Variation in the Stable Carbon Isotope Composition of Needle and Annual Rings in *Pseudotsuga menziesii* (Mirb.) Franco and its Relation to Water Use Needle Nitrogen Content and Productivity

by

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ABSTRACT

The variability of foliage stable carbon isotope composition ($\delta^{13}C$) in relation to needle nitrogen content and biomass production in coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) was investigated. The objectives were 1) to determine the variability in $\delta^{13}C$ of ten families of Douglas fir 2) to establish the relationship between needle nitrogen content and $\delta^{13}C$ 3) to establish the relationship between productivity and $\delta^{13}C$ 4) and to establish the relationship between $\delta^{13}C$ and tree water use. Needle and wood tissue were collected for anaylsis from ten half-sib families growing at two contrasting sites in southwestern Washington as part of a wide adaptability trial. Needles were also analzyed from seedlings representing the 10 families, that were subjected to an irrigated and drought treatment.

My research has shown that:

(1) Foliage $\delta^{13}C$ is influenced by both water and nitrogen stress. Whilst theory predicts that $\delta^{13}C$ should increase as the trees are subjected to water stress, it was found that the foliage $\delta^{13}C$ was more negative at the drier sites. This was because needle nitrogen had an overwhelming effect on $\delta^{13}C$ There was a positive, linear relationship between foliage $\delta^{13}C$ and nitrogen content across all sites.

(2) There was a positive correspondence between growth and foliage $\delta^{13}C$ within trees that were not subjected to severe water stress. The lack of any relationship between foliage $\delta^{13}C$ and growth within droughted seedlings and the increase in the root:shoot ratio under water stress suggests that carbon allocation patterns change under water stress. No relationships were found between the $\delta^{13}C$ of whole annual rings and growth. However, there was a significant relationship between the latewood $\delta^{13}C$ and latewood width. It is recommended that the annual tree ring should be delineated into its early and latewood components to evaluate relationships between $\delta^{13}C$ and other parameters.

(3) There was consistent ranking of families in terms of foliage $\delta^{13}C$ between the

irrigated and droughted seedlings and between the irrigated seedlings and the needles from the coastal Washington site suggesting that there is genetic variability in foliage $\delta^{13}C$. Therefore, there is a potential to use foliage $\delta^{13}C$ as a proxy indicator of long-term water use efficiency in Douglas-fir. However, annual ring $\delta^{13}C$ was influenced more by environmental rather than genetic factors. It may be that the annual ring $\delta^{13}C$ is a better indicator of tree water use over the long-term.

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A	assimilation rate (µmol m^{-2} s^{-1})
BAI	basal area increment (mm)
D	vapor pressure difference between leaf and air (MPa)
DBH	diameter at breast height (cm)
D_r	drainage (mm)
e	leaf water vapor pressure (MPa)
es	ambient water vapor pressure (MPa)
E	transpiration rate (mmol m^{-2} s^{-1})
E_i	evaporation of intercepted water (mm^{-d})
E_{max}	potential transpiration (mm^{-d})
E_s	ability of the soil to supply water to trees (mm^{-d})
E_t	actual transpiration (mm^{-d})
g	stomatal conductance (mmol m^{-2} s^{-1})
G	soil heat flux (W m^{-2})
I	intercepted water (mm)
k	unsaturated hydraulic conductivity (mm^{-d})
K	daily solar radiation (W m^{-2})
L*	long-wave radiation (W m^{-2})
N	needle nitrogen content (%N gm^{-1})
P	rainfall (mm)
Pi	intercellular CO2 partial pressure (MPa)
R	stable carbon isotope abundance ratio
Rn	net radiation
R_r	runoff (mm)
RuBp	ribulose bisphosphate
Rubisco	ribulose bisphosphate carboxylase-oxygenase
TDR	time domain reflectometry
WUE	water use efficiency
^{12}C	carbon-12
^{13}C	carbon-13
Δ	stable carbon isotope discrimination (‰)
$\delta^{13}C$	stable carbon isotope discrimination (‰)
α	proportionality constant in Priestly-Taylor relationship
ϕ	volumetric water content (m^3 m^{-3})

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CHAPTER ONE
GENERAL INTRODUCTION

Trees need an adequate water supply to grow. Research has shown that variations in seasonal and annual water availability is correlated with tree height and annual diameter growth (Spittlehouse,1985; Giles *et al.*, 1985; Robertson *et al.*,1990). Climatic variables (i.e. temperature, precipitation, solar radiation) also correspond to annual stem increments (Fritts, 1976; Francey and Farquhar, 1982; Leavitt and Long, 1989.) Because water deficits can develop during the summer months due to insufficient precipitation and high evaporative demand, trees often experience water stress during the growing season. Recent work has shown that growth is positively correlated to water use efficiency (defined as the unit of biomass gained per unit of water lost) in some commercial forest species (Aitken, 1993; Flanagan and Johnson, 1995; Sun *et al.*,1996). This is promising because tree breeders and researchers can then select for trees with higher water use efficiency without compromising yield.

Conventionally, plant water use efficiency is estimated by laborious physiological measurements or by using a combination of soil water balance, and/or evapotranspiration measurements and modelling, and dry weight determinations (Black, 1979; Federer, 1979; DeBruin, 1983; Giles et. al., 1985; Spittlehouse, 1985,1989; Spittlehouse and Childs, 1990; Spittlehouse and Goldstein, 1990). These methods are difficult and time-consuming. A new approach is needed for water use efficiency assessment that is relatively quick, easy and reliable. Within the last decade, stable carbon isotope ratios in plant tissue have been explored as a possible proxy indicator of long-term integrated water use efficiency in a wide range of crop species (Farquhar *et al.*, 1984; Hubick *et al.*, 1986, 1988; Masle *et al.*, 1988; Martin and Thortenson, 1988; Wright *et al.*, 1988; Hubick *et al.*, 1989; Virgona *et al.*, 1990; Hall *et al.*, 1992; Ehdaie and Waines, 1994). This is because water use efficiency and stable carbon isotope ratios are independently linked to intercellular CO_2 concentrations at the leaf level.(Farquhar et. al. 1982; Farquhar, 1989). Results show that stable carbon isotope ratios are correlated with water use efficiency in several species and are genetically variable. Because of the ease by which stable carbon isotopes can be measured, this technique is, therefore, a potentially powerful selection tool.

A number of studies have investigated the possibility of using the stable carbon isotope composition of plant tissue as a surrogate of water use efficiency in some commercial forest species (Aitken,1993; Flanagan and Johnson, 1995; Sun *et al.*, 1996). Generally, these studies show a strong correspondence between tree water use, productivity, and stable carbon isotope ratios in needle and wood tissue. Furthermore, the relationships are maintained over several growing seasons and at different sites. This suggests that stable carbon isotope ratios and hence, water use efficiency are under strong genetic control in some tree species.

This thesis explores variation in stable carbon isotope ratios in *Pseudotsuga menziesii.* Ten families of Douglas fir that are part of a wide adaptability trial set up by Weyerhaeuser Corp. and are growing at two contrasting sites in southern Washington, were selected for study. Variability in stable carbon isotope ratios were examined in needle tissue for the 1993 and 1994 growing seasons and in annual tree rings from 1988-1993. The isotope ratios were related to climatic variables for the time period under study and to tree water use determined from a simple soil water balance model. The main objectives of the study were:

1. To determine the extent of genetic variability in terms of stable carbon isotope ratios of needle and wood tissue.
2. To determine the relationship between tree water use and stable carbon isotope ratios of annual tree rings.
3. To determine the relationship between tree productivity and the stable carbon isotope ratios of needle and wood tissue.

CHAPTER TWO
LITERATURE REVIEW

2.1 Stomatal Conductance and Photosynthetic Capacity

Because carbon dioxide and water vapor both diffuse through stomata, carbon fixation by plants is inevitably accompanied by water loss. Water can be conserved through stomatal closure, but only at the expense of reduced carbon gain. As stomatal conductance (g) increases, carbon assimilation (A) increases asymptotically toward a maximum level set by the photosynthetic capacity, whereas transpiration (E) increases approximately linearly (Cowan, 1982). As a consequence, water use efficiency (WUE), the amount of carbon acquired per unit of water lost, decreases as g increases. Thus, for a given photosynthetic capacity, regulation of stomatal aperture controls the efficiency of water use.

The range of stomatal conductance varies such that it matches the photosynthetic capacity as it is determined by long-term environmental and plant factors (Schultz and Hall, 1982; Farquhar and Sharkey, 1982; Sharkey, 1985). For example, it is hypothesized that environmental and plant conditions that give rise to a low photosynthetic capacity per unit leaf area, may result in a small range of leaf conductance(Schultz and Hall, 1982). Regulation of stomatal aperture and CO_2 assimilation rate is strongly dependent upon the short-term responses of stomata to light, humidity, and temperature. There is evidence that stomatal responses to humidity and temperature may maximize daily WUE as described by the optimization theory of Cowan and Farquhar. (Cowan and Farquhar, 1977; Schultz and Hall, 1982; Cowan, 1982). This theory states that stomata behave so as to maximize the amount of carbon gained for a given amount of water lost by keeping the marginal cost of assimilating more carbon constant over the course of the day. The marginal cost is expressed as $dE/dA = \lambda$. (Cowan, 1982; Farquhar and Sharkey, 1982). Farquhar(1978) theorized that λ is kept constant if there is linear correspondence between photosynthetic capacity and concurrent stomatal conductance. Studies on numerous species, including *Vigna unguiculata* (Hall and Schultz, 1982), and Douglas-fir (Meinzer,1980) have shown that λ varies little under differing temperature or air humidity regimes. Generally, higher values of λ are associated with higher CO_2 assimilation rates for a given stomatal conductance which implies a higher intrinsic WUE. This has been observed mainly in C4 species and for some C3 species which have been subjected to drought (Schultz and Hall, 1982, Cowan, 1990).

Water use efficiency varies with water availability and atmospheric humidity. When water is abundant, many plants have high stomatal conductance to maximize carbon gain. However, as water becomes scarce, stomatal conductance decreases, resulting in increased WUE (Forseth and Ehleringer,1983; Comstock and Ehleringer,1984; Toft *et al.*,1989; Jones, 1992). It is well established that stomatal conductance decreases with increasing leaf and soil water deficits (Ong *et al.*,1985; Schultz, 1986; Grieu *et al.*,1988; Jones, 1992). Some studies suggest that stomata may close in direct response to soil drying and that this effect overrides leaf water status (Turner *et al.*, 1985; Gollan *et al.*,1986). Roots may sense limited water supply before the leaves do and this may induce ABA to be sent from the roots to the leaves. It is known that apoplastic ABA causes stomatal closure (Burschka *et al.*,1985; Raschke and Hedrich, 1985).

It is hypothesized that epidermal water loss and turgor in dry air signals the closure of stomata (Schultz, 1986; Weyers and Meidner, 1990). By using the isolated epidermis of the fern *Polypodium vulgare* and of lamb's lettuce, *Valerianella locusta, it was* clearly demonstrated that stomata react directly to changes in air humidity (Lange *et al.*,1971). Since then, it has been shown that stomatal responses to humidity occur in intact plant species from several ecological zones.(Schultz and Hall, 1982; Turner *et al.*,1985; Bunce, 1987). In particular, substantial regulation of transpiration by sensitive stomatal responses to humidity have been observed in conifers (Kaufmann 1982; Tinoco-Ojanguren and Pearcy, 1993; Angell and Miller, 1994). Douglas-fir is especially sensitive to vapor pressure deficits (Running, 1976; Meinzer, 1982; Grieu *et al.*,1988).

The biochemical limitations of WUE are set by the leaf photosynthetic capacity. This is the maximum net assimilation rate that can occur at a given intercellular CO_2 partial pressure (P_i) under optimum conditions. It depends on the activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco) and on the capacity for photosynthetic electron transport to regenerate ribulose bisphosphate (RuBp). At saturating light and high stomatal conductance, the regeneration of RuBp becomes limiting (von Caemmerer and Farquhar, 1981; Sharkey, 1985; Jones, 1992). At this point, electron transport capacity is producing NADPH and ATP at its maximum rate. Makino et. al.(1992,1994) found that light and CO_2 saturated photosynthesis is positively correlated with cytochrome f content. Maximum photosynthesis was also found to be limited by the capacity of starch and sucrose synthesis to regenerate P_i for photophosphorylation (Sharkey, 1985; Stitt, 1986; Makino, 1994; Stitt and Schulze, 1994). Under low stomatal conductance, assimilation rate is relatively independent of irradiance (von Caemmerer and Farquhar, 1981; Kirschbaum et. al. 1988; Jones, 1992). Instead, CO_2-limited photosynthesis tends to be linearly dependent on Rubisco activity (von Caemmerer and Farquhar, 1981; Makino et al. 1992, 1994; Stitt and Schultz, 1994).

There is a the strong positive relationship between photosynthetic capacity and leaf nitrogen content.(Field and Mooney, 1990). Consequently, WUE varies with nitrogen availability (Field and Mooney, 1990; Toft *et al.* 1989; Poorter and Farquhar, 1994). Convincing evidence exists to support the argument that nitrogen is mostly allocated towards Rubisco. The combination of low catalytic activity and competitive inhibition by oxygen suggests that the high levels of Rubisco found in leaves reflect nitrogen allocation to reduce assimilation rate limitation. This is reinforced by the high correlation between photosynthetic capacity and the total activity of Rubisco (Field and Mooney, 1990). In tobacco plants, Quick et. al. (1992) and Stitt and Schultz (1994) found that as the amount of Rubisco declined with decreasing leaf nitrogen content, there was a concomitant increase in control of photosynthetic rate by Rubisco. Makino et. al. (1992) also found that Rubisco activity increased with elevated leaf nitrogen levels in young, expanded leaves of spinach, bean, rice and pea.

