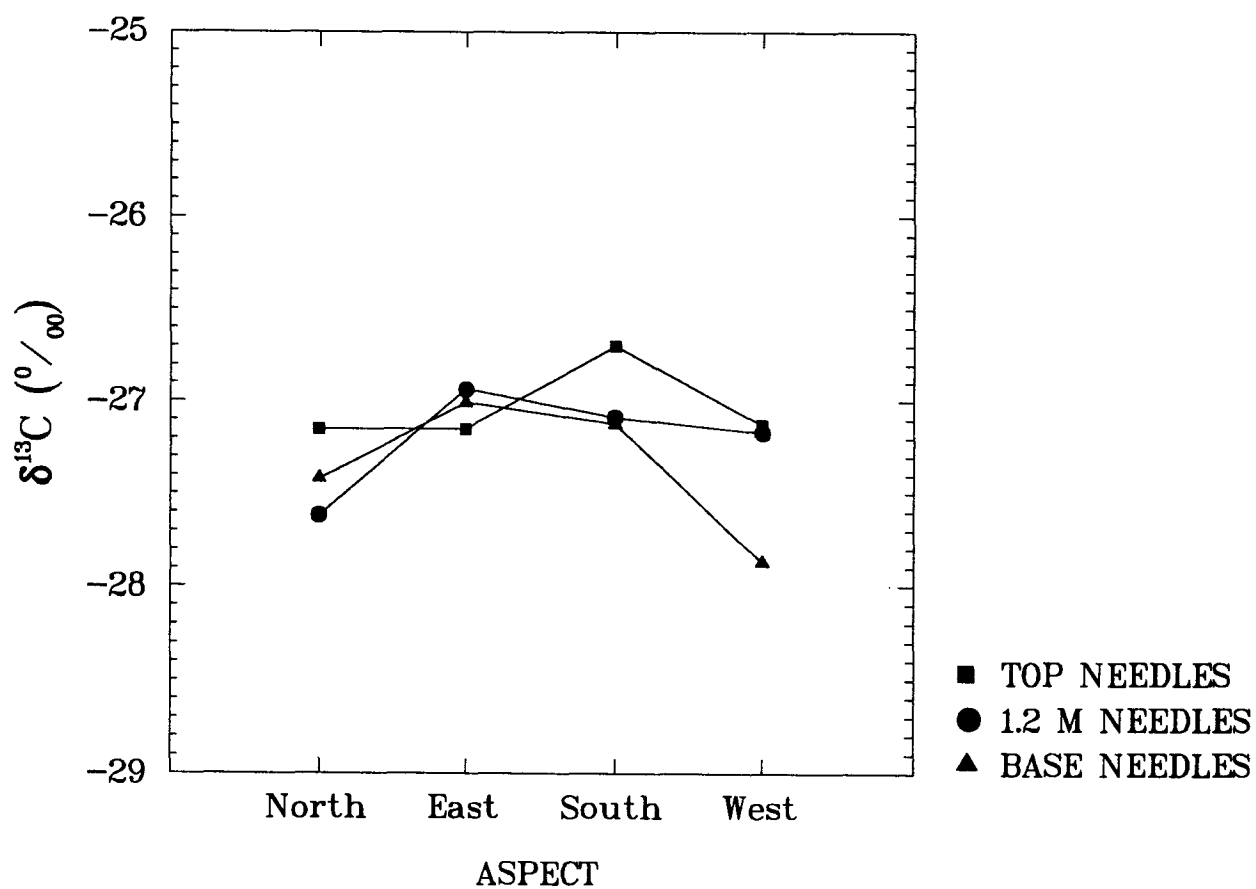


Fig. 11. Needle $\delta^{13}\text{C}$ trends with aspect and position in one juvenile *Pinus contorta* sapling. Isotopic compositions represent one sample. Needle $\delta^{13}\text{C}$ responds little to aspect and position in the tree.

Table 6. Summary statistics for $\delta^{13}\text{C}$ in *Pinus contorta* sapling provenances planted at 70 Mile House, Holden Lake and Salmon Lake.

	Provenance										
	1	13	30	44	57	61	69	71	95	100	104
<u>70 Mile House</u>											
LSM ^a	-24.754	-24.621	-24.867	-24.438	-25.144	-24.264	-24.787	-24.846	—	-24.518	-24.292
S E ^b	0.130	0.137	0.130	0.118	0.148	0.110	0.113	0.113	—	0.113	0.110
n ^c	13	12	14	16	10	18	17	17		17	18
<u>Holden Lake</u>											
LSM	-24.966	-24.942	-25.139	-24.960	-25.415	-24.533	-25.365	-24.777	-24.133	-24.837	-24.726
S E	0.128	0.137	0.127	0.132	0.128	0.128	0.125	0.125	0.209	0.132	0.128
n	17	15	18	16	17	17	18	18	10	16	17
<u>Salmon Lake</u>											
LSM	-25.748	-25.682	-26.120	-25.592	—	-25.213	-25.912	-25.764	-24.884	-25.467	-25.488
S E	0.132	0.152	0.118	0.132	—	0.132	0.122	0.122	0.127	0.136	0.130
n	15	11	18	15		15	17	17	16	14	15

^aLeast squares mean

^bStandard error of the LSM

^cMaximum=18

Table 7 summarizes ANOVA results (all factors random) for the data from 70 Mile House, Holden Lake and Salmon Lake, where all but provenances 57 and 95 were tested. Site, block (within site) and provenance variances were homogeneous (Bartlett's test). The analysis used 431 observations from a total of 486, since 55 trees were missing or dead. The model r^2 was 0.569. Site and provenance effects were significant ($\alpha=0.05$), while block (within site), site x provenance and block x provenance (within site) effects were not. Provenance $\delta^{13}\text{C}$ trends across sites are graphed in Figure 12.

The literature does not suggest specific tests to distinguish dependent (least squares) means, other than the Bryant-Paulson-Tukey test and conditional Tukey-Kramer test for adjusted means in one-way ANCOVAs (Day and Quinn 1989). I used Fisher's LSD test with an applied Bonferroni inequality to compare provenance LSMs (Miller 1981). The α level was decreased to 0.001 because 36 means comparisons were made for the analysis including all test sites; and 45, for the analyses including two sites. Lowering the comparisonwise error rate to 0.001 was an attempt to maintain the overall experimentwise error rate near 0.05, *e.g.*, $45 \times 0.001 = 0.045$. Bonferroni's procedure tested pairwise differences between sites, each site compared at a reduced α level of 0.01, again to keep the overall experimentwise error rate near 0.05. Holden Lake and 70 Mile House were different from Salmon Lake, but not different from each other.

Variance components derived according to the format in Table 7 showed that site and sampling error contributed most to the variation in $\delta^{13}\text{C}$ on a per sapling basis. Since the site x provenance component contributed nothing to the variation in $\delta^{13}\text{C}$ (in fact, it was negative), the test term used for means differences among populations was the block x provenance (within site) mean square (MS) rather than the site x provenance MS. The least significant difference (LSD) using block x provenance (within site) as the test term was 0.440 ‰. Table 8 shows provenance means test results. Provenances 61 and 104 were significantly different from provenances 69 and 30. As well, provenance 61 was different from provenances 71 and 1.

Table 7. ANOVA for provenance trials at 70 Mile House, Holden Lake and Salmon Lake. Variance components (VC) are presented as a percentage of total components.

Source	df	SS	MS	EMS	F Value	P > F	VC(%)
Site	2	80.5247	40.2623	$\sigma^2 + 7.7632\sigma_{BI*P(S)}^2 + 15.5264\sigma_{S*P}^2 + 69.8687\sigma_{BI(S)}^2 + 139.7374\sigma_S^2$	68.034	0.0208	47
Bl(S)	3	2.2591	0.7530	$\sigma^2 + 7.7764\sigma_{BI*P(S)}^2 + 69.9877\sigma_{BI(S)}^2$	2.032	0.1375	1
Prov	8	20.6984	2.5873	$\sigma^2 + 7.8192\sigma_{BI*P(S)}^2 + 15.6384\sigma_{S*P}^2 + 46.9151\sigma_P^2$	12.334	0.0001	8
S*P	16	3.3536	0.2096	$\sigma^2 + 7.8517\sigma_{BI*P(S)}^2 + 15.7034\sigma_{S*P}^2$	0.564	0.8809	0
Bl*P(S)	24	8.9348	0.3723	$\sigma^2 + 7.8828\sigma_{BI*P(S)}^2$	1.487	0.0673	3
Error	377	94.3909	0.2504	σ^2			41

Legend: BI=Block
S=Site
P=Provenance

Satterthwaite Approximations Used to Derive F Values:

Source: Site

Error: $0.9983*MS[Bl(S)] + 0.9887*MS(S*P) - 0.9848*MS[Bl*P(S)] - 0.0022*MS(Error)$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
2	40.2623	1.78	0.5918	68.034	0.0208

Table 7. (con't)

Satterthwaite Approximations Used to Derive F Values:

Source: Block(Site)

Error: $0.9865 \cdot \text{MS}[\text{B1} \cdot \text{P}(\text{S})] + 0.0135 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
3	0.7530	24.44	0.3706	2.032	0.1357

Source: Prov

Error: $0.9959 \cdot \text{MS}(\text{S} \cdot \text{P}) + 0.0041 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
8	2.5873	16.16	0.2098	12.334	0.0001

Source: Site*Prov

Error: $0.9961 \cdot \text{MS}[\text{B1} \cdot \text{P}(\text{S})] + 0.0039 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
16	0.2096	24.13	0.3718	0.564	0.8809

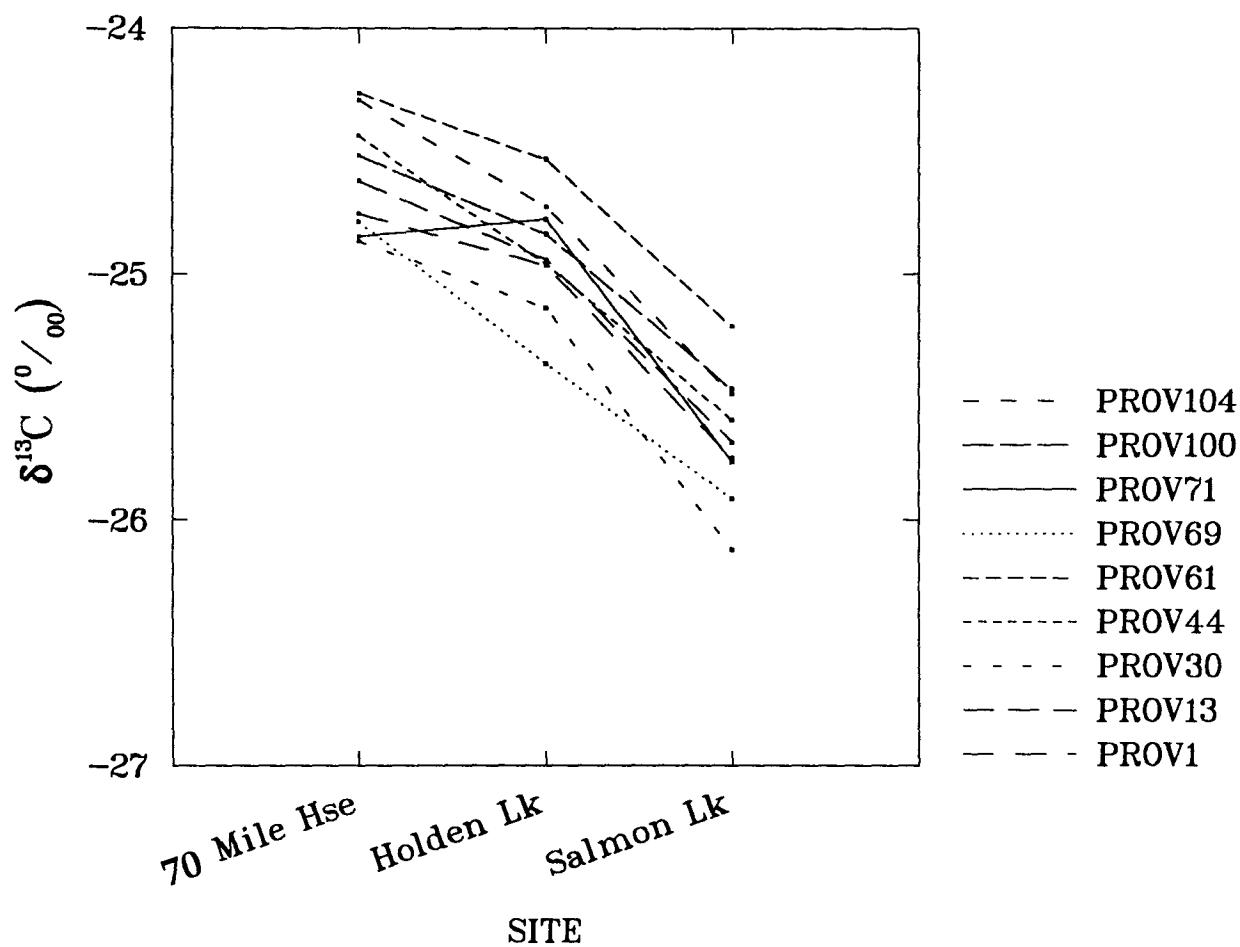


Fig. 12. Changes in provenance $\delta^{13}\text{C}$ least squares means among three sites – 70 Mile House, Holden Lake and Salmon Lake. Provenances respond similarly in degree and direction to changes in site moisture.