Other factors that affect leaf photosynthetic capacity include temperature, ambient oxygen concentration,nutrient deficiencies (i.e. phosphorus) and water stress. High temperatures tend to decrease photosynthetic capacity because of the increase in dark respiration and photorespiration(Jones, 1992) as well as the thermal denaturation of enzymes or alterations of compartmentation (Hallgren *et al.*,1991). It is thought that low temperatures depress assimilation rates because of damage to membrane lipids and subsequent conformational changes in membrane-associated enzymes and that interactions with light cause greater damage (Hallgren *et al.*,1991). Lowered oxygen concentrations increase photosynthetic capacity as less oxygen is then available to compete with CO_2 for Rubisco binding sites (Salisbury and Ross, 1992). Assimilation rates drop significantly with decreasing leaf phosphorus or potassium contents (McDonald *et al.*, 1991). Phosphorus deficiency may shift the ADP/ATP ratio during photophosphorylation resulting in a decreased RuBp-regeneration capacity.(Sharkey,1985). It may also perturb sucrose and starch metabolism since reactions involving fructose-1,6-bisphosphatase and sucrose phosphate synthase require P_i (Makino *et al.*,1994). Moderate water stress affects photosynthetic capacity by reducing growth, and protein and protochlorophyll synthesis (Jones, 1992; Salisbury and Ross, 1992). Lowered water potentials and high vapour pressure deficits can affect photosynthesis directly as well as causing stomatal closure (Bunce, 1984; Schultz, 1986; Bunce, 1987; Kirschbaum and Pearcy, 1988; Stitt and Schultz, 1994).

2.2 Mathematical Representation of Water Use Efficiency

Instantaneous WUE can be expressed as:

$$A/E = P_a(1-P_i/P_a)/1.6D \qquad [1]$$

(Farquhar, 1989)

where

A = assimilation rate

E = transpiration rate

D = vapor pressure difference between leaf and air

P_a = partial pressure of atmospheric CO_2

P_i = partial pressure of intercellular CO_2

1.6 = the ratio of the binary diffusivities of water and carbon dioxide in air

Since E is a function of stomatal conductance and D, A/E can be estimated from the ratio A/g under conditions where D does not vary and from P_i/P_a (determined by A and g) (Donovan and Ehleringer,1994). In general, instantaneous A/E varies over a growing season and differs among plants (Cowan, 1990). This is a consequence of the sensitivity of evaporation and stomatal conductance to D. Accordingly, g responds more quickly to changes in air temperature and atmospheric humidity than A (Shultz and Hall, 1982; Sharkey, 1985, Salisbury and Ross, 1992; Jones, 1992). Stomatal closure tends to increase the instantaneous WUE at the expense of absolute production.

Integration of instantaneous WUE can be achieved by including a term in the equation for carbon losses by respiratory processes and water losses by non-stomatal evaporation. Season-long WUE can then be calculated as:

$$WUE = P_a(1-P_i/P_a)(1-\theta_c)/1.6(D)(1+\theta_w) \qquad [2]$$

(Jones, 1992)

where

θ_c = carbon lost by respiration

θ_w = water evaporated from the soil or plant cuticle

Because season-long WUE is the assimilation weighted time integral of instantaneous WUE, it is well correlated with the season-long time integral of intercellular CO_2 partial pressure. As mentioned, for many species, including Douglas fir, there is a linear relationship between the maximum assimilation rate (i.e. photosynthetic capacity) and the coinciding stomatal conductance over the long-term within a given environment. (Wong *et al.*, 1979; von Caemmerer and Farquhar, 1981; Meizner, 1982; Bunce, 1987; McVetty *et al.*,1989). This suggests that the season-long time integral of intercellular CO_2 partial pressure remains relatively constant over the growing season. It follows that season-long WUE must also remain constant. Differences in WUE between plants growing in the same environment can then be attributed to genetic variation.

2.3 Stable Carbon Isotope Discrimination

Atmospheric CO_2 is composed of 98.89% ^{12}C and 1.11% ^{13}C (and 0.10-.011% ^{14}C). During photosynthesis, plants discriminate against ^{13}C (Farquhar *et al.*, 1982; O'Leary, 1988; Farquhar *et al.*,1989, Guy *et al.*,1993). This is because a) the diffusion coefficient in air is inversely proportional to the square root of molecular mass, and therefore, ^{13}C diffuses more slowly through stomata than ^{12}C and b) during carboxylation, chemical processes favor ^{12}C because of its lower mass, higher vibrational energy and weaker bond strength than ^{13}C. This leads to isotopic fractionation within the plant tissue. That is, as carbon is assimilated into plant tissues, a distinct isotopic composition evolves that is not in a steady-state equilibrium with the surrounding air(Farquhar *et al* .1982; O'Leary, 1988; Farquhar *et al.* 1989).

In 1982, Farquhar *et al.* showed that the stable isotope ratio of leaf tissue reflects the variation of internal CO_2 partial pressure within the leaf. They quantified discrimination by the following equation:

$$\Delta = a + (b-a)P_i/P_a \qquad [3]$$

where a is the discrimination due to the slower diffusivity of ^{13}C in air and is 1.0044 and b is the discrimination due to carboxylation and is 1.0029. Given that for most field conditions P_a is essentially constant, this equation can be used to calculate long-term, integrated P_i values. Studies have verified that the plant carbon isotope ratio corresponds well with long-term integrated values of P_i/P_a (Francey and Farquhar, 1982; Guy *et al.*,1983; Guy *et al.*,1985). P_i/P_a is important because it is a function of photosynthetic capacity and stomatal conductance. Changes in P_i/P_a and leaf carbon isotopic ratio are, therefore, a function of changes in either, or both, photosynthetic capacity and stomatal conductance (Farquhar *et al.*,1989).

Carbon isotope composition of plant tissue is usually expressed with reference to a standard as:

$$\delta^{13}C = [R(\text{sample})/R(\text{standard}) - 1] \times 1000 \qquad [4]$$

where R is the isotopic ratio $R = {}^{13}C/{}^{12}C$. The standard is carbon dioxide obtained from PDB, a limestone from the Pee Dee Formation in South Carolina. The non-dimensional unit of $\delta^{13}C$ is per mil (‰). The standard is given a value of 0 ‰; therefore, any sample which contains less ^{13}C than the standard will have a negative isotope composition. For example, the $\delta^{13}C$ for atmospheric CO_2 is about - 8.0 ‰.

Environmental influences upon stomatal conductance and photosynthetic capacity are reflected by the isotopic ratio of plant material. It has been shown that carbon isotopic composition of plant material is well related to the weather and soil moisture conditions experienced by the plant during growth (Farquhar *et al.*,1982; Francey and Farquhar, 1982; Guy *et al.*,1983,1986; Farquhar *et al.*,1988; MacDowell *et al.*,1989; White *et al.*,1990). There are also studies that relate annual variations in isotope composition in tree rings to temperature and rainfall during the year (Leavitt and Long, 1986,1989; Dupouey *et al.*,1993). Air pollution also affects the carbon isotope signature in plant material (Martin *et al.*,1988; Martin and Sutherland, 1990; Saurer *et al.*,1991). As well, a few studies suggest that nitrogen availability is correlated with the isotope composition (Toft *et al.* 1989; Raven and Farquhar, 1990; Stitt and Schultz, 1994).

2.4 Water use efficiency and Stable Carbon Isotope Discrimination

Because carbon isotope discrimination is proportional to integrated, long-term P_i/P_a, it can be used as a proxy indicator to season-long WUE. Season-long WUE can then be expressed as:

$$WUE = \frac{[P_a(b-d-\Delta)]/[(b-a)]\ (1-\theta_c)}{1.6\ D\ (1+\theta_w)}$$

This equation combines the relationship between WUE and P_i/P_a with the relationship between P_i/P_a and carbon isotope discrimination (Farquhar *et al.*, 1989; Jones, 1992).

Generally, there is a negative, but linear correspondence between WUE and Δ (Farquhar, 1989). Reduced stomatal conductance causes increases in WUE because the rate of photosynthesis is reduced proportionally less than the rate of transpiration (Cowan, 1982). At the same time, the decreased stomatal conductance causes P_i to fall, which results in lower isotope discrimination. This relationship has been confirmed empirically in several studies with potted plants including tomato (Martin and Thorstenson, 1988), poplar (Brugnoli *et al.*,1988), peanut (Hubick *et al.*,1986,1988; Wright *et al.*,1988), barley (Hubick and Farquhar, 1989); wheat (Condon *et al.*, 1990), sunflower (Virgona, 1990), cowpea (Ismail and Hall, 1992), red pine (Leavitt and Danzer, 1992), *Chrysothamnus nauseosus* (Donovan and Ehleringer, 1994), and interior spruce (Sun *et al.* 1996).

Farquhar *et al.*,(1989) pointed out that the relationship between WUE and Δ can be inconsistent when working with plants within a canopy. The boundary layer resistance of a canopy is higher than in potted plants. As stomatal conductance and therefore, transpiration decrease due to variation in D, more heat must be lost by sensible heat transfer. This causes leaf temperature and D to increase so that the effect of a decreased g on transpiration will be moderated. The higher the ratio of boundary layer resistance to stomatal resistance, the more this moderating effect is enhanced. With a sufficiently high ratio, the proportional reduction in transpiration caused by a decrease in g is no greater than the associated proportional reduction in assimilation. If the canopy boundary layer resistance to heat is very large, there is the possibility that a genotype with a greater g than another otherwise identical genotype will have a greater WUE, despite having greater isotope discrimination (Virgona *et al.*,1990). On the other hand, canopies with small leaves, such as coniferous forests or crops that have been subjected to early water stress, are more likely to be closely coupled to the atmosphere, and therefore "behave" more like isolated plants. Zhang *et al.* (1993) found a negative relationship between intrinsic WUE and Δ in 25 populations of Douglas fir growing in a "common-garden" environment and Delucia *et al.* (1988) sited a negative correspondence between WUE and Δ in jeffrey pine and ponderose pine growing in their natural, forested habitat. If the source of variation in Δ is the photosynthetic capacity, boundary layer effects are unimportant. For example, Wright *et al.* (1988) examined season-long WUE and productivity in several peanut genotypes growing within a well-watered crop and found a negative relationship between WUE and Δ, and productivity and Δ. This implies that the photosynthetic capacity contributed more to variation in Δ than stomatal conductance. Because differences in stomatal conductance are governed more by micrometeorological influences than differences in photosynthetic capacity (Farquhar, 1989), it is important to examine the underlying source of variation in isotope discrimination when studying plants within a canopy.

2.5 Genetics, Discrimination and Productivity

Considerable variation exists for Δ both among and within species (Briggs and Shantz, 1914; Martin *et al.*,1989; Jones, 1992). Since leaf carbon isotope ratios provide information about processes integrated over the whole life of a leaf, it is particularly useful for examining subtle genetic differences in photosynthetic characteristics (Farquhar, 1989). Genetic variation in carbon isotopic discrimination has been documented for a number of agricultural crops and tree species under well-watered and droughted conditions, and it has been suggested that it could be used as a selection criterion for improving WUE and productivity of commercial plants such as wheat (Farquhar and Richards, 1984), barley (Hubick and Farquhar, 1989), cowpea (Hall *et al.*,1993), peanut (Hubick *et al.*,1988), tomato (Martin and Thorstenson,1988), sunflower (Virgona *et al.*, 1990), Douglas-fir (Zhang *et al.*,1993), and spruce (Flanagan and Johnsen, 1995; Sun *et al.*,1996). Increased WUE might result in a greater yield in environments where plant growth is limited by a lack of adequate water.

To properly use carbon isotope discrimination as a selection tool for WUE, one must understand the relationship between variation in isotope discrimination and plant growth under field conditions. The growth of individual plants may be positively or negatively correlated with leaf discrimination depending on whether variation in discrimination is mainly due to variation in photosynthetic capacity or stomatal conductance (Farquhar *et al.*,1989). If photosynthetic capacity increases, and stomatal conductance remains constant, discrimination will be reduced, whereas growth will be enhanced because carbon is assimilated at a higher rate for a given P_i. This results in a negative correlation between growth and discrimination. In contrast, if stomatal conductance increases proportionally more than photosynthetic capacity, discrimination and carbon assimilation will be higher due to the increased P_i. Therefore, there is a positive correspondence between discrimination and growth.

The relationship between discrimination and productivity depends upon the species and can vary with growth conditions. Positive correlations between discrimination and growth have been found in several agricultural species including wheat (Condon *et al.*,1987; Richards and Condon, 1993), tomato (Martin and Thorstenson(1988), sunflower (Virgona *et al.*,1990) and bean (White *et al.*,1990). In bean, the strength of the relationship varied with drought. Droughted conditions yielded a significant correspondence between discrimination and growth, whereas there was no correlation under irrigated conditions.

Similar results were obtained with wheat. It was only on the driest site that there was a negative relationship between isotope discrimination and productivity. Even though yield is reduced in droughted conditions relative to well-watered conditions, it may be that selection for improved WUE may increase yield in a dry environment. Reduced stomatal conductance would decrease transpiration which in turn, would cause soil water to be depleted more slowly, thereby extending the growing season.

In contrast to the above studies, Sun *et al.* (1996) found a negative relationship between discrimination and productivity in spruce that did not change under different watering regimes. They attributed this to a greater variation in photosynthetic capacity than stomatal conductance. O'Leary (1988) points out that since CO_2 uptake is more limited by the rate of carboxylation of ribulose bisphosphate than by diffusion, breeding for plants with a more efficient Rubisco would result in plants with higher assimilation rates without sacrificing WUE. Flanagan and Johnsen (1995) also found a negative correspondence between discrimination and tree height in selected full-sib families of *Picea mariana* (that were genetically variable in terms of growth and discrimination) growing at a dry, poor-quality site in eastern Ontario. They suggested that trees with low discrimination and higher photosynthetic capacity relative to water loss have an advantage on water stressed sites, while trees with high discrimination values perform well on sites where water is not limiting.

Other studies with forest species have focused on the longer term relationships between isotope discrimination, climate, and water use. Livingston and Spittlehouse (1993) related carbon isotope ratios of tree rings (in Douglas fir) to seasonal accumulated transpiration and soil water deficits calculated from a forest soil water balance model. They noted a good positive correspondence between accumulated transpiration and isotope discrimination of whole annual rings at both an upper,wet site and a lower, dry site over a 17 year period. In a more recent study, Livingston and Spittlehouse (1996) significantly improved upon the results of the 1993 study by separating the annual rings into early and latewood components. They showed a stronger relationship between isotope discrimination of latewood and accumulated transpiration than between the whole annual ring and accumulated transpiration, especially at the drier site. Because they found that the association between growth and isotope discrimination was not as strong, they suggested that carbon isotope discrimination is a better indicator of water use in trees than of growth. This view is supported by Dupouey *et al.*, (1993) who found the variability in isotope discrimination in whole annual rings of beech trees was explained more by soil water availability than by radial growth. As well, Stewart *et al.* (1995) showed that in over 300 forest species, isotope discrimination varied along a 900 km rainfall gradient in response to soil moisture availability.

The fact that there is genetic variability in WUE and that this is reflected within the carbon isotope discrimination of plant tissue, as well as the fact that productivity and discrimination values are related, strengthens the idea that carbon isotope discrimination can be used as a surrogate for WUE in breeding and selection programs. This is particularly encouraging for the forestry industry. The difficulty in assessing WUE is exacerbated in commercial forest species because of the problems associated with evaluating water use by trees within a canopy and because of their large size and longer life span. Even though recent work on forest species supports using carbon isotope discrimination as an indicator of water use by trees, more study is needed.

2.7 Assessment of Evapotranspiration

Conventionally, soil water balance is used to estimate plant water use. Difficulties can arise when evaluating soil evaporation and drainage. Recently, a new method to accurately measure soil water content has been developed. This procedure involves time domain reflectometry (TDR). A high frequency pulse is sent down a probe that is buried in the soil (Hook *et al.,* 1992). The travel time of the pulse is linearly related to the volumetric soil water content. Sun (1996) found that evaluation of plant water use by TDR agreed well with accumulated transpiration based on the product of leaf area, D, and stomatal conductance. TDR measurements may provide a reliable, accurate means to assess plant water use in the field.

The difference between the water use of a single leaf or potted plant and the water use of a large vegetated area such as a forest or crop is one of scale. Canopies have much larger and more turbulent boundary layer conductances than leaves or single plants (Jones, 1992). When estimating evapotranspiration from forests or crops, attention must be given to the degree of coupling of the vegetation to the atmosphere. Coniferous forests typically have a higher degree of coupling to the atmosphere than agricultural crops because of the height and roughness of their canopies and therefore, the relatively increased turbulence within the boundary layer. Together with small leaf size, increased turbulence decreases boundary layer resistance to water vapor transfer. Concomitantly, evapotranspiration becomes more sensitive to leaf conductance and D (Jones, 1992).

There are many approaches to estimating evapotranspiration from forested areas. The Penman-Monteith equation combines energy balance components and vapor pressure gradients with physiological parameters (Monteith, 1981; Spittlehouse and Black, 1981; DeBruin and Holtslag, 1982; Jones, 1992). It takes into account the physiological control of evaporation in a canopy. Modifications to the model allow for differences in evapotranspiration associated with intercepted rainfall. However, to use this model, extensive micrometeorological data is needed along with measurements or estimates of canopy stomatal and boundary layer conductance. Spittlehouse and Black, (1981) claim that the major limitation on the use of the Penman-Monteith equation is the requirement for vegetation LAI (leaf area index) and stomatal and boundary layer conductance.

Some other methods or approaches used to assess evapotranspiration from a forest canopy include (i) Bowen ratio/energy balance method, (ii) eddy correlation, (iii) Priestly-Taylor equation, and (iv) soil water balance models. The Bowen ratio is the ratio of the sensible heat flux to the latent heat flux and can be included within the energy balance equation. Measurements of net radiation, soil heat flux, and the gradients of temperature and vapor pressure within the boundary layer are needed to calculate evapotranspiration. This approach assumes that the transfer coefficients to heat and water vapor within the boundary layer are equal and that there is no advective enhancement (Spittlehouse and Black, 1980; Jones, 1992). A large fetch is required to meet these assumptions and to allow for accurate measurements to be made above the canopy. However, no physiological or canopy surface information is needed.

Eddy correlation involves a direct measurement of the fluxes of water vapour above a canopy (Spittlehouse and Black, 1980; Jones, 1992). Assumptions and associated limitations for this technique are similar to those for the Bowen ratio method. Additional difficulties with this method are the complexity of anaylsis, the relatively high cost of instrumentation, and the limitations imposed on certain systems by the generally low wind speed above forests (Spittlehouse and Black, 1980; Jones, 1992). The Priestly-Taylor relationship is:

$$E_{max} = \alpha(s/s + \gamma)(Rn - G) \qquad [6]$$

where

E_{max} = potential evapotranspiration

α = proportionality constant

s = slope of the saturation vapor pressure curve (k/P_a)

γ = the pyschrometric constant (k/P_a)

Rn = net radiation (W/m^2)

G = soil heat flux (W/m^2)

α is a proportionality constant that equates the potential evapotranspiration to the energy available for evapotranspiration (Priestly and Taylor, 1972; Jones, 1992). It incorporates plant parameters such as stomatal conductance and leaf area index and ranges from 0.7 - 1.26 depending on surface control and advective enhancement (Jury and Tanner, 1975; Debruin, 1975). This equation best approximates evapotranspiration from large areas of vegetation well-supplied with water. It is especially useful in conjunction with models evaluating soil water availability and water balance (Spittlehouse and Black, 1981; Federer, 1982; DeBruin and Holtstag, 1982; Giles *et al.*,1985; Spittlehouse, 1989). Maximum and minimum air temperature, solar radiation and soil temperature are the only parameters needed for input into the model.

Soil water balance models are also a common method used to estimate evapotranspiration from canopies. The method requires the measurement or estimate of the root zone water content, precipitation, drainage, and run-off. Evapotranspiration is the residual term in the equation. Actual measurements of soil water content can be made with TDR, neutron scattering or gravimetric sampling (Spittlehouse and Black, 1980; Hillel,1980). Spittlehouse and Black (1981) modified the soil water balance model so that measurements of soil water content are not required. Instead, an estimate of the saturated soil water content in the early spring and some knowledge of the soil's depth and texture is needed. The negative feedback between root zone water content and stomatal conductance and drainage play a major role in the model.

2.8 Assessment of Tree Growth

Growth of trees within a forest canopy can be assessed in several ways. Common measurements include total height, height to the base of the live crown, diameter at breast height (DBH), and basal area (Brix, 1972; Robertson, 1990; Livingston and Spittlehouse, 1993; 1996). Total ring width, early and latewood width, and ring density and volume can be discerned from increment cores. Division of cores into separate annual rings and their respective earlywood and latewood components have been achieved by x-ray densitometry (Robertson, 1990), commercial tree ring analyzers (Livingston and Spittlehouse, 1994), and by sight (Leavitt and Long, 1991). X-ray densitometry is the least subjective technique available (Robertson, 1990). However, it is expensive.

Growth of individual trees within an even-aged stand can be extrapolated to the whole stand by averaging growth measurements (Robertson, 1990; Dupouey *et al.*,1993). In this way, total above ground carbon gain can be compared to the canopy water use. However, Spittlehouse (1985), Giles *et al.* (1985), and Robertson (1990), have shown that estimates of stand water use from a soil water balance model correlate well with growth data from individual trees.

CHAPTER 3

VARIATION IN NEEDLE STABLE CARBON ISOTOPE COMPOSITION AND ITS RELATION TO NEEDLE NITROGEN CONTENT AND BIOMASS PRODUCTION

3.1 INTRODUCTION

The ability to minimize the effects of drought is important to plants in that water stress tends to limit productivity. Water deficits frequently occur in the summer months in the Pacific Northwest and reduce tree productivity (Waring and Franklin, 1979). Foresters need, therefore, to select for more drought tolerant genotypes to increase survival and maintain yield. Water use efficiency (WUE) is defined as the amount of carbon gained by net assimilation relative to the amount of water lost by transpiration over a given period. Recent work has shown that long-term WUE and growth are positively correlated in some conifer species.(Flanagan and Johnsen, 1995; Sun *et al.*,1996). These results indicate that selection for improved WUE might not necessarily compromise yield. Water use efficiency is a function of the ratio of the net assimilation rate (A) to stomatal conductance (g). It follows that factors which vary stomatal conductance and/or photosynthetic capacity will determine how efficiently a plant utilizes its water source. For example, instantaneous WUE is highly affected by ambient vapor pressure deficits (D) since evaporation and stomatal conductance are very sensitive to changing D (Tan *et al.*,1977; Meinzer *et al.*,1982; Jones, 1992). When comparing genotypes for inherent or long-term WUE, it is therefore crucial that all genotypes are subjected to the same D (Farquhar *et al.*,1989).

Long-term WUE represents integrated instantaneous WUE. It includes carbon losses through respiratory processes and non-stomatal losses of water. Long-term WUE is difficult to assess. Whole or above-ground dry matter production is usually determined on potted plants and plant water use is estimated through changes in soil water content (Zavitkovski and Ferrell, 1968; Deleens *et al.*,1989; Virgona, 1990; White *et al.*,1990; Smit and van den Driessche, 1992). The method is labor-intensive and results can be ambigous. Evaluation of long-term WUE in forest tree species is even more difficult given their size, and long life-span. Therefore, there is a need for a quick, reliable method to accurately assess long-term WUE.

There are two naturally occuring stable carbon isotopes: ^{12}C and ^{13}C. ^{12}C exists in the atmosphere at an abundance of about 98.9% whereas the abundance of ^{13}C is about 1.1%. Plants discriminate against ^{13}C during photosynthetic gas exchange as discussed in Chapter 2, Section 2.3.

In 1982, Farquhar *et al.*,theorized that the discrimination against ^{13}C is proportional to the ratio of the intercellular CO_2 partial pressure (P_i) to the ambient CO_2 partial pressure (P_a). The ratio, P_i/P_a, is important because it varies with stomatal conductance and photosynthetic capacity. Thus, WUE is related to carbon isotope discrimination through its independant link to P_i/P_a.

The carbon isotope composition of plant tissue is usually determined to evaluate carbon isotope fractionation. (See Chapter 2, Section 2.3). Because differences in plant tissue $\delta^{13}C$ can be quite small, comparing $^{13}C/^{12}C_{plant}$ to a standard improves precision. The nondimensional units of $\delta^{13}C$ are then expressed in per mil. Carbon isotope discrimination is related to $\delta^{13}C$ by the expression:

$$\delta = (\delta^{13}C_{air} - \delta^{13}C_{plant})/(1 + \delta^{13}C_{plant}/1000) \quad [7]$$

(Farquhar, 1989).

It is well-established that long-term, integrated values of P_i/P_a and Δ are linearly related in many C3 species (Francey and Farquhar, 1982; Guy *et al.*, 1983,1985; Farquhar *et al.*,1989). Consistently, researchers have reported genetic variation in Δ and a negative linear relation between WUE and Δ in several crop species (Hubick *et al.*,1988; Martin and Thorstenson, 1988; Hubick and Farquhar,1989; Condon et.al,1990; Virgona, 1990; Ismail and Hall, 1992). Most studies also show a positive relation between productivity and $\delta^{13}C$ (Martin and Thorstenson, 1988; Virgona *et al.*,1990; White *et al.*,1990; Richards and

Condon, 1993).In contrast, Hubick *et al.* (1988) and Wright *et al.* (1988) found a negative relation between Δ and growth in peanut genotypes. Thus, in peanut, selection for low discrimination (high WUE) would not compromise yield.

Recently, the relation between Δ and WUE has been studied in a few commercial forest species to see if Δ could be used as a surrogate for long-term WUE in selection and breeding programs. A positive linear relation was found between P_i/P_a and Δ and between Δ and productivity in Douglas fir (Zhang *et al.*, 1993; Livingston and Spittlehouse, 1993;1996) and two species of spruce (Flanagan *et al.*, 1995; Sun *et al.*, 1996). Discrimination has also been shown to be genetically variable in Douglas fir, larch (Zhang et.al, 1993,1994), and spruce (Flanagan and Johnsen, 1995, Sun *et al.*,1996). These results suggest that Δ may be useful as a proxy indicator of integrated, long-term WUE in some commercial forest species.

The main objective of this chapter is to examine variability in foliage $\delta^{13}C$ in coastal Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco var.*menziesii*). Ten half-sib families of Douglas fir were selected from trees growing at two contrasting sites in southern Washington that were part of a wide adaptability trial established up by Weyerhaueser Corp. Genetic variability of needle $\delta^{13}C$ and the relationship between $\delta^{13}C$ and productivity were assessed for the 10 families within irrigated and droughted water treatments. In addition to water stress, the effects of nitrogen stress on $\delta^{13}C$ were examined because it is well-known that water stress and nitrogen deficiency affects photosynthesis (Field and Mooney, 1990) and a few studies have reported that leaf nitrogen content may influence carbon isotope discrimination (Toft *et al.*,1989; Stitt and Schultz, 1994; Sun and Livingston, 1996).

3.2 METHODS AND MATERIALS

TREE SOURCE

Five families came from a wet provenance source located in Twin Harbours, Washington. The other five families came from a dry provenance source near Vail, Washington. All ten families were growing at both experimental sites. The parental origin of the ten families are given in Table 3.1. All trees were outplanted in 1986.

Seeds from the same provenance sources were planted in May 1993 and grown in the greenhouse at the University of Victoria, until May 1994 when all seedlings were outplanted to field facilities. One family from the wet provenance source was omitted (1026 x 9131) due to a lack of seed.