Table 8. Summary of test results for differences in $\delta^{13}\text{C}$ among nine provenances over all sites (70 Mile House, Holden Lake, Salmon Lake).

Provenance	61	104	100	44	13	71	1	69	30
LSM	-24.670	-24.835	-24.941	-24.997	-25.082	-25.129	-25.156	-25.355	-25.375

Note: Underscores show homogeneous subsets (comparisonwise error rate=0.001).
Fisher's Least Significant Difference=0.440 ‰.

The provenance effect in the ANOVA (Table 7) remained significant at $\alpha=0.05$ when the block x provenance (within site) MS was used rather than the site x provenance MS to derive the F value. This was the case for the other analyses as well (Tables 9 and 11), with changes to test terms.

Least squares means were also compared using the Tukey test since the Fisher's LSD with a comparisonwise error rate of 0.001 might miss real differences among provenances. Tests based on the studentized range (Tukey) assume that the means are independent. In unbalanced designs this assumption is violated to some degree. The Tukey test (experimentwise error rate=0.05) found a few more significant differences than the Fisher's LSD test. The Tukey test determined that provenances 61, 104 and 100 were different from provenances 69 and 30. As well, provenance 61 was different from provenances 13, 71 and 1.

Table 9 summarizes ANOVA results (all factors random) for the data from 70 Mile House and Holden Lake, where provenance 57 and all other provenances except 95 were tested. The variances of main effects were homogeneous (Bartlett's test). The analysis used 321 observations from a total of 360 (model $r^2 = 0.369$). Provenance and block x provenance (within site) effects were significant ($\alpha=0.05$) while site, block (within site) and site x provenance effects were not. Provenance $\delta^{13}\text{C}$ trends with site are graphed in Figure 13.

Variance components (Table 9) showed that site and provenance contributed 11 and 15% respectively to the variation in $\delta^{13}\text{C}$ on a per sapling basis. Sampling error contributed 65%. The site x provenance component contributed nothing to the variation, therefore the test term used for means differences among populations was block x provenance (within site). The least significant difference using block x provenance (within site) as the test term was 0.625 ‰. Table 10 shows means test results. Provenances 61 and 104 were different from provenance 57. As well, provenance 61 was different from provenance 69. The Tukey test showed a few more differences among provenances. Provenances 61, 104, 100 and 44 were different from provenance 57. As well, provenances 61 and 104 were different from

Table 9. ANOVA for provenance trials at 70 Mile House and Holden Lake. Variance components are presented as a percentage of total components.

Source	df	SS	MS	EMS	F Value	P > F	VC(%)
Site	1	7.5938	7.5938	$\sigma^2 + 7.7682\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 15.5360\sigma^2_{\text{S}*P} + 77.6820\sigma^2_{\text{Bl}(\text{S})} + 155.3600\sigma^2_{\text{S}}$	9.263	0.1534	11
Bl(S)	2	2.0048	1.0024	$\sigma^2 + 7.8133\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 78.1330\sigma^2_{\text{Bl}(\text{S})}$	2.208	0.1383	2
Prov	9	19.1918	2.1324	$\sigma^2 + 7.8634\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 15.7270\sigma^2_{\text{S}*P} + 31.4540\sigma^2_{\text{P}}$	7.760	0.0027	15
S*P	9	2.4371	0.2748	$\sigma^2 + 7.8634\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 15.7270\sigma^2_{\text{S}*P}$	0.604	0.7783	0
Bl*P(S)	18	8.2361	0.4576	$\sigma^2 + 7.9526\sigma^2_{\text{Bl}*\text{P}(\text{S})}$	1.816	0.0232	7
Error	281	70.7883	0.2519	σ^2			65

Legend: Bl=Block
S=Site
P=Provenance

Satterthwaite Approximations Used to Derive F Values:

Source: Site

Error: $0.9942*MS[\text{Bl}(\text{S})] + 0.9879*MS(\text{S}*P) - 0.9768*MS[\text{Bl}*P(\text{S})] - 0.0053*MS(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
1	7.5938	1.30	0.8198	9.263	0.1534

Table 9. (con't)

Satterthwaite Approximations Used to Derive F Values:

Source: Block(Site)

Error: $0.9825 \cdot MS[B1 \cdot P(S)] + 0.0175 \cdot MS(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
2	1.0024	18.35	0.4540	2.208	0.1383

Source: Prov

Error: $MS(\text{Site} \cdot \text{Prov})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
9	2.1324	9	0.2748	7.760	0.0027

Source: Site*Prov

Error: $0.9888 \cdot MS[B1 \cdot P(S)] + 0.0112 \cdot MS(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
9	0.2748	18.23	0.4553	0.604	0.7783

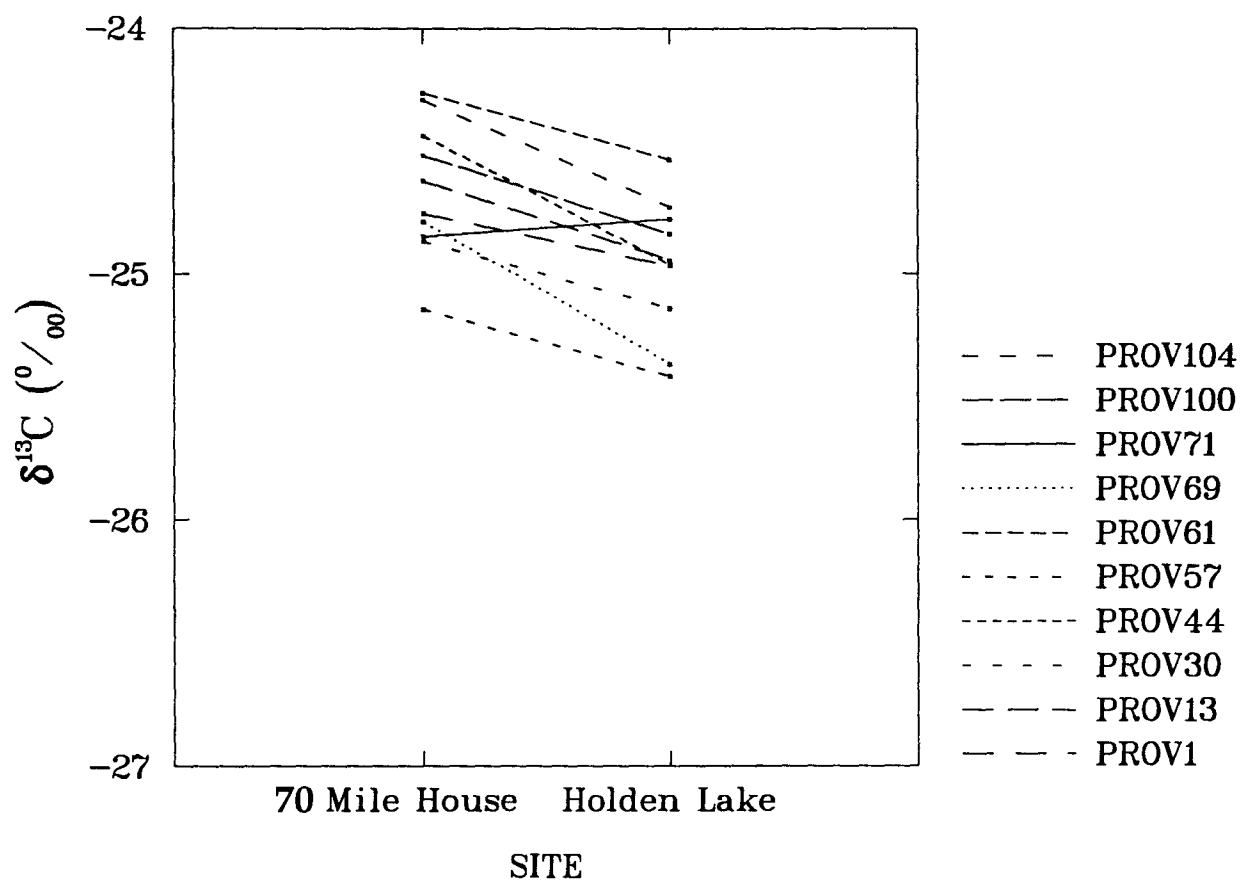


Fig. 13. Changes in provenance $\delta^{13}\text{C}$ least squares means between two sites – 70 Mile House and Holden Lake.

Table 10. Summary of test results for differences in $\delta^{13}\text{C}$ among ten provenances over two sites (70 Mile House, Holden Lake).

Provenance	61	104	100	44	13	71	1	30	69	57
LSM	-24.398	-24.509	-24.678	-24.699	-24.782	-24.812	-24.860	-25.003	-25.076	-25.280

Note: Underscores show homogeneous subsets (comparisonwise error rate=0.001).
Fisher's Least Significant Difference=0.625 ‰.

provenance 69. Provenance 61 was also different from provenance 30.

Table 11 summarizes ANOVA results for the data from Holden Lake and Salmon Lake (all factors random), where provenance 95 and all other provenances except 57 were tested. The variances of main effects were homogeneous (Bartlett's test). The analysis used 315 observations from a total of 360 (model $r^2 = 0.523$). Site, block (within site) and provenance effects were significant ($\alpha=0.05$); block x provenance (within site) and site x provenance effects were not. Provenance $\delta^{13}\text{C}$ trends with site are graphed in Figure 14.

The Bonferroni procedure was applied to pairwise comparisons between blocks (within sites). Although the F test found blocks different, pairwise comparisons did not. Huitema (1980) explains this apparent inconsistency. Frequency of false rejections of the null hypothesis is less with multiple comparison procedures than with the F test (Zar 1984).

Variance components showed that site and sampling error contributed most to the variation in $\delta^{13}\text{C}$ per sapling (43.5 and 41.5% respectively). Provenance contributed 14%. Since the site x provenance and block x provenance (within site) components contributed nothing to the variation, sampling error became the test term for means differences among provenances. Table 12 depicts means test results. The LSD was 0.399 ‰. Provenance 95 was different from all provenances except provenance 61. Provenances 61, 104 and 100 were different from provenances 30 and 69. As well, provenance 61 was different from provenances 1, 13 and 44. The Tukey test gave nearly the same result as Fisher's LSD test, the only difference being that provenance 61 was different from provenance 71 as well as provenances 1, 13 and 44.

Correlations between growth parameters (diameter, height, volume) and $\delta^{13}\text{C}$ were site- and provenance-dependent. (Growth measurements were not available for provenance 95). Significant correlations ($\alpha=0.05$) are discussed below. If α reflected the number of correlations tested (87), none would be significant (the "p" value required for a significant pairwise correlation would be 0.0006). All correlations are listed in Appendix 1.

Table 11. ANOVA for provenance trials at Holden Lake and Salmon Lake. Variance components are presented as a percentage of total components.

Source	df	SS	MS	EMS	F Value	P > F	VC(%)
Site	1	41.0215	41.0215	$\sigma^2 + 7.3049\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 14.6100\sigma^2_{\text{S}*P} + 73.0490\sigma^2_{\text{Bl}(\text{S})} + 146.1000\sigma^2_{\text{S}}$	63.954	0.0175	43.5
Bl(S)	2	1.2993	0.6497	$\sigma^2 + 7.3058\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 73.0580\sigma^2_{\text{Bl}(\text{S})}$	3.469	0.0501	1
Prov	9	26.3096	2.9233	$\sigma^2 + 7.5547\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 15.1090\sigma^2_{\text{S}*P} + 30.2190\sigma^2_{\text{P}}$	16.594	0.0001	14
S*P	9	1.5855	0.1762	$\sigma^2 + 7.5547\sigma^2_{\text{Bl}*\text{P}(\text{S})} + 15.1090\sigma^2_{\text{S}*P}$	0.954	0.5047	0
Bl*P(S)	18	3.2983	0.1832	$\sigma^2 + 7.6880\sigma^2_{\text{Bl}*\text{P}(\text{S})}$	0.692	0.8185	0
Error	275	72.8135	0.2648	σ^2			41.5

Legend: Bl=Block
S=Site
P=Provenance

Satterthwaite Approximations Used to Derive F Values:

Source: Site

Error: $0.9999*MS[\text{Bl}(\text{S})] + 0.9669*MS(\text{S}*P) - 0.9502*MS[\text{Bl}*P(\text{S})] - 0.0166*MS(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
1	41.0215	1.91	0.6414	63.954	0.0175

Table 11. (con't)

Satterthwaite Approximations Used to Derive F Values:

Source: Block(Site)

Error: $0.9503 \cdot \text{MS}[\text{Bl} \cdot \text{P}(\text{S})] + 0.0497 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
2	0.6497	20.82	0.1873	3.469	0.0501

Source: Prov

Error: $\text{MS}(\text{Site} \cdot \text{Prov})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
9	2.9233	9	0.1762	16.594	0.0001

Source: Site*Prov

Error: $0.9827 \cdot \text{MS}[\text{Bl} \cdot \text{P}(\text{S})] + 0.0173 \cdot \text{MS}(\text{Error})$

DF	MS	Denominator DF	Denominator MS	F Value	P > F
9	0.1762	18.93	0.1846	0.954	0.5047

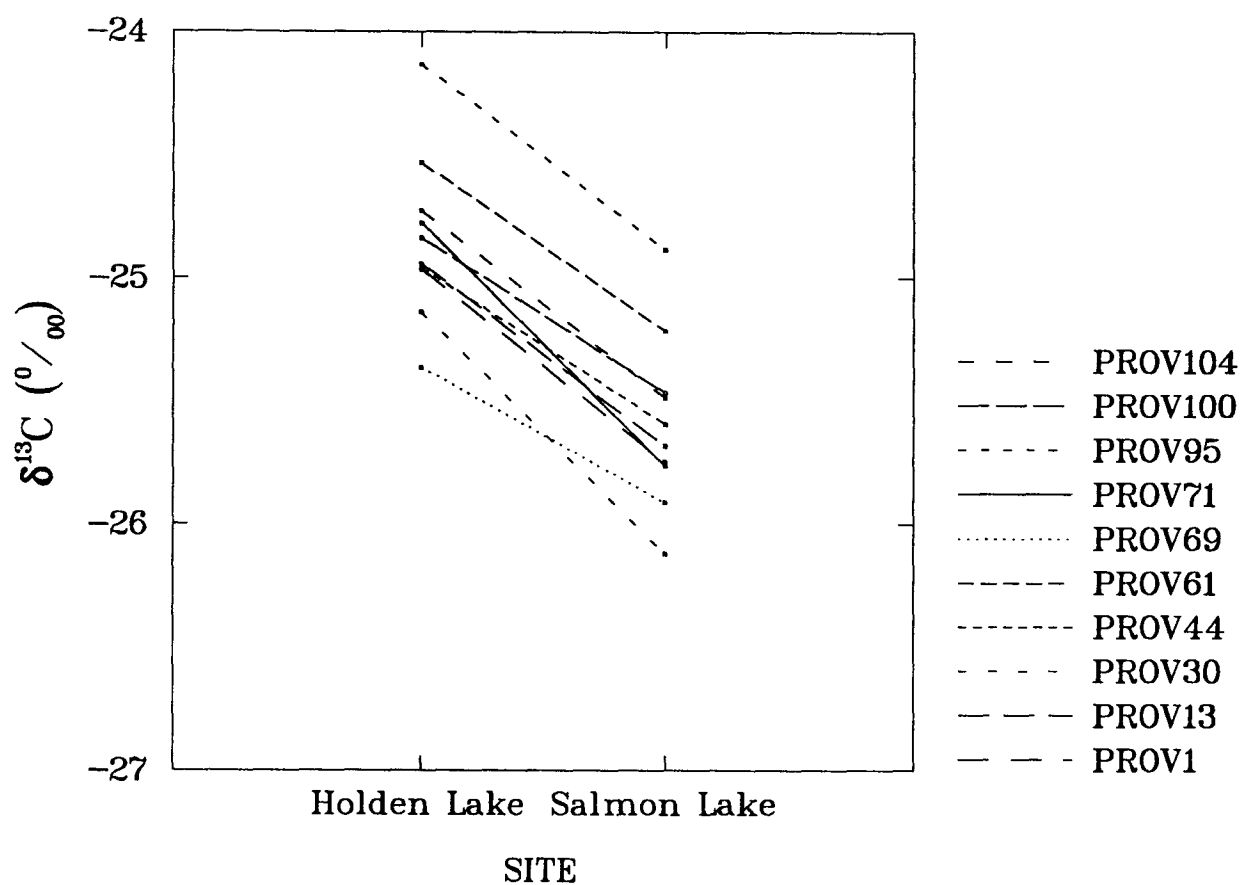


Fig. 14. Changes in provenance $\delta^{13}\text{C}$ least squares means between two sites – Holden Lake and Salmon Lake.

Table 12. Summary of test results for differences in $\delta^{13}\text{C}$ among ten provenances over two sites (Holden Lake, Salmon Lake).

Provenance	95	61	104	100	71	44	13	1	30	69
LSM	-24.508	-24.873	-25.107	-25.152	-25.271	-25.276	-25.312	-25.357	-25.629	-25.638

Note: Underscores show homogeneous subsets (comparisonwise error rate=0.001).
Fisher's Least Significant Difference=0.399 ‰.

At 70 Mile House, all growth variables and $\delta^{13}\text{C}$ were negatively correlated in provenance 71 (diameter: $r=-0.645$, $p=0.007$; height: $r=-0.720$, $p=0.002$; volume: $r=-0.679$, $p=0.004$). Provenance 104 showed positive correlations between all growth variables and $\delta^{13}\text{C}$ (diameter: $r=0.574$, $p=0.013$; height: $r=0.540$, $p=0.021$; volume: $r=0.547$, $p=0.019$). It appeared that in provenance 71, decreased WUE (measured relatively by $\delta^{13}\text{C}$) was accompanied by increased growth, while the opposite occurred in provenance 104. Interpretations had to be site specific, however. Provenance 71 showed positive correlations between growth and $\delta^{13}\text{C}$ at Holden Lake and Salmon Lake (non-significant).

At Holden Lake, height and $\delta^{13}\text{C}$ were positively correlated in provenance 30 ($r=0.463$, $p=0.053$). All growth parameters correlated positively with $\delta^{13}\text{C}$ in provenance 44 (diameter: $r=0.512$, $p=0.043$; height: $r=0.681$, $p=0.004$; volume: $r=0.554$, $p=0.026$), as well as in provenance 57 (diameter: $r=0.745$, $p=0.001$; height: $r=0.475$, $p=0.054$; volume: $r=0.671$, $p=0.003$). At Holden Lake, it appeared that growth improved with increased WUE in these provenances.

At Salmon Lake, diameter and volume correlated negatively with $\delta^{13}\text{C}$ in provenance 61 (diameter: $r=-0.652$, $p=0.008$; volume: $r=-0.596$, $p=0.019$). All growth parameters correlated positively with $\delta^{13}\text{C}$ in provenance 30 (diameter: $r=0.547$, $p=0.019$; height: $r=0.650$, $p=0.004$; volume: $r=0.556$, $p=0.017$), as well as in provenance 44 (diameter: $r=0.600$, $p=0.018$; height: $r=0.699$, $p=0.004$; volume: $r=0.672$, $p=0.006$). Decreased WUE was accompanied by increased growth in provenance 61, while the opposite occurred in provenances 30 and 44.

Provenances 13, 69 and 100 demonstrated no significant relationships between growth factors and $\delta^{13}\text{C}$ at any site.

Only one provenance had significant relationships between all growth variables and $\delta^{13}\text{C}$ on more than one site. In provenance 44, diameter, height and volume were positively correlated with $\delta^{13}\text{C}$ at Holden Lake and Salmon Lake.

Of interest was whether provenances with more positive $\delta^{13}\text{C}$ values (assumed to be more water-use efficient) produced higher volumes. Per site, correlations between provenance $\delta^{13}\text{C}$ LSMs and volume LSMs were positive: 1. 70 Mile House ($r=0.608$, $p=0.062$, $n=10$); 2. Holden Lake ($r=0.759$, $p=0.011$, $n=10$); 3. Salmon Lake ($r=0.689$, $p=0.040$, $n=9$). Considering average performance over three sites, the correlation between $\delta^{13}\text{C}$ LSMs and volume LSMs was 0.727 ($p=0.027$, $n=9$).

At the provenance level there were significant correlations ($\alpha=0.1$) between $\delta^{13}\text{C}$ and annual climate variables at all sites. At 70 Mile House, $\delta^{13}\text{C}$ correlated with mean temperature of the warmest month (mtwm) ($r=-0.794$). At Holden Lake, $\delta^{13}\text{C}$ correlated with mtwm ($r=-0.667$), mean summer precipitation (msp) ($r=0.737$), mean precipitation of the driest month (mpdm) ($r=0.715$) and mean annual precipitation (map) ($r=0.730$). The coastal provenance (95) had large influence at this site. Removing provenance 95 eliminated the significant correlations between isotope ratios and precipitation variables. At Salmon Lake, $\delta^{13}\text{C}$ correlated with mtwm ($r=-0.786$), mean annual temperature (mat) ($r=0.739$), msp ($r=0.750$), mpdm ($r=0.783$) and map ($r=0.753$). Removing provenance 95 eliminated the significant correlations between $\delta^{13}\text{C}$ and the precipitation variables.

At the provenance level, isotopic composition was related to geographic variables at two sites. At Holden Lake, the correlation between $\delta^{13}\text{C}$ and longitude was 0.822 ($p=0.023$) while that between $\delta^{13}\text{C}$ and elevation was -0.713 ($p=0.072$). At Salmon Lake $\delta^{13}\text{C}$ correlated with elevation ($r=-0.711$, $p=0.114$). These correlations were high ($\alpha=0.1$) due to the influence of provenance 95.