WASHINGTON FIELD SITES

Location of Washington Sites

Both sites are located in southern Washington. One site is a coastal site near Raymond, Washington (hereafter referred to as "Raymond") and the other site is situated in the foothills on the west side of the Cascade Mountains at Upper Bald Hills near Vail, Washington (hereafter referred to as "Vail"). Both sites are located at the same latitude (46 degrees 40 min) and are approximately 60 miles apart. The Raymond site has a 15.0 % slope with a NE aspect whereas Vail has a 7.0 % slope with a SW aspect.

Weather Stations

Automated climate stations were installed at both sites in March 1993 and removed in November 1994. Each station included a quantum sensor (Licor, Licor Inc., Lincoln, Nebraska), tipping bucket rain gauge (TR-525M, Texas Electronics, Lincoln, Nebraska), a relative humidity/temperature probe and a copper-constantin thermocouple. All instruments were connected to a datalogger (21X,Campbell Scientific Inc.,Logan,UT) which was programmed to record maximum and minimum air temperatures and total precipitation and solar radiation for every 12 hours as well as average hourly atmospheric relative humidity. The climate stations ran from April 1 to October 31 in 1993 and 1994. Daily maximum and minimum air temperatures and daily precipitation totals were also obtained for April to October inclusive from 1973-1994 from 2 state weather stations one of which was located within the town of Raymond and the other near the town of Vail.

In addition to the measured variables, the atmospheric vapor pressure deficit (D) was estimated using the expression:

$D = (e_s - e)$ [8]

(Jones, 1992)

where

$e(s) = f(m \exp\{nT/c+T\})$

f = an enhancement factor to convert the saturation pressure of pure water vapor to the saturation partial pressure of water vapor in moist air $(1.0007 + 3.46 \text{ x } 10^{-8})$

m = an empirical coefficient (0.061121)

n = an empirical coefficient (17.502)

T = air temperature in Celsius

z = an empirical coefficient (240.97)

It was assumed that the absolute water vapor content of the air remained relatively constant over the day; therefore, e was assumed to be equal to the saturation vapor pressure at the minimum night-time air temperature.

Water Balance Model

Water deficits were estimated for both sites. Water deficits are defined as the difference between potential and actual evaporation. To estimate evaporation, a well-tested water balance model described by Spittlehouse and Black (1981) and Spittlehouse (1989) was used. The model determines the daily root zone water content as:

$W_i = W_i-1 + P_i - ET_i - D_{ri} - R_{ri}$ [9]

where

W = the water content of the root zone for day i (mm)

P = rainfall (mm)

ET = evaporation (mm)

D_r = drainage (mm)

R_r = runoff (mm)

Climate variables such as rainfall, maximum and minimum air temperature, and solar radiation were used as the inputs to drive the model. These were derived from the climatic variables outlined above.

Evaporation is calculated as:

$$ET = E_t(1-I/E_i) + I \qquad [10]$$

where

E_t = transpiration on a dry day (mm d^{-1})

I = interception of rain by the canopy (mm d^{-1})

E_i = evaporation of intercepted water (mm d^{-1})

When there is no rain, interception is zero, and ET is equal to E_t

It is assumed that evaporation is driven by the radiant energy and its rate is the lesser of E_{max} or E_s. E_{max} is transpiration when water is not limiting and is calculated using the Priestly-Taylor relationship. (See Chapter 2, Section 2.7, Equation 6) E_{max} was expressed in mm d^{-1} by assuming that 2430 J of energy evaporates 1.0 mm of water.

E_s is the ability of the soil to supply water to the trees. It is:

$$E_s = b\phi_e$$

where

b = an experimentally determined constant. (mm d^{-1})

ϕ_e = extractable water content (mm)

ϕ_e is defined as $([W-W_{min}])/([W_{max}-W_{min}])$ and is dependent upon root zone stone content and soil texture. W_{max} is the soil water content at field capacity and was approximated at 0.26 m^3m^{-3} (i.e. volume of water per volume of soil) for both sites. This was considered a reasonable value because of the rainy spring and relatively shallow root zone depths at both sites. The soil was assumed to be at field capacity as of April 1. W_{min} is the soil water content when transpiration ceases. It was arbitrarily given a value of 0.8 m^3m^{-3} which corresponded to - 1.5 MPA on a general soil retention curve for sandy-clay loam soils (Salisbury and Ross, 1992).

The proportionality constant b is a parameter which depends on soil texture and water storage capacity. A value of 10 mm^{-d} was used as suggested in Spittlehouse, 1989.

α can vary between 0.6 - 11.0 depending on stomatal resistance and medium scale advective enhancement (McNaughton and Black, 1973; Spittlehouse and Black, 1981; Spittlehouse, 1989). In forest canopies, α tends to be lower when the canopy is dry due to increased canopy resistance. Values of 0.60 to 1.27 have been reported for various forest canopies under dry conditions (Spittlehouse, 1989). Conversely, on rainy days, α ranges from 1.3 to 11.0.(Spittlehouse and Black, 1981; Spittlehouse, 1989). Not only does stomatal resistance increase on rainy days (i.e.transpiration ceases when the canopy is wet), but additional energy becomes available for evaporation upon release of sensible heat as water condenses to form precipitation (Campbell, 1977). In this study, values of 1.3 and 0.80 were used for wet and dry conditions, respectively. These values were assumed to be typical of Douglas fir canopies based on studies by McNaughton and Black (1973), Spittlehouse and Black, (1981) and Spittlehouse (1989). They were expected to remain constant over the growing season (Spittlehouse, 1989).

The evaporation of intercepted water is given by E_i which is equivalent to E_{max} under wet conditions (i.e.α equals 1.3). The amount of intercepted water is related to the leaf area index and is determined by:

$$I = fP^l \quad \text{for} \quad P > P_c \qquad [11]$$

$$I = P \quad \text{for} \quad P \leq P_c \qquad [12]$$

where

P_c = maximum amount of rainfall that is intercepted (mm^{-d})

P = amount of rainfall (mm^{-d})

f, l = empirical constants (dimensionless)

The values of f,l and P_c were 0.6, 0.6, and 0.3 mm^{-d} respectively. These values are shown elsewhere to be typical for a young, established Douglas fir stand(Spittlehouse and Black, 1981; Giles *et al.*, 1985; Spittlehouse, 1989).

Net radiation was evaluated using:

$$Rn = (1-r)K + L^* \qquad [13]$$

where

r = albedo or reflectivity of the surface (dimensionless)

K = daily solar radiation (Wm^{-2})

L* = daily long-wave radiation (Wm^{-2})

r was assumed to be 0.12 which has been determined to be an average value for coniferous forests (Jarvis *et al.*,1976). L* was calculated from:

$$L^* = (c + d(K/K_{max}))e_v(e_a-1)\sigma T^4 \qquad [14]$$

where

c,d = constants (dimensionless

K_{max} = maximum solar radiation for the day (Wm^{-2})

σ = Stefan-Boltzmann constant (5.67 x 10-8 $Wm^{-2}K^4$)

e_a = clear-sky atmospheric emissivity (dimensionless)

e_v = emissivity of the vegetation (dimensionless)

T = daily mean air temperature (K)

Jarvis *et al.*, (1976) suggest a value of 0.96 for e_v. $e_a$was determined from average daytime air temperature using the Jackson-Idso formula (Idso and Jackson, 1979):

$$e_a = 1 - (0.261 \exp(0.00077(T -273)^2)) \qquad [15]$$

K_{max} was calculated from:

$K_{max} = 0.73Ko$ [16]

where Ko equals the extraterrestrial irradiance (1370 Wm^{-2}) Others have found that the coefficient 0.73 can be applied with minor variation over a range of irradiances by comparing solar radiation values on clear days with the appropriate value of Ko (List, 1971; Giles *et al.*, 1985). The values of c and d were set at 0.1 and 0.9 respectively. This was done to improve the estimation of low Rn without significantly affecting the higher values as recommended by Spittlehouse and Black, (1981).

Solar radiation was measured at both sites for the 1993 and 1994 growing seasons (See Section 3.2). However, data was not readily available for 1973-1992. Solar radiation was modelled for this time period using known solar-earth geometrical relationships using day of the year, declination angles, and latitude of the sites. These determinations are shown in Appendix A.

For simplicity, soil heat flux was not calculated.

The drainage component of the water balance equation was derived from:

$k = k_r(\phi/\phi_r)^c$ [17]

where

k = the unsaturated hydraulic conductivity (mm^{-d})

k_r = the saturated hydraulic conductivity at a reference (high) average root zone water content (mm^{-d})

ϕ = the average root zone water content for the day (mm)

ϕ_r = the reference average root zone water content (mm)

c = constant (dimensionless)

As in Spittlehouse and Black, (1981) and Livingston and Spittlehouse (1993) values of 100, 0.30, and 14.8 were used for K_r, ϕ_r, and c respectively. For a range of soil textures, Spittlehouse and Black (1981) point out that the calculation of drainage using the unsaturated hydraulic conductivity characteristic is adequate in freely draining, coarse soils. Both soils at Raymond and Vail were considered to be freely draining, based on the percentage of sand and stones and the absence of greyish mottles within the soil profile. As well, the soils from both sites were a sandy, clay loam texture.

Runoff was calculated from the residual of the water balance equation on days where soil water content exceeded 0.26 m^3m^{-3} Generally, runoff was a minor component of the water balance model. This was because at both sites, the soil had a high infiltration capacity and that the surface was completely covered by either low-level vegetation or by a thick layer of leaf litter. At Raymond, the canopy was fully closed by April 1993 and the magnitude of the basal area and height of the trees created a densely packed stand. Little vegetation existed at the soil surface; instead, a thick layer of leaf litter was present. In contrast, Vail had an open canopy structure and as a result, the soil surface was covered by low-level vegetation such as salal.

Soil Analysis

A general soil survey by Weyerhaeuser Corp. was completed at both sites prior to this study. Soil from Raymond is from the Astoria soil series whereas soil from Vail is from Wilkeson association, soil series Maschel.

Soil texture and rooting depth were estimated to define the soil water storage capacity at both sites. Rooting depth was gauged by digging pits (approximately 1 m by 1 m) in the soil until bedrock was uncovered, and measuring the depth of each hole. Six pits were dug at each site; the pits were randomly distributed throughout the site. Depth measurements were averaged over the six pits.

From each pit, grab samples of soil were taken from a lateral wall at a depth of 0.30 m and a few centimetres from the bottom. At Raymond, an additional sample was taken from each pit at a depth of about 0.60 m. A textural analysis using the finger assessment method of McRae (1988) was subsequently performed on all samples. Stoniness (by volume) was assessed by a water displacement technique. Stones were removed from a known volume of soil, and placed in an equivalent volume of water. The volume of water displaced by the stones was measured and compared to the total volume of soil. Stone size was determined by measuring the width of each stone greater than 2.0 mm.

Soil structure was roughly approximated by examining the lateral walls of the pits and comparing aggregate size and shape to a scale developed by McRae (1988). Porosity was also approximated by estimating the diameter of any visible pores greater than 0.5 mm within soil aggregates in the lateral walls. An arbitrary scale also suggested by McRae was used to establish the general coarseness of the soil.

EXPERIMENTAL DESIGN

Washington Sites

Five families from the wet provenance source and five families from the drier provenance source were growing at both the Raymond and Vail sites. The trees were outplanted in 1986. Approximately 7000 trees were planted in 6.0 acres at both sites. Each site was divided into six blocks. Within each block, there were 4 trees/family interspersed randomly among other families of Douglas fir not involved in this study. Therefore, there were 24 trees/family arranged in a completely randomized block design at each site. There was a total of 240 trees per site studied.

Field Study at the University of Victoria

Stratified seeds for all families were sown in May 1993 and grown in the greenhouse until May 1994. Three seeds per family were placed within a styrofoam well filled with a peat:vermiculite:perlite mixture at 3:1:0.33 v/v/v. A slow release fertilizer with N:P:K in 17:7:10 proportions and a micromix of micronutrients was added. There were 45 wells per styrofoam block; therefore, there were 135 seeds planted for each family. The blocks were randomly arranged on a warm, sand-filled bed and watered every 2 hours with an automated mist spray for 8 hours/day. In the first few weeks of August, the seedlings were thinned to one seedling per well and then left to grow over the winter months. The seedlings were hand-watered once every two days. In March 1994, the blocks were placed outside on a gravelly surface to acclimate the seedlings to outdoor conditions. On May 1 1994, 20 seedlings from each family were randomly selected and transplanted to buried sand-filled stove-pipes (1 m long, 0.20 m in diameter) at the field facilities at the university. These cylinders provided a well-defined volume of soil that allows for accurate assessment of soil water balance and root growth. The 200 seedlings were randomly distributed throughout the field and hand-watered every other day to field capacity for six weeks. This allowed the seedlings to become established within their new environment before stress treatments began. In addition, the seedlings were fertilized once a week with a liquid fertilizer containing 150 ppm of NPK at 10:52:17 proportions and trace amounts of micronutrients.

To evaluate the soil's field capacity, unplanted sand-filled cylinders were saturated with water and left to drain. To minimize evaporation from the soil surface, the sand was topped with 1-2 cm of gravel and the cylinders were covered with black plastic. The soil water content was measured once a day using time domain reflectometry (TDR) until no change in water content was detected. The results from the 5 cylinders were subsequently averaged and this value was used as an indication of field capacity in the other cylinders.

Irrigation and droughted treatments began on June 13,1994 and continued until October 6,1994 when the trees were harvested. Ten seedlings per family were selected for each treatment. Irrigated seedlings were hand-watered as needed (i.e.3 times per week) to maintain the soil at field capacity. Droughted seedlings were hand-watered once a month to field capacity. The seedlings were given additional water when the volumetric soil water content fell below 3% In both treatments, the soil water content was measured with TDR 3 times a week.