Since isotope ratios were influenced by several climate variables, regression modelling determined the factors contributing most to the prediction of $\delta^{13}\text{C}$. The geographic variables were not applied to the regression equations, since longitude and elevation were highly correlated with precipitation. Latitude was highly correlated with mean annual temperature. As well, longitude, latitude and elevation were highly correlated with each other. Initially a common model was sought for all sites. One model including

mtwm and msp was appropriate for sites with provenance 95, while another including mat and msp was suited for 70 Mile House and the other sites without provenance 95. The comparison was necessary, since provenance 95 had excessive influence.

Collinearity problems were small for the climate factors. Per site, mtwm and msp (at the provenance level) had a correlation of about 0.318 ($p < 0.05$). The correlation became negative when provenance 95 was included. The r value for mat and msp was about 0.311 ($p < 0.05$) without provenance 95. Including 95 increased the correlation to 0.477 at Holden Lake and 0.696 at Salmon Lake (both at $p < 0.05$).

To find models suitable for the complete set of data points (with and without provenance 95), indicator variables representing site (S_1 , S_2) were included: $S_1=0$, $S_2=0$ for 70 Mile House; $S_1=1$, $S_2=0$ for Holden Lake; and $S_1=0$, $S_2=1$ for Salmon Lake. The advantage of having replication with regression was the opportunity to test for lack of fit of the models (Ott 1988; Bergerud 1990). The best fitting regression model for the data set including provenance 95 was $\delta^{13}\text{C} = -22.607 - 0.365S_1 - 1.133S_2 - 0.158\text{MTWM} + 0.001\text{MSP}$ ($r^2=0.450$; $p < 0.0001$; $df=5, 284$). The lack-of-fit test was done by separating the residual sums of squares into two parts: a lack-of-fit sums of squares ($df=14$) and a within group sums of squares called the pure error ($df=271$) (see Appendix 3). The test for lack-of-fit was significant ($\alpha=0.05$); the model was inadequate for predictive purposes. However the model was useful in describing a relationship between $\delta^{13}\text{C}$, temperature and precipitation. The best fitting model for the data set without provenance 95 was $\delta^{13}\text{C} = -27.970 - 0.380S_1 - 1.103S_2 + 1.756\text{MAT} + 0.015\text{MSP} - 0.007\text{MAT}*\text{MSP}$ ($r^2=0.476$; $p < 0.0001$; $df=5, 258$). The test for lack-of-fit was not significant (Appendix 3), indicating that the model was adequate for predictive purposes. Standardized coefficients from the regression output showed that the mat variable had six times the influence on $\delta^{13}\text{C}$ as the msp variable and that the interaction between the two had the greatest influence. The equation showed that the slope of the relationship between $\delta^{13}\text{C}$ and mat depends on msp and vice versa. The two variables interact to negatively affect isotopic composition. For example, depending on what

the msp is, WUE can increase or decrease with increasing temperature. The equation appropriate to 70 Mile House shows that the change in mean $\delta^{13}\text{C}$ per $^{\circ}\text{C}$ increase in mat is given by $1.756 - 0.007\text{MSP}$. For 300 mm msp, the estimated change in $\delta^{13}\text{C}$ per $^{\circ}\text{C}$ increase in mat is -0.344‰ . For 150 mm msp, the estimated change in $\delta^{13}\text{C}$ per $^{\circ}\text{C}$ increase in mat is $+0.706\text{‰}$. Under dry conditions $\delta^{13}\text{C}$ is more positive, indicating higher intrinsic WUE. It is apparent also that as mat decreases, WUE will increase under conditions of increasing moisture.

4.3 Seedling Experiment

Summary statistics (Table 13) show little variation among provenance biomass means other than shoot/root ratio. The range for shoot weight was 0.186 g; for root weight, 0.076 g; for shoot+root weight, 0.207 g; and for shoot/root ratio, 0.448. The range for $\delta^{13}\text{C}$ was 1.648 ‰.

Shoot variances were heterogeneous (Bartlett $\chi^2=27.985$, $p=0.002$; Cochran's C statistic $0.1947 >$ Cochran's critical value 0.1678). These heterogeneities were not remedied by transformations (log, square root, $1/y^2$, y^2). Residuals analysis showed nine outliers. Removing one (1.316 g, prov. 95) decreased variance heterogeneity (Bartlett $p=0.01$; Cochran's C statistic $0.1663 <$ Cochran's critical value 0.1678). Since non-parametric (Kruskal-Wallis) analysis of the complete data set gave the same result as ANOVA on the data set, minus the outlier, no further changes were made to shoot data prior to the F test. As well, the outlier was removed from the remaining data sets. Table 14 shows ANOVA results for seedling data. Shoot means were significantly different ($\alpha=0.05$, $p=0.001$). Tukey's test (Table 15) found provenance 30, with lowest shoot weight, different from provenances 61, 1, 13, 44 and 57. Provenances 69, 71, 104, 95 and 100 formed subsets with provenances 61, 1, 13, 44 and 57, as well as provenance 30.

Root variances were heterogeneous (Bartlett $\chi^2=19.170$, $p=0.038$). The Cochran test, less sensitive to non-normality than Bartlett's test, showed homogeneity (Cochran's C

Table 13. Summary statistics for growth parameters of eleven *Pinus contorta* seedling provenances.

	Provenance										
	1	13	30	44	57	61	69	71	95 ^a	100	104
<i>Shoot Weight (g)</i>											
Mean	0.753	0.753	0.575	0.741	0.740	0.761	0.673	0.654	0.622	0.620	0.654
S D	0.189	0.216	0.172	0.249	0.141	0.189	0.158	0.174	0.276	0.180	0.224
<i>Root Weight (g)</i>											
Mean	0.262	0.257	0.247	0.228	0.257	0.235	0.257	0.253	0.186	0.224	0.209
S D	0.058	0.078	0.052	0.076	0.061	0.085	0.067	0.075	0.096	0.080	0.089
<i>Shoot + Root Weight (g)</i>											
Mean	1.015	1.010	0.821	0.969	0.997	0.995	0.930	0.907	0.808	0.844	0.863
S D	0.220	0.274	0.205	0.314	0.174	0.254	0.199	0.225	0.358	0.246	0.298
<i>Shoot / Root Ratio</i>											
Mean	2.964	3.056	2.348	3.280	2.984	3.494	2.727	2.756	3.703	2.936	3.391
S D	0.736	0.748	0.596	0.655	0.637	0.978	0.760	0.841	1.066	0.678	0.935
<i>δ¹³C</i>											
Mean	-31.628	-31.861	-31.939	-31.660	-31.870	-31.630	-31.966	-31.882	-30.318	-31.698	-31.803
S D	0.868	0.627	0.628	0.848	0.559	0.806	0.647	0.905	0.943	0.809	0.852

^an=29 from a maximum of 30

Table 14. One-way ANOVAs on growth parameters for *Pinus contorta* seedling data.

	Source	df	SS	MS	F Value	P > F
<i>Shoot Weight</i>	Between Prov	10	1.304	0.130	3.246	0.0010
	Within Prov	318	12.776	0.040		
<i>Root Weight</i>	Between Prov	10	0.172	0.017	3.044	0.0010
	Within Prov	318	1.800	0.006		
<i>Shoot + Root Weight</i>	Between Prov	10	1.860	0.186	2.824	0.0020
	Within Prov	318	20.949	0.066		
<i>Shoot/Root Ratio (Natural Logarithms)</i>	Between Prov	10	4.895	0.490	7.525	<<0.0001
	Within Prov	318	20.688	0.065		
$\delta^{13}C$	Between Prov	10	62.058	6.206	10.168	<<0.0001
	Within Prov	318	194.093	0.610		

Table 15. Summary of Tukey test results for means differences among seedling shoot weights (g).

Prov	61	1	13	44	57	69	71	104	95	100	30
Mean	0.761	0.753	0.753	0.741	0.740	0.673	0.654	0.654	0.645	0.620	0.575
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Note: Underscores show homogeneous subsets (experimentwise error rate=0.05).

statistic $0.1429 < \text{Cochran's critical value } 0.1678$). Therefore, data were not changed prior to ANOVA, which gave a significant result for root means ($p=0.001$). Provenance 95, with lowest root weight, was different from provenances 1, 13, 57, 69 and 71 (Table 16).

Shoot+root variances were heterogeneous (Bartlett $\chi^2=26.703$, $p=0.003$; Cochran's C statistic $0.1763 > \text{Cochran's critical value } 0.1678$). Transforming data or removing two extreme outliers did not correct the problem. Since non-parametric analysis produced the same result as ANOVA, the data were not changed. Although ANOVA gave a significant result for shoot+root growth ($p=0.002$), Tukey's test revealed no means differences.

Shoot/root ratio variances were heterogeneous (Bartlett $\chi^2=20.784$, $p=0.023$). Cochran's C statistic, 0.1623 , was less than Cochran's critical value, 0.1678 , but the difference was too small to discount a heterogeneity problem. Homogeneity was achieved by natural log transformation of the data (Bartlett $p=0.858$). The F test was significant ($p<<0.0001$). The Tukey test (Table 17) showed that provenances 95, 61 and 104 were different from provenances 71, 69 and 30. As well, provenance 95 was different from provenances 1 and 100.

Isotope variances were homogeneous (Bartlett $\chi^2=17.225$, $p=0.070$) and significantly different ($p<<0.0001$). Tukey's test (Table 18) found two subsets: (provenance 95) and (provenances 1, 13, 30, 44, 57, 61, 69, 71, 100, 104).

Correlations between growth variables of all provenances' pooled seedlings ($n=329$) and $\delta^{13}\text{C}$ were poor: 1. Shoot mass ($r=0.014$, $p=0.807$); 2. Root mass ($r=0.041$, $p=0.464$); 3. Shoot+Root ($r=0.023$, $p=0.684$); 4. Shoot/Root ratio ($r=0.016$, $p=0.772$). Individually, few provenances showed significant correlations ($\alpha=0.05$) between growth variables and $\delta^{13}\text{C}$ (see Appendix 2). If α reflected the number of correlations tested (44), none would be significant (the "p" value required for a significant correlation would be 0.001). Shoot weight, as well as shoot/root ratio and $\delta^{13}\text{C}$ correlated significantly in provenance 57 ($r=-0.364$, $p=0.048$ and $r=-0.369$, $p=0.045$ respectively). The shoot/root ratio and $\delta^{13}\text{C}$ correlation in provenance 69 was $r=-0.467$ ($p=0.009$). Root weight, as well as

Table 16. Summary of Tukey test results for means differences among seedling root weights (g).

Prov	1	13	57	69	71	30	61	44	100	104	95
Mean	0.262	0.257	0.257	0.257	0.253	0.247	0.235	0.228	0.224	0.209	0.189
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Note: Underscores show homogeneous subsets (experimentwise error rate=0.05).

Table 17. Summary of Tukey test results for means differences among seedling shoot/root ratios (natural logarithms).

Prov	95	61	104	44	13	57	1	100	71	69	30
Mean	1.270	1.214	1.185	1.168	1.087	1.070	1.057	1.051	0.974	0.969	0.822
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Note: Underscores show homogeneous subsets (experimentwise error rate=0.05).

Table 18. Summary of Tukey test results for means differences among seedling $\delta^{13}\text{C}$ values.