In both treatments, seedlings were well-fertilized for the duration of the experiment. In June, the fertilizer was changed to 20:20:20 NPK and supplemented with magnesium, sulphate, and calcium. The new fertilizer was applied to sustain growth and minimize nutrient stress. The irrigated seedlings received fertilizer in liquid form at 150 ppm NPK once a week while the droughted seedlings received 200 ppm NPK in liquid form once a month.

NEEDLE ISOTOPE COMPOSITION ANALYSIS

Washington Sites

Needles were harvested from the top terminal leaders of all 240 trees at Raymond and Vail in mid-October in 1993 and 1994. The needles were oven-dried at 70 degrees Celsius for 48 hours and then ground to a fine powder using a mortar and pestle after rapid freezing by liquid nitrogen. Samples were subsequently analyzed for carbon isotope composition on a MAT 252 Isotope Ratio Mass Spectrometer (Finnigan MAT San Jose, CA) with an elemental analyzer (NA 1500NC, Fisons Instruments, UK). Carbon isotope composition was calculated using equation 4. (See Chapter 2 Section 2.3.) Acetanilide, with a carbon isotope composition of -30.54 ‰ relative to PeeDee belemnite (PDB) was used as a working laboratory standard. Duplicate analysis of the working standard and of a sample showed precision to be within 0.15 ‰.

Seedlings at University of Victoria

In March 1994, needles were collected from the top terminal leaders of the greenhouse grown seedlings. Ten seedlings/family were sampled. In mid-October 1994, all seedlings planted in the field were harvested and needles from the top terminal leaders of these trees were collected. All needle tissue was oven-dried at 70 degrees Celsius for 48 hours and then ground into a fine powder for ensuing carbon isotope composition analysis. Grinding and isotope analysis were similar to the procedures outlined in the above section.

NEEDLE NITROGEN CONTENT ANALYSIS

Washington Sites

Needles from the 1994 harvest were analyzed for nitrogen content. The amount of nitrogen in the needle tissue was determined on a MAT 252 Isotope Ratio Mass Spectrometer (Finnigan MAT San Jose, CA) with an elemental analyzer(NA 1500NC, Fisons Instruments, UK) and expressed as a percentage on a leaf dry-weight basis. Reproducability of the technique was 0.02%.

Seedlings at University of Victoria

Needles from the October 1994 harvest were analyzed for nitrogen content for both the irrigated and droughted treatments. The same procedure was applied as for the needles from the Washington sites.

MORPHOLOGICAL MEASUREMENTS

In May 1994, 10 greenhouse grown seedlings/family were measured for above-ground height and diameter and then separated into roots and shoots. The seedlings were oven-dried at 70^{o} Celsius for 48 hours and then roots and shoots were weighed to obtain root:shoot ratios and total dry weight. In October 1994, all seedlings in the field were harvested; the same parameters were obtained as for the greenhouse grown seedlings.

STATISTICAL ANALYSIS

Water Balance Analysis

To evaluate each site in terms of its general water availability, the water balance was analyzed for the growing seasons (i.e. April 1-October 31) of 1973 -1994 for both sites. Water deficits were evaluated as the difference between E_{max} and E_t on days without rain. The total growing season water deficit for both sites was obtained by summing the daily water deficit for April through October. Total precipitation was also evaluated from the sum of daily rainfall for April through October. Growing season average vapor pressure deficits were calculated from the average of the daily average vapor pressure deficit over a twelve hour period. Comparisons between sites were made by performing t-tests between weather variables from both sites and by linear regression.

To verify that the weather data from the state weather stations was applicable to both sites, weather data collected from the small climate stations at each site, was compared to the weather data obtained from the state weather stations for 1993 and 1994. Results are shown in Appendix B.

Washington Sites

For 1993 and 1994, the needles from the 4 trees/block were combined and treated as a composite sample for both sites. Data was analyzed as a completely randomized block design with six replicates/family. ANOVA was used to establish differences between families in terms of carbon isotope composition and to determine any differences between sites. Regression analysis was performed to evaluate relationships between needle nitrogen content and carbon isotope composition and to determine if the ranking of the families of Douglas fir remained constant from site to site and from year to year. Results were also compared to data collected from the seedlings at the field facilities at the University of Victoria.

Regression analysis was performed to determine the relation between carbon isotope composition data for the 1993 data from Raymond and the greenhouse grown seedlings and for the 1994 data from Raymond and the irrigated treatment at the university. As well, plots of needle nitrogen content versus needle carbon isotope composition across all sites (i.e. Raymond, Vail and the university) were undertaken. Needle intercellular $C0_2$ partial pressure (P_i) was estimated from the carbon isotopic composition values using:

$\Delta = (\delta^{13}C_p - \delta\ ^{13}C_a)/(1 + \delta\ ^{13}C_p)$ then

$P_i = \Delta(P_a)/(a + (b-a))$ (Farquhar *et al.*, 1989)

A Plot of P_i versus N for individual trees from Raymond and Vail was done to determine the extent of the relationship between leaf nitrogen content and P_i.

Seedlings at University of Victoria

Data collected from the seedlings growing in the greenhouse and in the field was analyzed as a completely randomized design. 10 seedlings/family were evaluated for morphological measurements, carbon isotope composition, and leaf nitrogen content in all treatments. ANOVA was performed to test for differences between families in terms of height, diameter, dry weight, and root:shoot ratios as well as for carbon isotope composition. ANOVA was also used to evaluate carbon isotope composition and morphological differences between the irrigated and droughted treatments. Regression analysis was completed between the irrigated and droughted treatment in terms of isotope composition to see if ranking between families was maintained across treatments. Regression analysis was also used to find the extent of the relationship between productivity and needle isotope composition.

3.3 RESULTS AND DISCUSSION

WASHINGTON SITES

Weather Variability

Figure 3.1a shows the year-to-year variability in growing season rainfall totals at both sites for 1973-1994. There was considerable variability within sites and no significant difference between sites over the 20 year period. In contrast, Table 3.2 and Figures 3.1b and 3.2, show there were highly significant differences between sites in terms of growing season soil water deficits totals and average vapor pressure deficits (D). Vail consistently had larger soil water deficits than Raymond. This can mainly be attributed to the smaller root zone water storage capacity. Vail had a much shallower root zone depth than Raymond (i.e. 0.7m versus 1.2m). Even though the total growing season rainfall was similar at both sites, more of the precipitation at Vail would have been lost by drainage and runoff than at Raymond. Because the soil had a relatively low water holding capacity, soil water deficits quickly developed when there was little rain. The problem was further exacerbated by high vapor pressure deficits. Figure 3.2 shows that, on average, Vail always experienced higher vapor pressure deficits than Raymond during the growing season. High evaporative demand would have reduced the soil water content rapidly because of increased evaporation. In contrast, Raymond had a deeper root zone depth and lower vapor pressure deficits than Vail. More rain would have been stored between rainfall events. The lower evaporative demand meant that soil water deficits developed slowly and were less intense as evaporation would have been reduced.

It is well established that gas exchange is highly sensitive to vapor pressure deficits in Douglas fir (Tan *et al.*,1977; Meinzer, 1982; Grieu *et al.*, 1988). In general, stomatal conductance tends to decrease curvilinearly as D rises. Therefore, the trees at Vail would likely have had lower average stomatal conductance and concurrently, lower net assimilation rates on a given day than the trees at Raymond. Over prolonged periods this would have caused a reduction in growth.

Morphological Characteristics

The impact of the water stress experienced by the trees at Vail is reflected by the different size of the trees and the difference in the canopy closure between sites. The canopy at Raymond was fully closed and very little understory growth was evident. This suggests that the trees at Raymond had an adequate supply of resources throughout the growing season and experienced low water stress. Conversely, the canopy at Vail was open and a lush understory of salal was present. Larger water deficits at Vail limited tree growth such that canopy closure was incomplete. Resource availability could have become limited under these circumstances, as different species would have competed for water and nutrients. Black *et al.* (1979) and Spittlehouse (1985) noted that a salal undergrowth in thinned stands of young Douglas fir accounted for 30-60% of the transpired water from the stand depending on soil dryness. Evans and Ehleringer (1994) found that inorganic nitrogen tended to accumulate under inter-canopy brush resulting in lower soil nitrogen concentration near the trees within a Pinus/Juniperus mixed canopy. Limited nutrient availability at Vail would have had additional deleterious effects on tree growth.

There is ample evidence that suggests that water stress reduces growth in Douglas fir. For example, higher root:shoot ratios, decreased height and basal area, lower leaf surface area, and increased leaf specific weight have all been found under dry conditions (Brix, 1972; Joly *et al.*,1989; Smit and Van Den Driessche, 1992). Giles *et al.* (1985) found that the site index for Douglas fir was positively correlated to the growing season soil water deficits. Spittlehouse (1985) found a good correspondence between the annual basal area increment index and soil water deficit in a young Douglas fir stand on the eastern coast of Vancouver Island. Table 3.3 shows the mean height and diameter at breast height (DBH) of all trees at both sites. The mean height and DBH of the trees at Vail were 36% and 28% less respectively, than the trees at Raymond. Higher coefficients of variation in DBH and height at Vail also shows that the trees endured a more unpredictable, arid environment.

CARBON ISOTOPE COMPOSITION

Leaf Nitrogen Content and $\delta^{13}C$

Figures 3.3a and 3.3b shows the average carbon isotope composition from needle tissue harvested from all families at each site in 1993 and 1994. All families at Vail had a significantly lower (more negative) needle $\delta^{13}C$ than the families at Raymond. This was unexpected since plants grown in a drier environment would be predicted to have a (less negative) $\delta^{13}C$ than plants growing in a wet environment (Farquhar, 1989). The expected trend was shown by the seedlings growing at the research facilities at the University of Victoria. Needles were harvested from the irrigated and droughted seedlings in 1994. Needles from the droughted seedlings had significantly higher $\delta^{13}C$ than the needles from the irrigated seedlings.

The less negative $\delta^{13}C$ of the needles from the droughted seedlings showed that under soil water deficits, a lowered stomatal conductance resulted in a lower P_i and higher water use efficiency at a given photosynthetic capacity. Conversely, the exceptionally negative $\delta^{13}C$ of the needles from Vail indicated that the P_i was exceedingly high, despite the fact that high vapor pressure and soil water deficits should have caused the needles to have a lower stomatal conductance. This suggests that, for a given P_i, the CO_2 assimilation rate was very low and/or the sensitivity of stomata to high vapor pressure deficits or soil water deficits was decreased. Therefore, it was hypothesized that the trees growing at Vail were experiencing a resource limitation in addition to water stress that adversely affected the photosynthetic capacity and/or stomatal sensitivity. Because the trees at Vail were stunted in growth relative to Raymond and needles from the trees were somewhat chlorotic, it was suspected that the trees were suffering from a nitrogen deficiency.

In October 1994, needles from all families growing at Raymond and Vail and all seedlings growing under both water treatments at the university were analyzed for needle nitrogen content. All needles from Vail had significantly less nitrogen content (per percent dry weight basis) than the needles from Raymond (Table 3.4). All the seedlings grown at the university under both watering regimes had needle nitrogen contents above 2.0%. Figure 3.3a and 3.3b shows that there is a trade-off in resource use efficiency. The droughted seedlings at the university exhibited an increase in WUE at a high leaf nitrogen content while the water-stressed trees from Vail exhibited a decrease in WUE at a low leaf nitrogen content. At low nitrogen availability, nitrogen is utilized more efficiently at the expense of water, whereas at high nitrogen availability, water use efficiency increases. Others have found an inverse relationship between nitrogen use efficiency and water use efficiency in conifers (Sun and Livingston, 1996; Field *et al.*, 1983).

Figure 3.4 shows that there was a strong linear correspondence between average needle nitrogen content and $\delta^{13}C$ across all the trees studied. These results support the argument that a lowered leaf nitrogen content opposes an increase in WUE. On the other hand, it demonstrates that $\delta^{13}C$ reflects both instantaneous WUE and leaf nitrogen content. It could be that the more negative $\delta^{13}C$ at lower leaf nitrogen levels partly reflect a higher accumulation of starch inside the chloroplasts. Under low nitrogen availability, Fichtner *et al.* (1993) and Stitt and Schultz (1994) showed that in tobacco leaves, up to 40% of the photosynthate accumulates in the leaves as starch. Brugnoli *et al.* (1988) showed that starch has a more negative $\delta^{13}C$ than structural carbon or sugars.

Within an individual site, there was no significant relationship between needle $\delta^{13}C$ and nitrogen content (data not shown). Nevertheless, there was a significant correlation between the estimated intercellular CO_2 partial pressure (P_i) and the leaf nitrogen content for individual trees from Raymond and Vail ($r^2 = 0.48$. $p < 0.05$), as shown in Figure 3.5. This means that at a given stomatal conductance, the assimilation rate declined at lower leaf nitrogen levels. Stitt and Schultz (1994) showed that the slope of the $\delta^{13}C$ versus measured P_i plot increased in tobacco plants with a lower nitrogen supply. That is, P_i was higher than usual in plants with low leaf nitrogen contents. Similarly, earlier studies found that $\delta^{13}C$ values became increasingly more negative as leaf nitrogen levels declined in cold desert plants (Gebauer *et al.*,1987; Toft *et al.*,1989). It seems that low leaf nitrogen contents consistently result in an increase of intercellular CO_2 partial pressure. I propose that this increase in P_i is primarily due to a decrease in photosynthetic capacity.

It is well-known that leaf nitrogen content is strongly correlated with photosynthetic capacity in C3 species. As leaf nitrogen content increases, net assimilation rate at any P_i is enhanced (von Caemmerer and Farquhar, 1981; Makino *et al.*, 1994). Moreover, the increase in photosynthetic capacity with nitrogen content is believed to be due to the amounts of stromal enzymes and thylakoid proteins that require nitrogen. In particular, Rubisco is a large "consumer" of leaf nitrogen. Makino *et al.* (1992,1994) found that Rubisco content per unit leaf nitrogen increased with increasing leaf nitrogen content in rice, spinach, bean, and pea. The amount of total leaf nitrogen incorporated into Rubisco was species-specific ranging from 28-37%. Furthermore, they found that at a limiting P_i, the photosynthetic rate reflects the in vivo Rubisco activity. Similarly, Quick *et al.* (1992) demonstrated that the amount of Rubisco in tobacco plants was reduced as the leaf nitrogen content decreased and that this was associated with an increase of control over the rate of photosynthesis. They implied that there is a specific reduction in the amount of Rubisco compared with other components of the photosynthetic machinery. Stitt and Schultz (1994) found that there was a good correspondence between the amount of Rubisco and instantaneous WUE. These studies suggest that a decrease in the amount of Rubisco is the primary cause of reduced photosynthetic rates in nitrogen-limited conditions. Therefore, the nitrogen deficiency in the needles from Vail could have reduced the photosynthetic capacity by primarily limiting the amount of Rubisco.

The reason why there is a higher proportion of nitrogen partitioned into Rubisco under low and high leaf nitrogen contents, in some species is unclear. Some researchers have argued that the balance between the in vivo Rubisco and electron transport capacities remains constant in several C3 species.(von Caemmerer and Evans, 1991; Makino *et al.*,1992; 1994). In some species, the relative increase in Rubisco with increasing leaf nitrogen content is required to keep this balance because of the presence of a CO_2-transfer resistance between the intercellular air space and the carboxylation site (i.e. mesophyll resistance) (Makino et.al, 1992; 1994). In other species, (such as wheat), others have found that at higher leaf nitrogen contents, activity of Rubisco is largely independent of leaf nitrogen and that the mesophyll resistance tends to decrease due to an increase in carbonic anhydrase activity and chloroplast surface area.(Makino et.al, 1992). On the other hand, some researchers claim that under high leaf nitrogen contents, there is a luxury additional investment of nitrogen into Rubisco. The balance between Rubisco and electron transport capacities is not maintained at high nitrogen content. Therefore, Rubisco has also be considered as a nitrogen storage protein. This would be beneficial in that it allows for a slightly higher water use efficiency and for photosynthesis to respond to temporarily high irradiance (Quick *et al.*,1992; Stitt and Schultz, 1994).

The relationship between leaf nitrogen content and photosynthetic capacity is not only a reflection of Rubisco activity and photosynthetic processes *per se*, but is also a function of leaf structure and processes such as translocation and storage of photosynthates. Nitrogen availability undoubtedly affects these processes to some degree. For example, it is well known that leaf area and specific leaf area (leaf area per dry weight) decrease at low nitrogen availability (McDonald *et al.*,1991; Salisbury and Ross, 1992; Stitt and Schultz, 1994). It has also been reported that root growth decreases more slowly than shoot growth at low nitrogen availability and that this corresponds to a shift in dry-matter allocation in favour of roots (McDonald *et al.*,1991, Stitt and Schultz, 1994). Thus, nitrogen availability may decrease photosynthetic capacity by changing source-sink relationships and by decreasing total available photosynthetic tissue. These facts cannot be ruled out at Vail for growth was definitely stunted at this site (see Table 3.3). It may be that dark and photorespiration may have increased at Vail due to high air temperatures. This could have resulted in less carbohydrates been allocated for growth and also could have increased P_i.

Stomatal conductance at Vail may not have necessarily decreased as much as it should have under the prevailing water stress. This would have increased P_i more than usual relative to trees that experienced water stress at a high nitrogen content. Quick *et al.* (1992) found that tobacco plants grown under saturated light and 70 % relative humidity exhibited an unexpected increase in stomatal conductance under low nitrogen availability whereas the stomatal conductance of plants grown in medium or high nitrogen availability was independent of nitrogen. It could be that low soil nitrogen availability inhibits effective communication between the root and shoot via chemical messengers such as abscisic acid.

However, a common symptom of plant nutrient deficiency (particularly nitrogen) is a reduced rate of leaf transpiration and this is typically associated with elevated levels of leaf ABA (Weyers and Meidner, 1990). Thus, it seems more likely that the stomata at Vail would have had increased sensitivity to water stress under low leaf nitrogen contents due to a synergistic interaction between increasing P_i attributable to a lower photosynthetic capacity and ABA.

In addition to nitrogen stress, the magnitude of the water stress at Vail could have directly affected the photosynthetic capacity. Interactions between high irradiance and water stress may cause photoinhibitory damage at photosystem II (Jones, 1992). As well, moderate water stress significantly reduces leaf area by decreasing cell enlargement and cell division through changes in cell wall extensibility (Hinckley *et al.*, 1990; Salisbury and Ross, 1991; Jones, 1992). It has been reported that net assimilation rates decline with leaf water potential in apple trees (Jones, 1992).

Variability in Needle $\delta^{13}C$

Figure 3.6a and 3.6b shows the variability associated with needle $\delta^{13}C$ for all families at Raymond and Vail. Generally, Vail experienced higher variability in terms of $\delta^{13}C$ than Raymond for 1993 and 1994. This is reflected by the standard deviation associated for each family. However, the total range of $\delta^{13}C$ at Raymond for both years was higher than at Vail. (i.e. 5.19 ‰ and 5.21‰ at Raymond versus 4.05‰ and 4.94‰ at Vail for 1993 and 1994, respectively). This shows that both sites had high microsite differences between individual trees.

There was no significant difference between the 1993 and 1994 mean $\delta^{13}C$ at Raymond or Vail, despite 1994 being a warmer, drier year than 1993. At Vail, the 1994 $\delta^{13}C$ total mean was slightly higher (-29.10‰) than in 1993 (-29.8‰). Higher soil water deficits and D would have resulted in an overall lower stomatal conductance and P_i, which in turn, would be expected to have caused the less negative $\delta^{13}C$ in 1994. At Raymond, the higher soil water deficits and D in 1994 may have significantly affected stomatal conductance. The mean $\delta^{13}C$ in 1994 was slightly lower than in 1993 (i.e. -27.9‰ and -27.72 ‰ respectively). It could be that the larger soil water storage capacity and the fully closed canopy at Raymond moderated any changes in stomatal conductance.

The variability in terms of needle $\delta^{13}C$ for all families grown at the university of Victoria are shown in Figure 3.7a, 3.7b and 3.7c. There was no significant difference between the total mean $\delta^{13}C$ of the irrigated seedlings (-27.39‰) or the greenhouse grown seedlings (-27.28‰), despite a high standard deviation for some families from the greenhouse. The total mean $\delta^{13}C$ for the droughted seedlings was significantly higher (-25.68‰) than for the irrigated seedlings. This shows that under water stress and adequate nitrogen supply, stomatal conductance decreases enough to substantially reduce P_i and subsequently decrease carbon isotope discrimination in these families of Douglas fir.

Table 3.5 shows that there were significant differences between two families in terms of needle $\delta^{13}C$ within the irrigated treatment at the university. However, significant differences were not observed between any family within the droughted treatment or the greenhouse grown seedlings. There were also no significant differences between families within Raymond or Vail. It is probable that the variable responses of individual trees to microsite differences masked any genetic differences. This was certainly true at Vail where drought and nutrient stress and soil heterogeneity caused highly variable physiological responses within individual trees. In comparison, the smaller standard deviations associated with each family growing at Raymond and within the greenhouse at the university indicates that these trees were subjected to a more homogeneous environment. Nevertheless, Raymond and the greenhouse grown seedlings experienced a large range in $\delta^{13}C$ between individual trees (i.e. 5.19‰ and 5.10‰ respectively) showing that microsite differences were large enough to increase the variability of $\delta^{13}C$ between trees enough to obscure genetic differences. Within the irrigated and droughted treatment at the university, however, microsite differences were minimized. All seedlings were grown in the same soil type (sand) and all were watered and fertilized in the same manner. Thus, variability between individual seedlings was reduced and genetic differences surfaced within the irrigated treatment. The reason why genetic differences were not observed between families within the droughted treatment is unclear. It could be due to the fact that 10 seedlings did not survive the water stress which may have influenced statistical testing by lowering the degrees of freedom.

There was a significant correlation between family mean $\delta^{13}C$ of 1994 needles from Raymond and of the irrigated seedlings from the university ($r^2 = 0.36$; $p < 0.05$). When family 2 was excluded from the regression analysis, there was a highly significant correspondence between family mean $\delta^{13}C$ from the irrigated and droughted seedlings.($r^2 = 0.73$; $p < 0.01$).(See Figures 3.8a and 3.8b). These results show that for some families, ranking of $\delta^{13}C$ was maintained at Raymond in 1994 and within the watering treatments at the university and supports the fact that needle $\delta^{13}C$ are genetically variable in Douglas fir. However, ranking of $\delta^{13}C$ was not maintained across Raymond and Vail for either year or across Raymond and the greenhouse grown seedlings in 1993; nor was ranking of $\delta^{13}C$ maintained across years within Raymond or Vail (data not shown). The lack of consistent ranking suggests that there is a genotype x environment interaction. Because of the consistent ranking of $\delta^{13}C$ observed between the well-fertilized irrigated and droughted genotypes, the genotype x environment interaction is probably due to soil nutrient availability. This is especially applicable to Vail where highly variable needle nitrogen contents in 1994 probably reflected high soil nutrient heterogeneity. Even though the environment was more homogeneous at Raymond and within the greenhouse, microsite differences in nutrient availability could have obscured genotypic differences in carbon isotope discrimination. Hall *et al.* (1993) reported that for cowpea genotypes, ranking of $\delta^{13}C$ was inconsistent for genotypes grown in highly contrasting environments. This could explain the inconsistent ranking observed in this study between Vail and Raymond. Vail had highly variable nutrient availability as compared to Raymond. Similarly, the greenhouse grown seedlings had continually high soil nutrient status whereas nutrient availability at Raymond was more variable. Thus, despite genetic differences, ranking was not maintained between sites.

In contrast, Sun *et al.* (1996) found no significant genotype x environment interaction with respect to foliage$\delta^{13}C$ for 10 families of white spruce. They found that neither water stress, nitrogen stress nor the combination of the two stresses substantially changed the ranking of $\delta^{13}C$ of the 10 families. However, each seedling experienced relatively the same nitrogen and/or water stress within each treatment. For example, every seedling within the nitrogen stressed treatment received the same amount of nitrogen and were well-watered. Similarly, every seedling within the water stressed treatment were watered the same way and received adequate nutrition. Therefore, it could be that microsite differences between seedlings were minimized in all treatments and genetic differences between families became apparent. On the other hand, it could be that the foliage $\delta^{13}C$ of white spruce is under stronger genetic control than Douglas-fir.

The genetic variability of $\delta^{13}C$ detected in this study suggests that it may be possible to use $\delta^{13}C$ as a surrogate for integrated, long-term water use efficiency in Douglas fir. Many studies have shown that $\delta^{13}C$ is genetically variable and negatively correlated with long-term water use efficiency in several crop species (Farquhar *et al.*, 1989; Virgona *et al.*,1990; Ismail and Hall, 1992; Wright *et al.*,1993) and some conifer species (Zhang *et al.*,1994; Flanagan *et al.*, 1995; Sun *et al.*,1996). Recently, there have been a few studies that have indicated genetic variability between interior and coastal varieties of open-pollinated Douglas fir in terms of $\delta^{13}C$ (Zhang *et al.*,1993; Aitken, 1993). These studies have also shown a weak, but positive correlation between tree size and $\delta^{13}C$. This means that productivity may not be compromised in more water use efficient trees. Therefore, if $\delta^{13}C$ is heritable, selection for less negative $\delta^{13}C$ (high WUE) might be a productive and feasible avenue for increasing the productivity of Douglas fir.

Productivity versus $\delta^{13}C$

Table 3.6 shows that there were highly significant differences ($p<0.001$) between the irrigated and droughted seedlings grown at the university in terms of family means of height, diameter, dry weight and root:shoot ratios. All seedlings were significantly smaller in the droughted treatment than in the irrigated treatment, indicating that water stress reduced overall growth in all families. Root:shoot ratios were significantly higher in the droughted seedlings. During drought, more fixed carbon could have been allocated to the roots relative to the shoots or it is also possible that the growth rate of the shoots was affected more by drought than the growth rate of the roots. Overall, stomatal conductance could have been reduced during drought, causing a reduction in both transpiration and CO_2 uptake giving rise to smaller seedlings. This is supported by the fact that $\delta^{13}C$ was less negative in all families in the drought treatment indicating a lower P_i due to decreased stomatal conductance.

There were no significant differences between families of seedlings in terms of height, diameter, or dry weight in either wet or dry conditions. However, there were significant differences in the root:shoot ratio between families within the drought treatment. Thus, the allocation patterns of fixed carbon during drought is likely genetically variable and may contribute to improved drought hardiness in some families of Douglas fir. There was no significant correlation between the root:shoot ratio and family mean $\delta^{13}C$ within the drought treatment (data not shown). However, family 8 and family 6, which had the least negative $\delta^{13}C$ (highest WUE) showed the highest root:shoot ratios. Interestingly, these two families consistently had the least negative $\delta^{13}C$within the irrigated and droughted treatment at the university, and within Raymond in 1994. It could be that in times of low soil water availability, stomatal conductance decreases in response to decreasing soil water content by a chemical signal from the roots (i.e. such as ABA). In turn, more fixed carbon is allocated to the roots whereby the subsequent increase in the root system can be used to better exploit the available soil water. It is known that for some species, stomatal conductance is sensitive to soil water deficits (Grieu *et al.*, 1988; Jones, 1992) and that ABA does cause stomates to close (Jones, 1992).