	95	1	61	44	100	104	13	57	71	30	69
Prov Mean	-30.318	-31.628	-31.630	-31.660	-31.698	-31.803	-31.861	-31.870	-31.882	-31.939	-31.966
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Note: Underscores show homogeneous subsets (experimentwise error rate=0.05).

shoot/root ratio, and $\delta^{13}\text{C}$ correlations were significant in provenance 71 ($r=0.405$, $p=0.026$ and $r=-0.410$, $p=0.024$ respectively). Correlations were positive as well as negative, depending on provenance and growth variable tested. All provenances showed positive correlations between root weight and isotope ratio. All but provenances 61 and 104 showed negative correlations between shoot/root ratio and $\delta^{13}\text{C}$. Correlations between population averages of $\delta^{13}\text{C}$ and root weight, as well as shoot/root ratio were significant ($r=-0.754$, $p=0.007$ and $r=0.680$, $p=0.021$ respectively). Interestingly, the correlations between $\delta^{13}\text{C}$ and biomass variables (root, shoot/root) for seedlings within populations were opposite in sign to correlations using seedling population means. Correlations between population averages of $\delta^{13}\text{C}$ and shoot weight, as well as total weight were insignificant ($r=-0.188$, $p=0.580$ and $r=-0.386$, $p=0.241$ respectively).

Of interest was the correlation between seedling and sapling $\delta^{13}\text{C}$. Over all sites (minus provenances 57 and 95), seedling $\delta^{13}\text{C}$ means and sapling $\delta^{13}\text{C}$ LSMs correlated by 0.690 ($p=0.040$, $n=9$) (Fig. 15). At Holden Lake, where all provenances were tested, r equalled 0.781 ($p=0.005$, $n=11$) (Fig. 16). At Salmon Lake and 70 Mile House the correlations were 0.812 ($p=0.004$, $n=10$) and 0.591 ($p=0.072$, $n=10$), respectively. Spearman's rank correlations did not improve any relationships other than the finding for 70 Mile House, where the correlation coefficient became 0.673 (significant at $\alpha=0.05$).

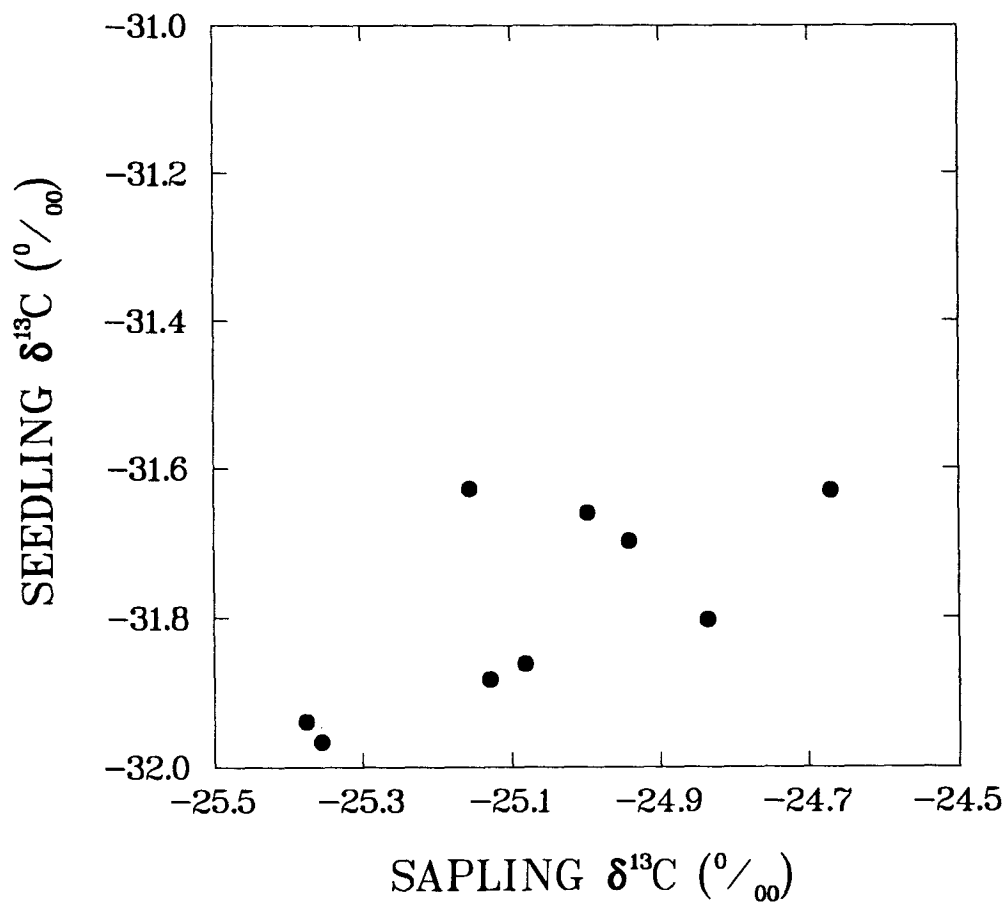


Fig. 15. *Pinus contorta* greenhouse seedling $\delta^{13}\text{C}$ means plotted against sapling least squares means averaged over three sites – 70 Mile House, Holden Lake and Salmon Lake. Provenances 95 and 57 are not included. Correlation coefficient $r=0.690$ ($p=0.040$).

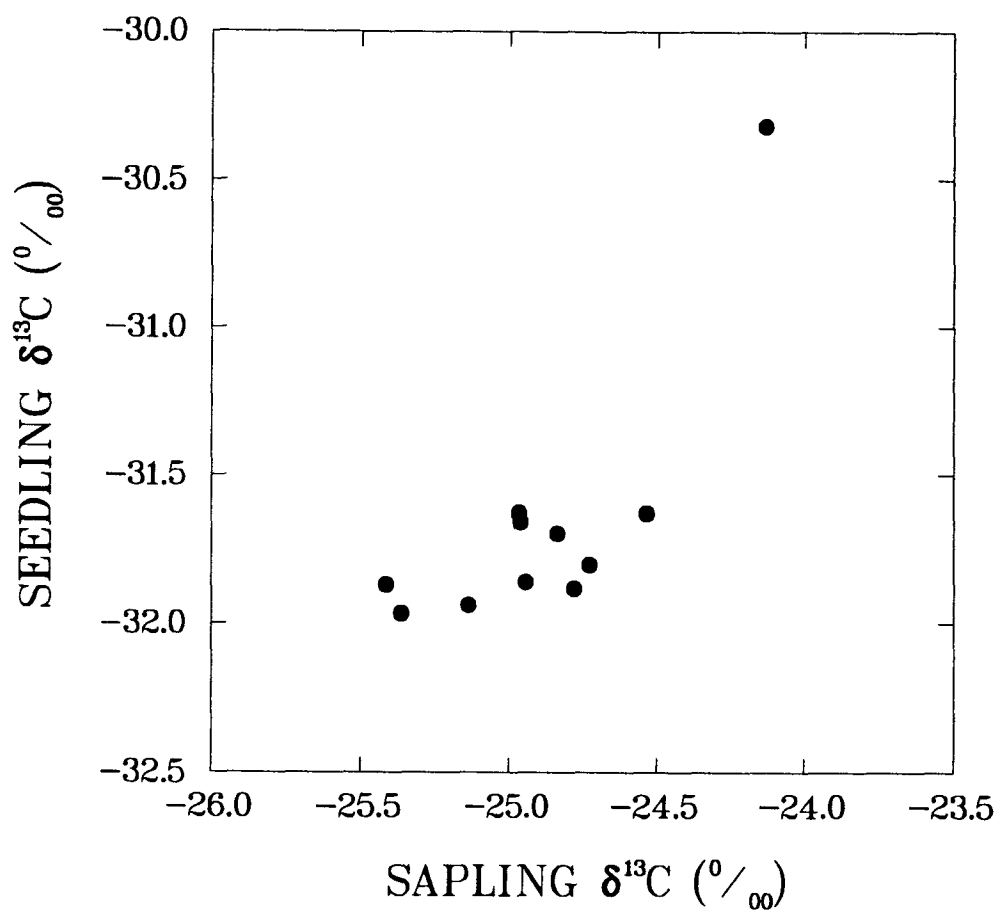


Fig. 16. *Pinus contorta* greenhouse seedling $\delta^{13}\text{C}$ means plotted against sapling least squares means at Holden Lake (where all provenances were tested). Correlation coefficient $r=0.781$ ($p=0.005$). The outlier is provenance 95.

5.0 Discussion

This study is based on the assumption that $\delta^{13}\text{C}$ correlates with WUE in trees. Traditional measures of WUE in agricultural crops have been significantly correlated with $\delta^{13}\text{C}$, but these calibrations have not been done for trees. Recently, however, instantaneous WUE (A/E) and intrinsic WUE (A/g) have been positively correlated with $\delta^{13}\text{C}$ in *Pinus* seedlings and tropical forest shrubs (Elsik *et al.* 1992; Jackson *et al.* 1992).

5.1 Determination of Sampling Methods for Field Trials

Intra- and inter-sapling $\delta^{13}\text{C}$ variation has not been reported. My data show that carbon isotope trends in saplings generally follow those for mature trees. This suggests that sampling methods developed for mature trees may be applied to younger trees. However, saplings usually show less $\delta^{13}\text{C}$ variation within and among individuals. The results of Freyer and Belacy (1983) and Francey (1981) suggest there is little circumferential variation in the early decades of growth, prior to crown enlargement and the development of associated microenvironmental differences.

Around the trunk, the mean stump wood variance within saplings is 0.09 ‰ (1989 samples, n=5 trees). At 1.2 m, the variance is 0.09 ‰. For needles, the mean intra-sapling variance (lowest branches) is 0.68 ‰ (1989 samples, n=5 trees). The circumferential variance for $\delta^{13}\text{C}$ in wood of mature trees has not been reported, although a range of 0.5 to 1.2 ‰ at ≈ 1.5 to 2.5 m height is given for *Pinus edulis* (Leavitt and Long 1986). In the *Pinus contorta* saplings, at about the same height, the range is 0.03 to 1.10 ‰. For needles of mature trees, Leavitt and Long (1986) cite studies showing variances of 0.38 to 0.46 ‰ in *Juniperus* species and 0.38 ‰ in *Pinus edulis*.

For *P. contorta* saplings, the intra-site variance in stump wood (1989) is 0.12 ‰ (four observations per tree, n=5 trees). The variance in needles (lowest branch) is 0.26 ‰ (four observations per tree, n=5 trees). Intra-site variance in wood of mature trees has not been reported. However, the range for $\delta^{13}\text{C}$ among eight *P. edulis* has been given as 2.0 to

3.0 ‰ (Leavitt and Long 1984). Each tree was represented by a pooled sample of four orthogonal cores comprised of seventeen 5-year ring groups. The range of $\delta^{13}\text{C}$ among saplings (16 data points per tree representing four orthogonal cores) is 0.34 to 0.98 ‰. The variance in needles for mature *Juniperus* species (two to four trees per site) is given as 0.38 ‰ (Arnold 1979, cited in Leavitt and Long 1986).

To find a sampling technique to accurately reflect field $\delta^{13}\text{C}$, I used a statistical method lacking rigor. There were no replicates within years, making the error term irretrievable in all analyses of variance. However, sufficient information was gained about $\delta^{13}\text{C}$ variation within and among the saplings at Juliet Creek. The information was adequate to select a technique lending itself to less sampling error than alternative ones (*e.g.*, taking east-west cores or using needles instead of wood as the sampling material). The chosen field sampling technique was to obtain north-south wood stem cores.

In Table 3, the assumption of non-significance for the three-way interaction used as the error term is questionable. However, figures 4 and 5 validate the F tests determined by that error term. In Tables 4 and 5, the three-way interactions are probably insignificant. The two-way interactions comprising them are insignificant in all but one case; therefore, the F tests are considered valid.

In the saplings, the tree $\delta^{13}\text{C}$ effect (stump wood) as well as tree x aspect interactions were significant. This finding supported Leavitt's and Long's (1984) recommendation that at least four orthogonal cores from each of four trees be sampled to accurately represent site $\delta^{13}\text{C}$ values.