There was no significant correlation between needle $\delta^{13}C$ of the irrigated or droughted seedlings and root:shoot ratio, diameter or dry weight (data not shown). However, there was a significant relationship between $\delta\ ^{13}C$of the irrigated seedlings and height ($r^2 = 0.62$ $p < 0.05$). (See Figure 3.9a). This suggests that an increase in long-term WUE corresponds to an increase in productivity in Douglas fir. Flanagan *et al.*(1995) found that height was positively correlated with $\delta\ ^{13}C$ in black spruce. However, they found that this relationship was only significant on water-limited sites. Similarly, studies on wheat have shown that the relationship between growth and $\delta^{13}C$ can change when plants are grown on different quality sites. Condon and Richards (1993) reported that the positive relationship between $\delta^{13}C$ and growth in wheat genotypes broke down under well-watered conditions. However, in white spruce, Sun *et al.* (1996) found that growth and $\delta^{13}C$ were positively correlated under both irrigated and droughted treatments.

There was no significant relationship was found between $\delta^{13}C$ of the droughted seedlings and height (see Figure 3.9b) Again, it may be that under well-watered conditions, seedlings allocate more photosynthate to shoot growth whereas under droughted conditions, the same seedlings allocate more photosynthate to roots. Thus, the relationship between height and $\delta^{13}C$ changes under different watering regimes.

The positive relation between $\delta^{13}C$ and height further shows that at a given stomatal conductance, P_i is lower due to an increase in photosynthetic capacity. This means that photosynthetic capacity varies between families of Douglas fir. Based on the Farquhar *et al* .(1989) model of stable carbon isotope discrimination during photosynthetic gas exchange, $\delta^{13}C$ will be higher when photosynthetic capacity is increased, if stomatal conductance remains constant. The higher photosynthetic capacity will also translate into higher growth, if all other things remain constant. Thus, $\delta^{13}C$ should be positively correlated with growth when variation in carbon discrimination is due to changes in photosynthetic capacity. The photosynthetic capacity could have increased within the irrigated treatment by more fixed carbon being allocated to leaf growth. Increases in photosynthetic enzymes such as Rubisco could also have contributed to an improved photosynthetic capacity.

There was no significant relationship between height and needle $\delta^{13}C$ for the trees at Raymond or Vail.(data not shown). This could be due to the fact that height measurements were only assessed in March 1993, whereas $\delta^{13}C$ were assessed in October 1993 and 1994. Additionally, the high variability between families within Vail could have obscured the relationship between $\delta^{13}C$ and growth.

3.4 CONCLUSIONS

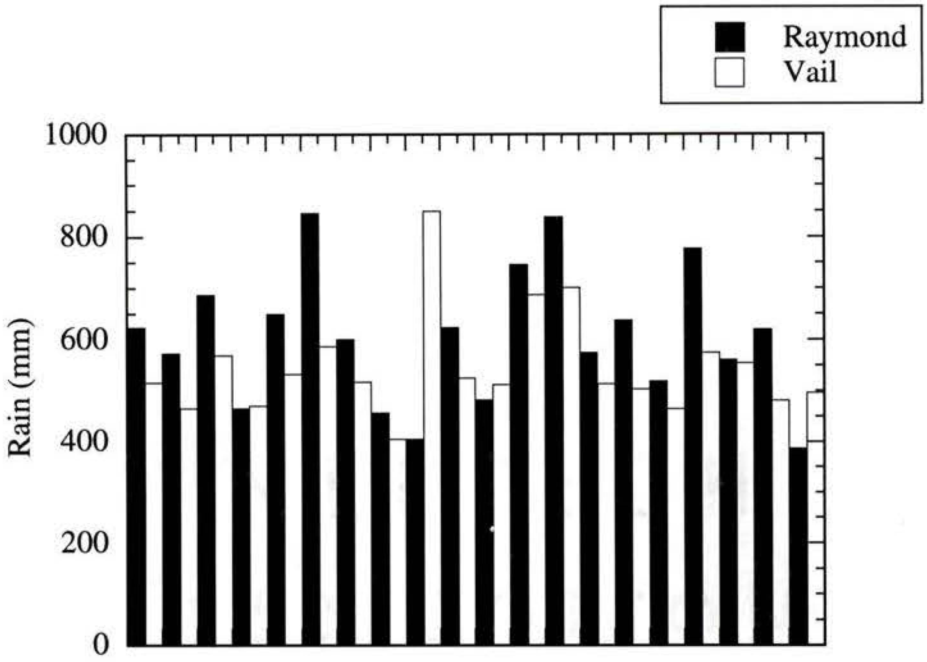
Three major conclusions can be drawn from this study. First, foliage $\delta^{13}C$ is influenced by both water and nitrogen stress. The unexpectedly highly negative $\delta^{13}C$ of the needle tissue from Vail, despite high water deficits at the site, was the result of nitrogen stress. Tissue nutrient status should be addressed when interpretating measurements of $\delta^{13}C$. Second, the consistent ranking between the irrigated and droughted seedlings and between the irrigated seedlings and the needles from Raymond in 1994 in terms of $\delta^{13}C$ shows that stable carbon isotope discrimination is genetically variable in Douglas fir. However, the genetic variability was only evident when the families were growing within a relatively homogeneous environment. Nevertheless, this suggests that there is a potential to use foliage $\delta^{13}C$ as a proxy indicator to long-term water use efficiency in Douglas fir.

Third, there is a positive correspondence between growth and foliage $\delta^{13}C$ in a low water stress environment. This suggests that there is genetic variation between families of Douglas fir in terms of photosynthetic capacity. Therefore, selection for high foliage $\delta^{13}C$ would correspondence to high growth. The lack of a significant relationship between growth and $\delta^{13}C$ within a droughted environment signifies that fixed carbon allocation patterns may change under water stress. The fact that the root:shoot ratio significantly increased within the drought treatment and that the root:shoot ratio was genetically variable, suggests that more photosynthate is allocated to roots during water stress and that allocation patterns vary between families of Douglas fir. Therefore, gas exchange characteristics and morphological changes contribute to water use efficiency in Douglas fir.

Figure 3.1a. The variability in rainfall (mm) for the 1973-1994 growing seasons at Raymond and Vail sites. (Raymond represented by black bars; Vail represented by white bars).

Figure 3.1b. The variability in water deficits (mm) for the 1973- 1994 growing seasons at Raymond and Vail sites. (Symbols same as above).

a)


Raymond
Vail
1000
800
600
400
200
0
Rain (mm)

b)

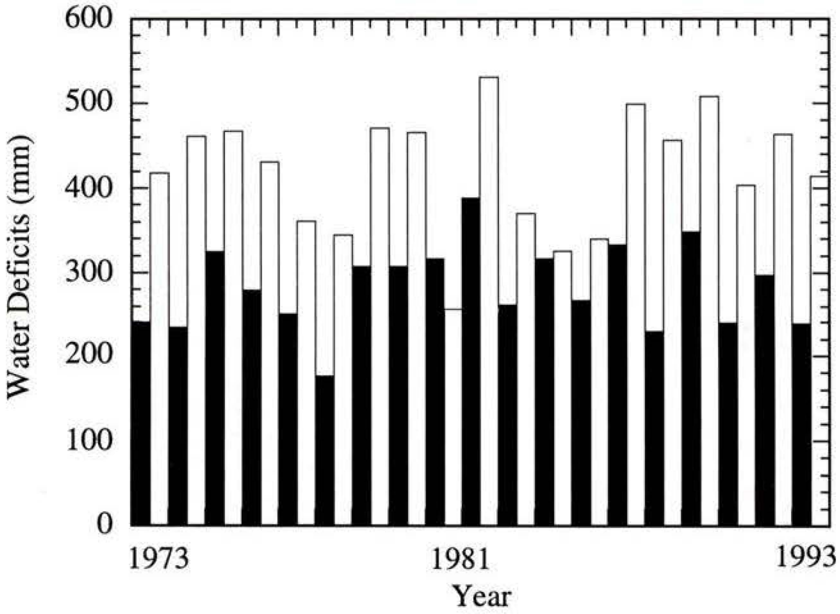

600
500
400
300
200
100
0
Water Deficits (mm)
1973
1981
1993
Year

Figure 3.2 The average growing season vapour pressure deficit [D (kPa)] for Raymond and Vail sites for 1973-1994. The average daily D was averaged from April 1 - October 31 for each year.

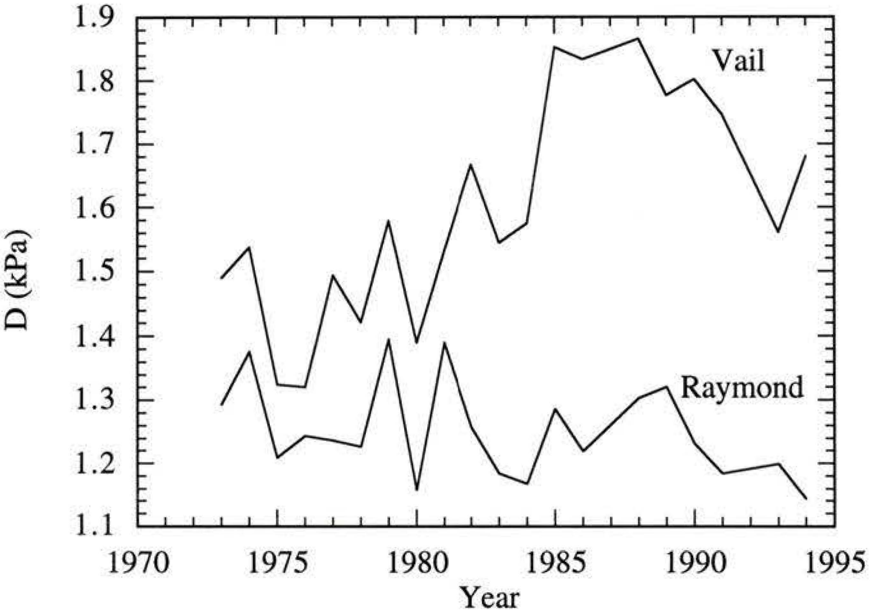
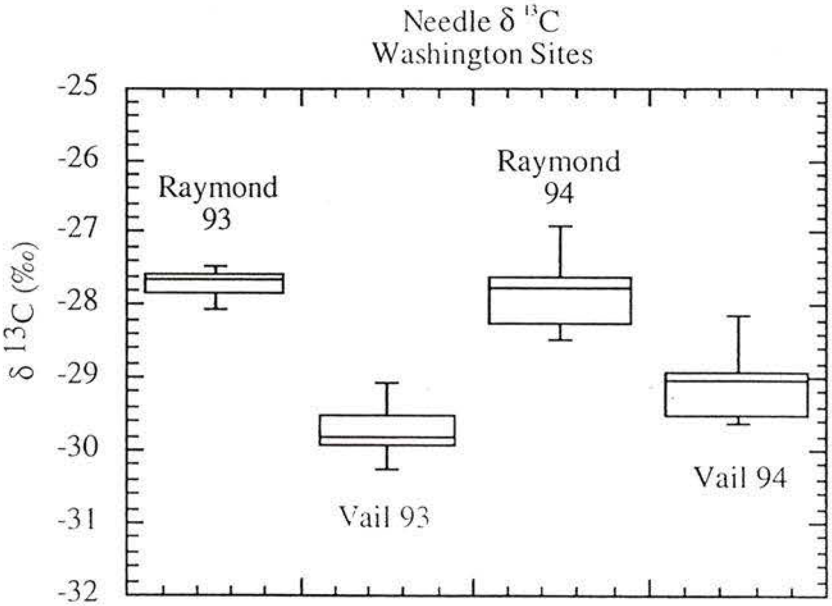

1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
D (kPa)
1970
1975
1980
1985
1990
1995
Year
Vail
Raymond

Figure 3.3a The variability in needle $\delta^{13}C$ for Raymond and Vail in 1993 and 1994. The width of the box represents one standard deviation. The error bars represent the range in $\delta^{13}C$ for the site. The line represents the median $\delta^{13}C$.

Figure 3.3b The variability in needle $\delta^{13}C$ for the seedlings growing at the field facilities at the University of Victoria. Symbols are the same as in Figure 2.3a

a)


Needle δ 13C
Washington Sites
δ 13C (‰)
-25
-26
-27
-28
-29
-30
-31
-32
Raymond 93
Vail 93
Raymond 94
Vail 94

b)

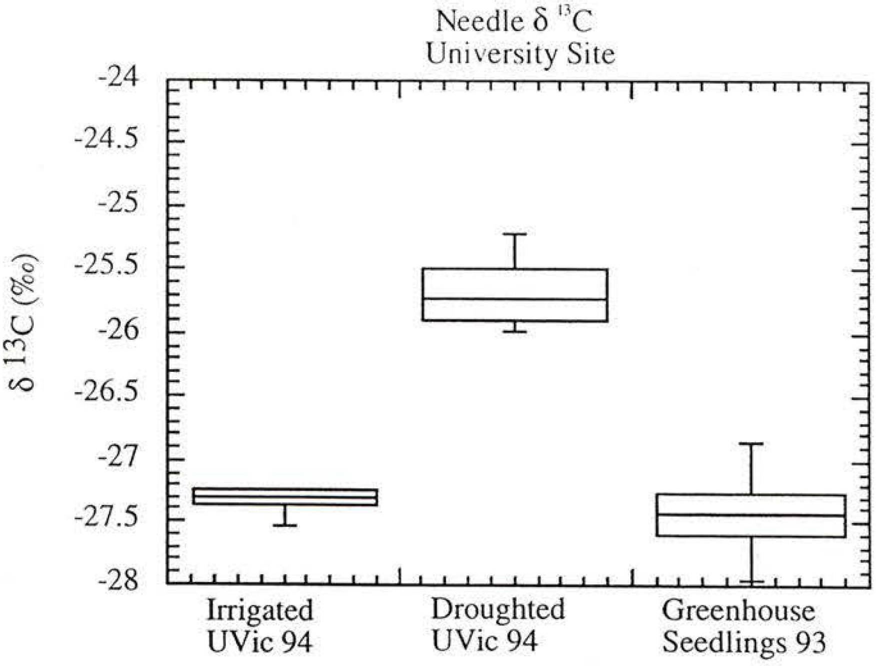

Needle δ 13C
University Site
δ 13C (‰)
-24
-24.5
-25
-25.5
-26
-26.5
-27
-27.5
-28
Irrigated UVic 94
Droughted UVic 94
Greenhouse Seedlings 93

Figure 3.4 The relation between needle nitrogen content (% N gm $^{-1}$) and $\delta^{13}C$ across all sites. Error bars represent one standard deviation.