To determine what influenced the tree $\delta^{13}\text{C}$ differences, I tested correlations between tree $\delta^{13}\text{C}$ and features which might contribute to the variation. Isotopic compositions for wood based on total observations per tree ($n=16$) had no significant correlations with height, age, percentage canopy cover, number of neighbors within a 3 m radius or average proximity to neighbors. The same lack of correlation was found for $\delta^{13}\text{C}$ means from north and south aspects combined ($n=8$). Isotopic compositions in both cases were positively correlated with

diameter (for all data points, $r=0.891$, $p=0.043$, $n=16$; for north and south cores, $r=0.873$, $p=0.053$, $n=8$). Needle $\delta^{13}\text{C}$, derived from averaging 1989 north and south values at stump level correlated positively with diameter ($r=0.937$, $p=0.019$), height ($r=0.941$, $p=0.017$) and average proximity to neighbors ($r=0.918$, $p=0.028$). From the average of four circumferential values (1989), needle $\delta^{13}\text{C}$ correlated positively with proximity to neighbors ($r=0.983$, $p=0.003$). Needle $\delta^{13}\text{C}$ at stump level is related to certain features, whereas wood $\delta^{13}\text{C}$ is not. The difference may be artificial since wood $\delta^{13}\text{C}$ is estimated from more data points than needle $\delta^{13}\text{C}$. Using wood samples from 1989 only, as was done for needles, shows a significant negative correlation between circumferential wood $\delta^{13}\text{C}$ and percentage canopy cover ($r=-0.932$, $p=0.021$). The correlations between needle $\delta^{13}\text{C}$ and tree height, as well as proximity to neighbors, and that between wood $\delta^{13}\text{C}$ and percentage canopy cover may reflect a shading effect. In 1984, Leavitt and Long compared $\delta^{13}\text{C}$ trends over 5 year intervals in mature *P. edulis*. Using four orthogonal cores per tree, they found no relationship between absolute $\delta^{13}\text{C}$ among trees and age, height, diameter, crown shapes or proximity to neighbors.

Tree x aspect effects may have been due to variable shading from neighbors. Saplings had neighbors of different crowns and heights as well as at varying distances and directions. Measuring duration or intensity of irradiance at each aspect over the growing season may have illuminated the "variable shading - variable $\delta^{13}\text{C}$ " hypothesis. However, the assumption would have had to be a relatively constant irradiance difference among aspects (within and among trees) over many years. Trees 3 and 4 contributed most to the interaction effect. They had very suppressed growth relative to the others. Many of their lower branches had been browsed. Trees 3 and 4 had taller neighbors to the east-south-east than the others and therefore, less estimated open canopy. Their south sides were more negative in $\delta^{13}\text{C}$ than their north sides, a trend opposite to that in the others.

Stomatal and photosynthetic effects influence the relationship between $\delta^{13}\text{C}$ and plant irradiance. The sunny side of a plant is likely to be more drought-stressed than the shady

side. Leaf stomates will remain more closed in the sun than in the shade relative to photosynthetic rate. Intercellular p_i/p_a in the sunlit leaves will be less than that for shaded leaves. With a lower p_i/p_a , discrimination against ^{13}C by photosynthetic enzymes will be less than that occurring at a higher p_i/p_a . Increased $\delta^{13}\text{C}$, positively related to intrinsic WUE, will be found in leaf samples from the sunlit side. Also, decreased illumination of a plant will result in decreased photosynthetic capacity. If photosynthetic capacity is relatively higher on the illuminated side (stomatal control being constant), p_i/p_a will decrease in the intercellular spaces of sunlit leaves. Leaf samples from more illuminated sides of the plant will record higher $\delta^{13}\text{C}$ values than those from less illuminated areas.

The saplings at Juliet Creek were relatively open-growing. There appeared to be no soil respiratory CO_2 effect on needle $\delta^{13}\text{C}$ at stump level (Table 5). It was likely, therefore, that wood $\delta^{13}\text{C}$ accurately reflects the physiological functioning of the trees rather than within-canopy gradients in $^{13}\text{CO}_2$. This assumption was applied to the field trials, where saplings were also relatively open-grown.

It appears that circumferential $\delta^{13}\text{C}$ in sapling stump wood follows no particular trend with aspect whereas $\delta^{13}\text{C}$ in leaves changes more obviously with aspect. If photosynthates are laid down spirally in tree trunks, they may reflect $\delta^{13}\text{C}$ values of carbon fixed in leaves at different sides of the tree. Some degree of spiral grain is a normal feature of wood formation in trees (Harris 1989). A spiral consistent in direction and degree throughout a stem is seldom seen. Leavitt's and Long's finding (1982) that needles and wood followed similar $\delta^{13}\text{C}$ trends on a monthly basis during one growing season were based on samples from one aspect only. An explanation for the different needle and wood $\delta^{13}\text{C}$ trends might be Schleser's (1992) finding that large contributions of carbon lower in the tree may originate from the top of the tree. Since a $\delta^{13}\text{C}$ gradient in leaves with height is not reflected in trunk wood (Schleser 1992), it is expected that circumferential values of $\delta^{13}\text{C}$ for needles and wood at nearly the same height will not correlate well.

The accuracy of the F test for the aspect effect (wood, Table 3) can be questioned since one of the test terms (tree x aspect) is significant. Figure 8 supports the finding of a non-significant aspect effect by showing similar trends for $\delta^{13}\text{C}$ values in wood over aspects. Analyses of variance on the tree, aspect and material effects (Table 4) and on the aspect, position and material effects in one year (Table 5) support the finding of a non-significant aspect effect for wood $\delta^{13}\text{C}$ as well.

Leavitt and Long (1984) recommend a reliable sampling technique for tree-ring isotope studies reconstructing climate and past atmospheric CO_2 . In studies like this one, however, extensive sampling may not be possible or necessary. The sampling protocol I established for *P. contorta* saplings may not apply to all species and environments but is reasonable for population studies on young pines. Wood is a more appropriate sampling material than needles since $\delta^{13}\text{C}$ variation within and among trees is smaller in wood than in needles. Some studies have used needles from tree tops but in this study the inter-sapling $\delta^{13}\text{C}$ variance shown by top needles (1989) was 0.83 ‰ (one data point per sapling), compared with a variance of 0.13 ‰ for 1989 stump wood (north and south aspects averaged per sapling).

5.2 Determination of Genetic Variation in WUE Among Sapling Provenances

There are genetic differences in relative WUE (determined by $\delta^{13}\text{C}$) among *Pinus contorta* populations. This genetic variation validates Rehfeldt's premise that *P. contorta* populations are adapted to small parts of the species' range.

Geography appears to have little influence on WUE (Fig. 17). Differences in WUE appear related to climate of origin. Leavitt and Long (1983) related $\delta^{13}\text{C}$ in *Pinus edulis* to temperature. Read and Farquhar (1991) found a significant negative correlation between Δ and precipitation, as well as temperature for *Nothofagus* species. Since temperature and precipitation were highly correlated (>0.6), they could not regress the two against Δ . However, they found the negative relationship between Δ and summer rainfall ecologically

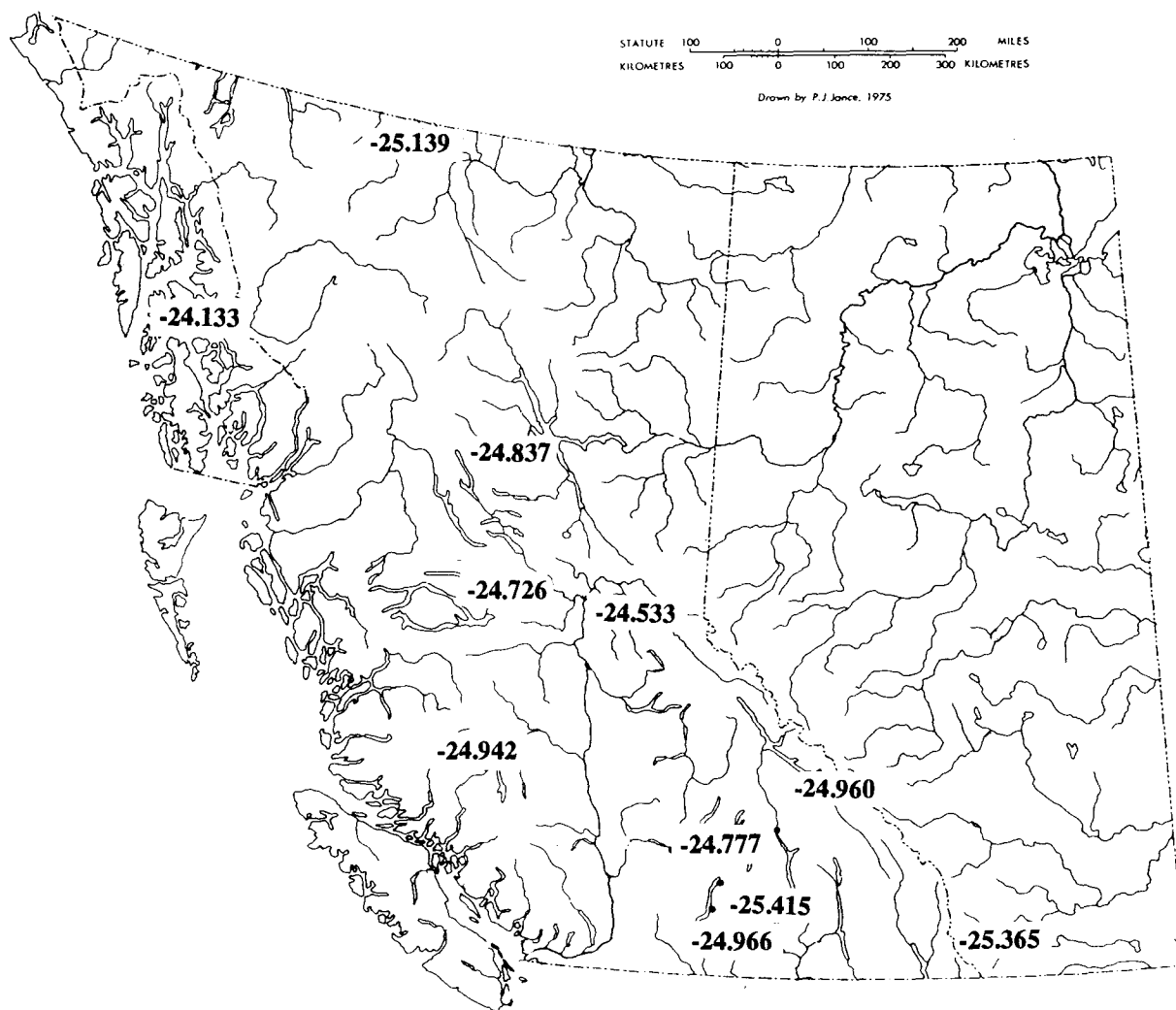


Fig. 17. Geographical trends for $\delta^{13}\text{C}$ in *Pinus contorta* in British Columbia and Alberta. The $\delta^{13}\text{C}$ values are the least squares means obtained for Holden Lake, where all provenances were tested. The positions of the $\delta^{13}\text{C}$ values on the map correspond to provenance locations.

meaningful since, for some species, the rainfall totals during the dry months exceeded the evaporative load and much soil water remained. This led Read and Farquhar (1991) to suggest that the high discrimination of species in drier climates with marked seasonal temperatures resulted from adaptations allowing continued photosynthesis during warm but relatively dry periods.

The regression model for the complete data set in this study shows a similar trend between Δ and summer rainfall as found by Read and Farquhar (1991) (temperature remaining constant). But higher seasonal temperatures (rainfall constant) give lower $\delta^{13}\text{C}$ values or higher Δ , the opposite trend found by Read and Farquhar (1991). Read and Farquhar (1991) used a wider temperature range than was used in this study. If both temperature and summer rainfall are varied to estimate $\delta^{13}\text{C}$, the model for this study indicates that increased temperatures with lower rainfall give lower $\delta^{13}\text{C}$ values. This model supports Read's and Farquhar's (1991) suggestion that the relationship between Δ and temperature is likely influenced by rainfall regimes. As well, indications are that populations have adapted to continue photosynthesis during warm but relatively dry periods. The data are poorly fitted to this model, however, and interpretations are inconclusive.

Modelling the relationship between climate and $\delta^{13}\text{C}$ without the coastal influence gives a model with adequate fit. The model predicts that under low summer moisture conditions WUE improves with increasing annual temperature, as expected in dry environments. In this case increased WUE may be due to selection for lower stomatal conductance in response to increasing vapor pressure deficit. Less water will be transpired relative to CO_2 assimilated. Under cool annual temperatures WUE also improves (to a lesser extent) with increasing summer precipitation. Increased WUE may be due to selection for lower stomatal conductance in normally humid environments to avoid dessication in unexpected drought. This model implies that the evolution of improved intrinsic WUE in *P. contorta* var. *latifolia* populations in British Columbia is related to complex interactions

between temperature and precipitation and their effect on biophysical and physiological processes in trees.

It appears that populations within the two varieties of the species have evolved high WUEs under very different conditions. The coastal provenance (var. *contorta*) maintains a high relative WUE similar to provenance 61 (var. *latifolia*) from the wet cool Sub-boreal Spruce subzone. The subzone has less than half the precipitation of the coastal location and a slightly higher summer temperature (msp=345 mm, mtwm=14.3 °C, mat=2.6 °C) (Meidinger and Reynolds 1991). To better understand the relationship between climate and $\delta^{13}\text{C}$ for the species we would require a class size spanning the species' range, as well as more appropriate climatic and atmospheric estimates. Coastal provenances in areas of summer drought should be sampled and the relative WUEs of interior populations (*e.g.*, 61, 104 and 100) should be compared with coastal populations on very wet maritime test sites.

Crucial origin site characteristics which would affect WUE (*e.g.*, soil moisture, nutrients, vapor pressure deficit) were not available for regression analyses. Potential evapotranspiration (PE) was not estimated with climate parameters since Thornthwaite's method (1948) applies only to regional analyses. Complex formulas requiring more data have been devised for local studies. Ideal estimates of PE would require weighing lysimeters (Muller and Oberlander 1984). Atmospheric data such as solar radiation, humidity and wind velocity would have allowed rigorous estimates of PE.

The most notable test site factor to elicit changes in $\delta^{13}\text{C}$ was moisture. The test sites were in biogeoclimatic subzones distinguished mainly by precipitation (Table1). Holden Lake and 70 Mile House were in the dry cool Interior Douglas-fir subzone while Salmon Lake was in the moist cool Sub-boreal Spruce subzone. The drier subzone had higher temperatures than the wetter subzone. Factors such as tree leader damage, number of cones, degree of shading (particularly to the south), disease, girdling or browsing showed no obvious trends with $\delta^{13}\text{C}$. More than one factor acted on most trees. Signs of severe disease, girdling or browsing were most useful in determining whether to eliminate outliers. Site

nutrient status may have been influential. Although mean $\delta^{13}\text{C}$ was not significantly different between 70 Mile House and Holden Lake, the slightly more positive values at 70 Mile House may have been related to its richer soil as well as decreased moisture.

Differences in WUE among some populations can be readily linked to climate and geography while others cannot. Provenance collections 100 and 104 have similar WUEs, differing from that of provenance collection 69. Provenances 100 and 104 are in close proximity and have similar mean temperatures of the warmest month and summer rainfall. [As seedlings, both populations had 36 stomates mm^{-2} leaf area (Illingworth 1973). Stomates are functional and likely to indicate an adaptive response]. Provenance 69 is located further south and has a warmer climate with more precipitation. This population had 35 stomates mm^{-2} in the seedling stage. [Illingworth (1973) measured stomatal densities on the provenance seedlings when they were two years old. Stomatal counts were grouped and averaged by broad geoclimatic region; *e.g.*, the stomatal density for provenance 69 (in the 'southern foothills') was the average of three provenances in that region. An important point is that average stomatal counts for the provenance collections are likely to have changed over time in response to environment as well as tree age]. In the context of scant site and climate information, provenance 30 appears to be an anomaly. Provenances 30 and 100 have almost identical summer rainfalls whereas provenances 30 and 69 do not, yet provenances 30 and 69 have almost identical WUEs. However provenances 30 and 69 have more similar temperatures (warmest month) than provenances 30 and 100. Provenances 30 and 69 likely grow in harsh conditions since provenance 69 is a high elevation source while provenance 30 is a far northern source. Some research indicates that provenances from the Yukon and northern British Columbia are genetically very different from the rest of the var. *latifolia* (Ying pers. comm. 1993). Morphologically, as seedlings, provenances 30 and 69 had 34 and 35 stomates mm^{-2} leaf area, respectively (Illingworth 1973). The stomatal count for provenance 100 was 36 mm^{-2} .

In the field trials and seedling study, provenance 95's performance stands out. This population originates in a very wet coastal environment and yet is the most water-use efficient of the provenances. In the seedling stage, provenance 95 had high stomatal density (45 mm⁻²). Illingworth (1973) found a significant difference between stomatal densities of coastal and inland regions. One explanation for provenance 95's high WUE in different environments may be that this provenance has very responsive stomates to moisture deficits. Relating leaf Δ to climate parameters in *Nothofagus*, Read and Farquhar (1991) found the lowest Δ values (or highest WUE) in species from regions without soil or air moisture deficits. The reason for this was unclear; they offered Körner's and Bannister's (1985) suggestion that stomatal sensitivity to humidity would be the most protective mechanism against sudden dessication in a normally humid environment. High WUE in wet climates, therefore, might reflect the costs associated with alternative mechanisms for avoidance of water stress. Provenance 95 appears to exemplify this.

With adequate water, such as in the greenhouse, provenance 95's high WUE may have resulted from decreased stomatal aperture instead of increased photosynthetic capacity. In this case, high WUE restricted yield. On the other hand, provenance 61's high WUE may have resulted from increased photosynthetic capacity at a given stomatal conductance. In this case, high WUE in adequate water led to relatively high yield. This is conjecture, since physiological measurements which might have helped explain the differences in yield associated with high WUE were not done.

In the field, increased $\delta^{13}\text{C}$ is not a reliable predictor of increased growth or yield within *P. contorta* provenances. But correlating productivity at particular sites with population averages ($\delta^{13}\text{C}$) shows significant correlations between high productivity and high WUE. Carbon isotopic composition may have practical use as a predictor of site yield. Selecting the most water-use efficient individual may not be as important as selecting the most water-use efficient population where long term productivity is concerned. In a *P. contorta* plantation water is shared among individuals. It is assumed that the more water-use

efficient trees will leave water for those requiring more moisture. Overall growth will be promoted by the faster growing less water-use efficient individuals. One assumption underlying this premise is that reproduction on the plantation prior to harvest will not occur, so the genetic composition of the population will not change. *Pinus contorta* seedlings do not establish under the canopy. The other assumption is that there will not be an abundance of other vegetation to compete for the water left by the more water-use efficient pines. In the case where only the most water-use efficient individuals of several populations are selected, the plantation may or may not ultimately produce a higher yield since high WUE is not always correlated with high yield in individuals.

Stronger relationships between $\delta^{13}\text{C}$ and yield in saplings may have been gained by using test sites close to the seed sources studied or sites favorable to the provenances' phenological and photoperiodic traits. Some populations were stunted or growing erratically on the interior test sites. Provenance 13 from the coast-interior transition region (Ying *et al.* 1989) was very susceptible to frost and foliage disease in the interior environment (Ying pers. comm. 1993). The fast growing populations 44 and 57 (Ying *et al.* 1985) showed significant positive correlations between $\delta^{13}\text{C}$ and yield at Holden Lake but different results at 70 Mile House. Repeated frost injury to trees in Block 51 at 70 Mile House resulted in a negative correlation between $\delta^{13}\text{C}$ and yield in population 57 (insignificant) while a small positive correlation (insignificant) remained in population 44. Fast growing populations are particularly susceptible to frost injury (Ying pers. comm. 1993).

Pest problems may have contributed to the variable correlations between $\delta^{13}\text{C}$ and yield. These factors could have increased sampling errors in growth measurements. At Salmon Lake about 23% of the trees were damaged by porcupines and small mammals. The very different correlations between $\delta^{13}\text{C}$ and yield for populations 61 and 104 at this site may have been largely due to the severe porcupine damage to population 104 (Ying pers. comm. 1993). Although growth in population 44 was slowed by porcupine damage, there was a rather high and significant correlation between $\delta^{13}\text{C}$ and yield in this population. It appears

that significant, positive correlations can exist despite adverse circumstances. Explanations remain elusive in these cases. Testing higher numbers of individuals per provenance may have given stronger correlations. Alternating provenance individuals systematically in lines or randomizing them throughout blocks may have prevented the rather selective destruction to certain plots.

Growth under contrasting field temperature and moisture regimes has led to changes in ecotypic $\delta^{13}\text{C}$. Evidence of a plastic response is shown by a significant site effect (Schlichting's definition 1986). Variation in $\delta^{13}\text{C}$ is similar among ecotypes; *i.e.*, ranking is maintained from site to site. The similarity in degree and direction of response has implications for studies trying to predict the species' response to rapid global warming. Woodwell (1989) suggests that by the middle of the next century, temperatures will rise by 0.5 to 1.0 °C per decade in middle and high latitudes. In this study, populations 61, 104, 100 and 44 maintained relatively high WUEs as well as yields compared with other populations on climatically different field sites. This pattern could hold over all climates. This prediction would require validation by research involving a wide range of climates on replicated sites. To most accurately assess the species' plasticity in unpredictable conditions, rapidity of response would have to be measured. Response time may differ between seedlings and saplings. In the field, especially, short term variation in WUE may be more easily determined by gas exchange measurements or by measuring $\delta^{13}\text{C}$ of the leaf soluble sugar fraction in needles.

Reciprocal transplants could offer useful information about plasticity but an acclimation period following planting would have to be considered in interpreting the outcome of physiological assessments. Previous site effects, especially drought, could determine the metabolism of trees for several years following transplantation, thus confounding assessments for plasticity of response to rapid changes in environment.

In *P. contorta*, the relationship between high WUE and survival is not clear. The relationship is confounded by many factors not measured in this study, *e.g.*, herbivory. Over

all sites, provenances 69 and 30 have the lowest relative WUEs and also the highest and third highest survival rates, respectively. Their performance is in contrast to *Populus* species where high WUE has been shown to promote survival in moisture-stressed environments (Dickmann *et al.* 1992). Provenance 61 follows the trend in poplars. With the highest WUE over all sites, it has the highest survival as well.

One aspect of the experimental design that could hinder finding true population differences is that provenances were planted in plots rather than randomized throughout blocks. Certain microsites may be associated with certain plots, making $\delta^{13}\text{C}$ a measure of microsite rather than provenance performance. This may have resulted in the significant block x provenance interaction shown in the analysis of variance for 70 Mile House and Holden Lake (Table 9). Yield data supported this as well. Fewer population differences were found in this analysis than in the other two. It is also known that block 51 at 70 Mile House was on a flat site prone to frost (Ying pers. comm. 1993) and would perhaps be best analyzed as a separate site. Due to the block x provenance effect, the finding of a non-significant site x provenance effect may not be valid. However, Figure 13 supports a non-significant site x provenance effect. The non-significant block effect can also be questioned. Examining block means with associated standard errors reveals that blocks are different at 70 Mile House but not at Holden Lake.

5.3 Seedling Experiment

Of 11 seedling populations representing all provenance collections tested, only the coastal one is different in relative WUE. Favorable growth conditions may have nullified expression of other genetic differences. Alternatively, measuring performance at 5 months may have not allowed time for full expression of genetic differences. Plant development may influence variation in $\delta^{13}\text{C}$ but little research has been done on trees.

Read and Farquhar (1991) hypothesized that species in dry summer climates (*Nothofagus*) have evolved physiological and/or morphological mechanisms to allow

continued water uptake and high stomatal conductance during mild water deficits. *Nothofagus* seedlings with low WUE had lower shoot/root ratios than the more water-use efficient ones. *Pinus contorta* seedlings showed the opposite trend in nine out of eleven provenances, similar to that for *Aestivum triticum* (Masle and Farquhar 1988). The more water-use efficient seedlings partitioned more assimilate to roots than the less water-use efficient ones. The positive correlation between root growth and $\delta^{13}\text{C}$ for all provenances supported this as well. However, provenances 61 and 104, relatively high in WUE, demonstrated the trend found by Read and Farquhar (1991). To accurately interpret the results of $\delta^{13}\text{C}$ analyses, assimilate partitioning as well as background physiology must be measured. Seedling stomatal conductance and photosynthetic measurements were begun to help explain these differences but structural and mechanical problems with facilities and equipment precluded their completion.

The correlation between $\delta^{13}\text{C}$ and root mass as well as shoot/root ratio for seedling populations followed an opposite trend to that found within populations. For the most part, correlations within populations were very low and not statistically significant, meaning that individuals with high or low WUE performed essentially the same. Regardless, an interpretation to explain the different trends among and within populations might be that the within population correlations between high root growth and high WUE largely reflect response plasticity to microenvironment while the correlation between high root growth and low WUE among populations mainly reflects an evolved response to origin habitat. The seedlings in the greenhouse were not competing for soil water but may have been affected by humidity changes. The high elevation populations 69 and 71 had the highest within-population correlations between increased root growth and increased WUE. High WUE as well as increased root growth would provide flexibility in adjusting to possible future drought. Either mechanism or a combination of the two could be developed as necessary. On a population basis, it seems reasonable to expect that population 95 would grow proportionally the least root mass to shoot mass, having originated in a very wet coastal

climate. Population 61 from a wet, cool environment in the Sub-boreal Spruce zone performs similarly. Both have high intrinsic WUEs. The less water-use efficient populations 69, 71 and 30 originating in harsh environments (high elevation or northern interior) allocate proportionally more biomass to roots than shoots. Accessing more soil water would be a means to avoid dessication while achieving growth during a short growing season. Indications are that in seedlings, $\delta^{13}\text{C}$ may detect differences in allocation patterns more readily than in total biomass.

More conclusive relationships between $\delta^{13}\text{C}$ and yield in seedlings may have arisen if the seedlings had been grown over two or three seasons out of doors. The illumination in the greenhouse was far from adequate for the establishment of *P. contorta* seedlings in their natural environment. They set bud earlier than expected, about one month before they were harvested. This may have been due, in part, to a long photoperiod (18 hours) which has been reported to sometimes cause early bud set in greenhouse-grown pines (Charleson, pers. comm. 1990).

The good correlations between seedling $\delta^{13}\text{C}$ and that of saplings suggest that nursery performance may be a useful predictor of field performance. Although the correlations were significant, seedling rankings may have been random since only one provenance was clearly different in $\delta^{13}\text{C}$. Studies with older seedlings over several years under well-defined drought conditions may give more sound results. Nursery shoot weights as well as total biomass were very poor (and insignificant) predictors of sapling volumes. Indirectly, $\delta^{13}\text{C}$ of seedlings shows more potential for predicting sapling yield than seedling growth variables.

If seedlings offer a means to predict relative WUE of saplings, the seed may do so as well. However, the seed would have to be collected from one site in one year. An important point is that the $\delta^{13}\text{C}$ of the seed would give a more accurate measure of the mother plant's relative WUE than seedling or sapling WUE.

6.0 Conclusions

Stable carbon isotope analysis has detected genetic variation in WUE among populations of *Pinus contorta* saplings. The genetic differences are related to provenance (habitat of origin), temperature and precipitation. There are no genetic differences in plasticity among populations. These results suggest that *P. contorta* provenances may respond to environmental changes in British Columbia by maintaining their ranking in relative WUE.

The relationship of WUE to biomass increment in controlled environments as well as in nature, is not clear. High WUE is associated with low or high productivity, depending on the population, its growing conditions, and its physiological and morphological attributes. However, correlating yield at particular sites with mean population $\delta^{13}\text{C}$ shows a positive correlation between high productivity and high WUE. To understand differences within and among populations, the physiological and morphological bases for high productivity must be measured (Dickmann *et al.* 1992). By testing the WUE response of provenances in controlled multiple factor experiments, we may improve our understanding of the adaptive nature of high or low WUE in *P. contorta* seedlings.

Early selection for improved WUE as indicated by $\delta^{13}\text{C}$ may be possible in *P. contorta*. However we do not know whether the significant correlation between $\delta^{13}\text{C}$ in seedlings and juveniles is repeatable.

Stable carbon isotope analysis offers a simple means to assess genetic differences in WUE among populations of large trees, as well as genotype x environment interactions. From a yield aspect, it is not clear whether high WUE is more advantageous than low WUE in promoting survival or productivity. It will be important for future investigators to examine the relevance of using $\delta^{13}\text{C}$ analysis as a selection tool for practical purposes in woody species. Stable carbon isotope analysis of plant tissue may be more valuable as a predictor of site yield than as a means to understand functional differences among tree populations.

7.0 Recommendations for Further Research

Several goals could be pursued in future studies:

1. Improving the models relating environmental factors to the evolution of differences in WUE among wild populations of *P. contorta*. Precise climatic, atmospheric and site characteristics of origin habitats would be required, the most important being those relating to air and soil moisture. Populations from the climatic range of the species should be tested. The provenance trials in British Columbia were not set up to assist in modelling the evolution of WUE according to climate. Seed sources were not chosen with particular attention to their vicinity to climate stations, nor were detailed micrometeorological data collected. Air moisture, soil moisture carrying capacity and nutrients were not documented. Using these trials for further research would require sampling the highest number of populations tested (total=140) that could be matched with acceptable climate station data. Biogeoclimatic subzone climate data could be used but would introduce more error. Costs could be reduced by pooling cores within populations in each block, giving two samples per population per site for carbon isotope analysis.

2. Pursuing the global warming problem. The ability of seedling populations to adjust their WUEs to rapid climate changes in ecologically sensitive areas in British Columbia could be assessed. This would require test sites in the boreal forest and the transition zones, e.g., Boreal White and Black Spruce-Alpine Tundra, Mountain Hemlock-Parkland. Micrometeorological data should be recorded. Physiological responses and assimilate partitioning should be measured on reciprocal transplants.

3. Determining more conclusively the relationship between improved WUE and yield as well as fitness (surviving to produce viable seed). Stronger correlations between $\delta^{13}\text{C}$ and yield for mature trees may be found on sites with optimal growth conditions for the populations tested. Individuals from certain populations will show stronger relationships between $\delta^{13}\text{C}$ and yield than individuals from others. For example, populations selected for

plantation renewal in tree improvement programs may show stronger positive relationships between $\delta^{13}\text{C}$ and yield than wild populations originating from marginal sites in British Columbia. An important distinction must be made between working to understand the variation in WUE within populations vs. the variation in WUE among populations. The variation in WUE within populations is often not addressed in the literature. If seedling establishment and productivity as related to WUE should be the focus, $\delta^{13}\text{C}$ vs. yield relationships can be measured in multi-factor experiments with regulated moisture, temperature and nutrients. For seedlings, changes in assimilate partitioning in response to environment may be more informative than overall growth responses. Improved WUE may be more strongly related to changes in allocation patterns rather than biomass.

4. Continuing to test the correlation between seedling and sapling $\delta^{13}\text{C}$ in other populations and families, as well as in other tree species. At some level, seedling $\delta^{13}\text{C}$ may be a reliable indicator of long-term performance.

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Appendix 1. Correlations between $\delta^{13}\text{C}$ and yield and growth variables at each test site (provenance 95 excluded).

70 Mile House

		Diameter	Height	Volume
Provenance	1	0.239	0.231	0.126
	13	-0.449	-0.493	-0.326
	30	-0.212	-0.342	-0.231
	44	0.125	-0.045	0.131
	57	-0.456	-0.596	-0.456
	61	0.038	0.025	0.063
	69	0.330	0.386	0.302
	71	-0.645*	-0.720*	-0.679*
	100	-0.002	-0.0001	0.082
	104	0.574*	0.540*	0.547*

Holden Lake

		Diameter	Height	Volume
Provenance	1	-0.028	0.037	-0.090
	13	-0.133	-0.092	-0.011
	30	0.345	0.463*	0.337
	44	0.512*	0.681*	0.554*
	57	0.745*	0.475*	0.671*
	61	0.079	0.338	0.151
	69	0.268	0.355	0.375
	71	0.114	0.068	0.119
	100	0.176	0.219	0.098
	104	0.273	0.235	0.233

Salmon Lake

		Diameter	Height	Volume
Provenance	1	0.319	0.482	0.324
	13	-0.141	0.082	-0.033
	30	0.547*	0.650*	0.556*
	44	0.600*	0.699*	0.672*
	61	-0.652*	-0.458	-0.596*
	69	-0.128	-0.118	-0.048
	71	0.233	0.268	0.276
	100	0.292	0.285	0.346
	104	0.267	-0.027	0.191

Note: None of the correlations are truly significant.

*Correlations significant at $\alpha=0.05$ (no correction for number of correlations tested).

Units of measurement: diameter, mm; height, cm; volume, m³.

Appendix 2. Correlations between seedling $\delta^{13}\text{C}$ and biomass variables, as well as shoot/root ratio.

		Shoot	Root	Shoot+Root	Shoot/Root
Provenance	1	-0.161	0.200	-0.085	-0.350
	13	0.021	0.145	0.057	-0.171
	30	-0.106	0.171	-0.046	-0.219
	44	-0.048	0.002	-0.037	-0.030
	57	-0.364*	0.037	-0.281	-0.369*
	61	0.253	0.059	0.208	0.120
	69	-0.171	0.341	-0.021	-0.467*
	71	0.199	0.405*	0.288	-0.410*
	95	-0.066	0.045	-0.041	-0.096
	100	0.181	0.206	0.200	-0.107
	104	0.332	0.306	0.340	0.016

Note: None of the correlations are truly significant.

*Correlations significant at $\alpha=0.05$ (no correction for number of correlations tested).

Units of measurement: shoot, g; root, g; shoot+root, g.

Appendix 3. Regression analyses of $\delta^{13}\text{C}$ vs. temperature and precipitation (for provenances 13, 30, 57, 69, 95, 100 and 104), including the tests for lack-of-fit of the models.

Source	df	SS	MS	Calculated F Value	F Value
<i>Complete Data Set Including Provenance 95</i>					
Regression	4	67.725	16.931	58.383	2.37
Residual	285	82.698	0.290		
Lack-of-fit	14 ^a	9.759	0.697	2.591	1.70
Within Groups	271	72.939	0.269		
<i>Complete Data Set Excluding Provenance 95</i>					
Regression	5	63.816	12.763	46.923	2.21
Residual	258	70.242	0.272		
Lack-of-fit	11 ^b	2.921	0.266	0.974	1.80
Within Groups	247	67.321	0.273		

^adf=290-4-1-271 (according to Ott 1988, p 369)

^bdf=264-5-1-247