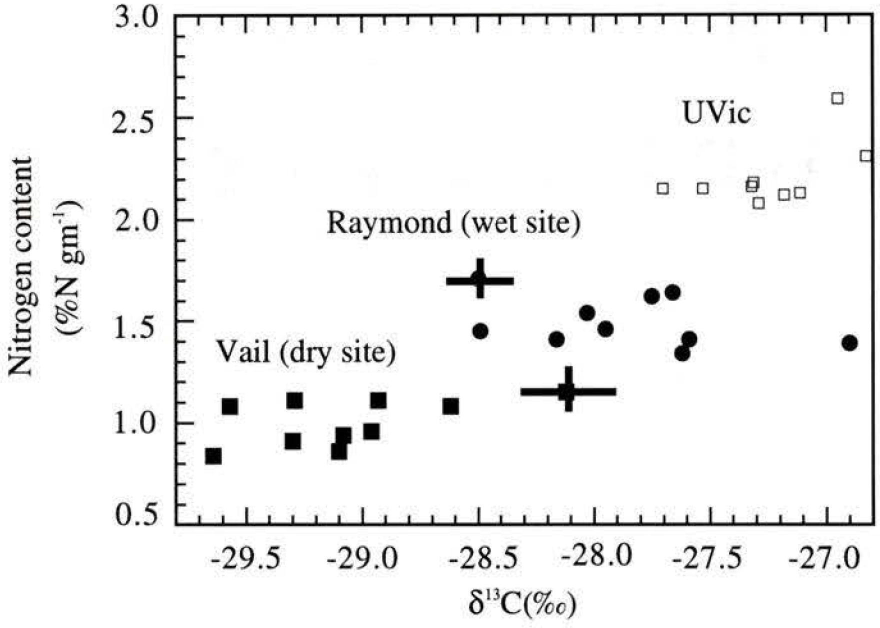

3.0
2.5
2.0
1.5
1.0
0.5
Nitrogen content
(%N gm^{-1})
UVic
Raymond (wet site)
Vail (dry site)
-29.5
-29.0
-28.5
-28.0
-27.5
-27.0
δ^{13}C(‰)

Figure 3.5 The relation between the needle intercellular CO_2 partial pressure (μbar) and the needle nitrogen content (% N gm^{-1}). Regression equation is $y = 271.62 - 26.36X$. $r^2 = 0.48$. ($p<.05$)

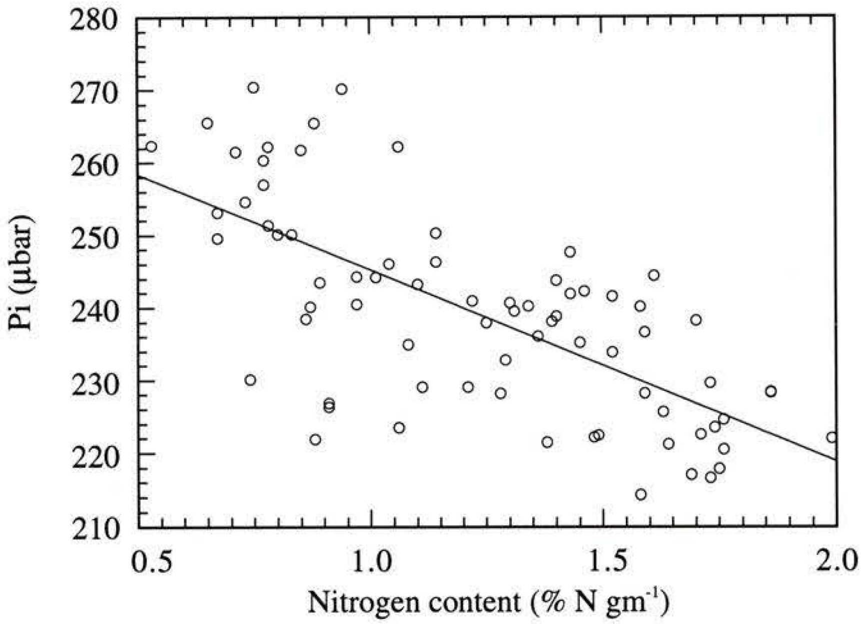
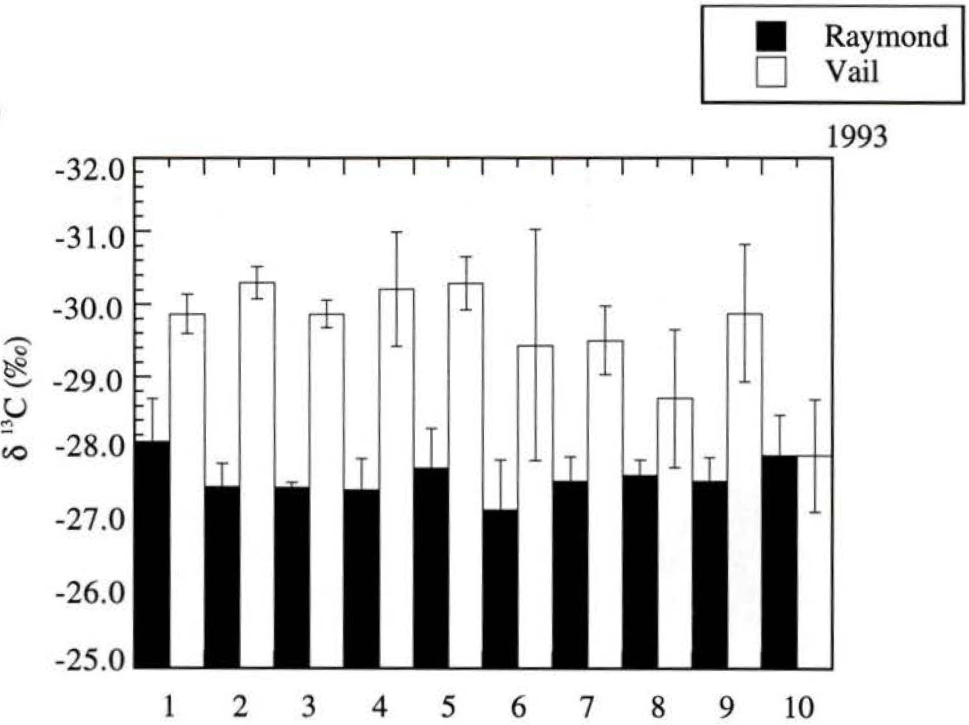

280
270
260
250
240
230
220
210
Pi (µbar)
0.5
1.0
1.5
2.0
Nitrogen content (% N gm^{-1})

Figure 3.6a The family variability in needle $\delta^{13}C$ for 1993 at Raymond and Vail. Error bars represent one standard deviation. (Raymond is represented by black bars; Vail is represented by white bars).

Figure 3.6b The family variability in needle $\delta^{13}C$ for 1994 at Raymond and Vail. Error bars represent one standard deviation. (Symbols as above).

a)


■ Raymond
□ Vail
1993
δ 13C (‰)
-32.0
-31.0
-30.0
-29.0
-28.0
-27.0
-26.0
-25.0
1
2
3
4
5
6
7
8
9
10

b)

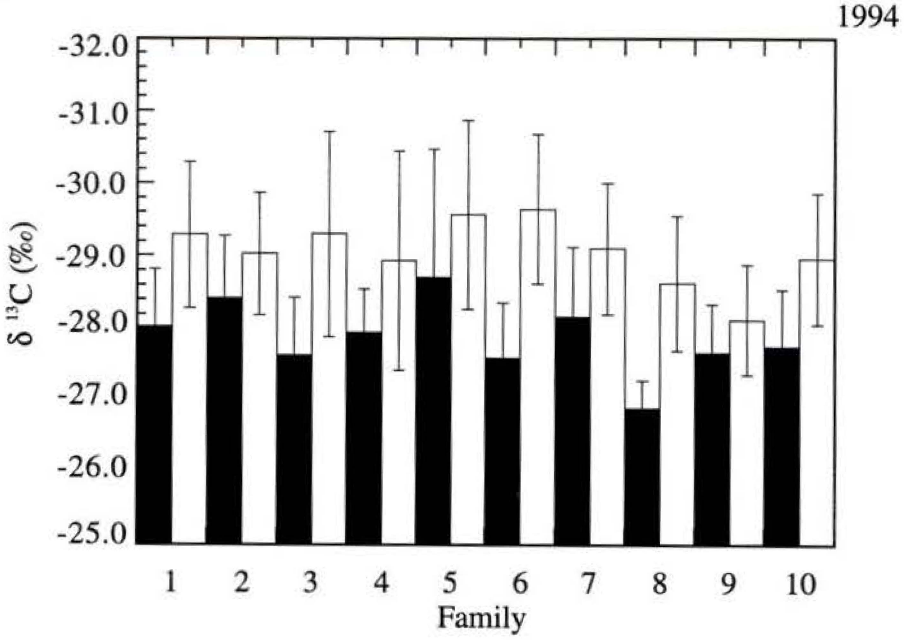

1994
δ 13C (‰)
-32.0
-31.0
-30.0
-29.0
-28.0
-27.0
-26.0
-25.0
1
2
3
4
5
6
7
8
9
10
Family

Figure 3.7a The family variability in needle $\delta^{13}C$ for the greenhouse grown seedlings. Error bars represent one standard deviation.

Figure 3.7b The family variability in needle $\delta^{13}C$ for the irrigated seedlings growing at the field facilities at the University of Victoria. Error bars represent one standard deviation.

Figure 3.7c The family variability in needle $\delta^{13}C$ for the droughted seedlings growing at the field facilities at the University of Victoria. Error bars represent one standard deviation.

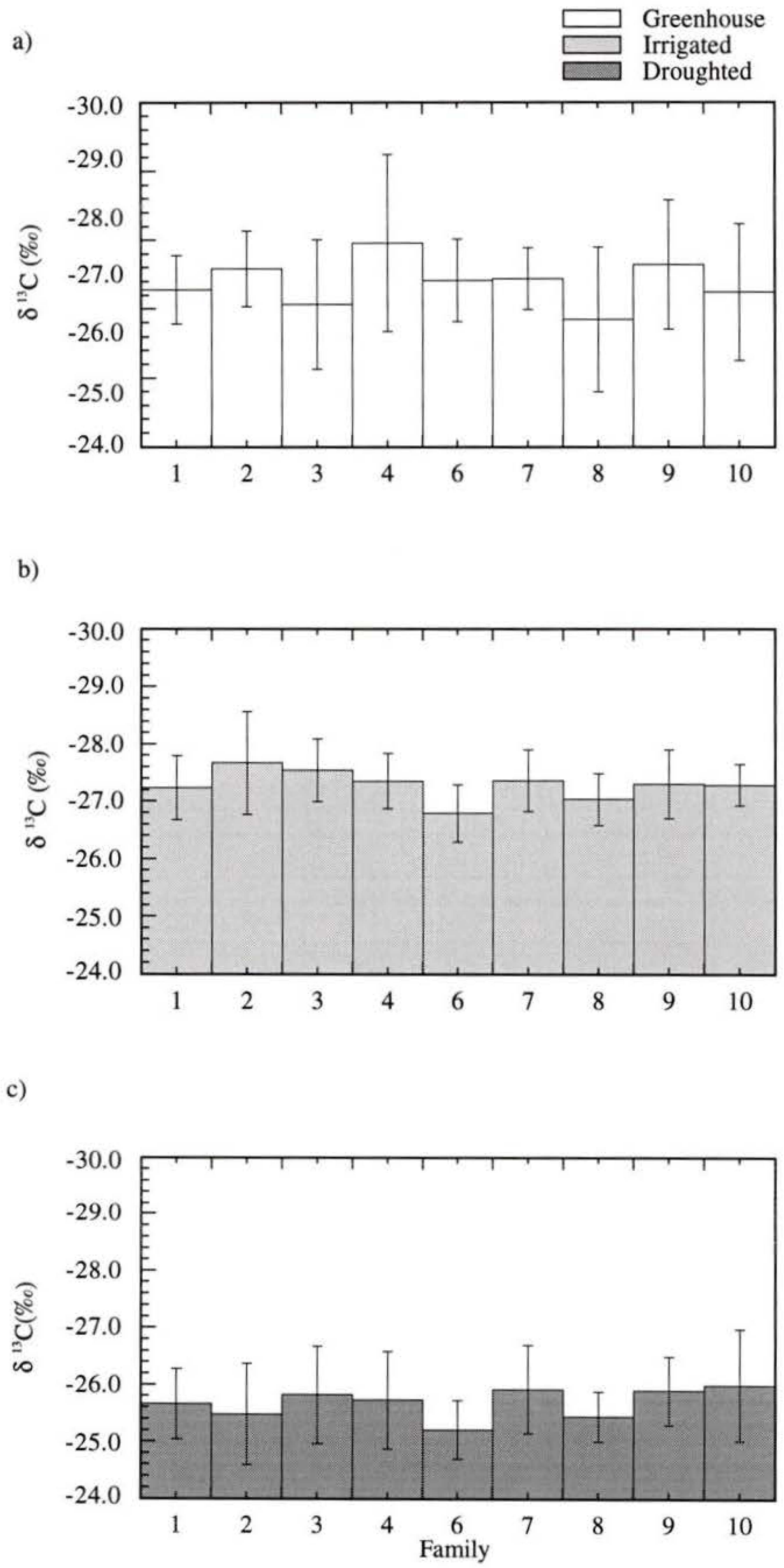
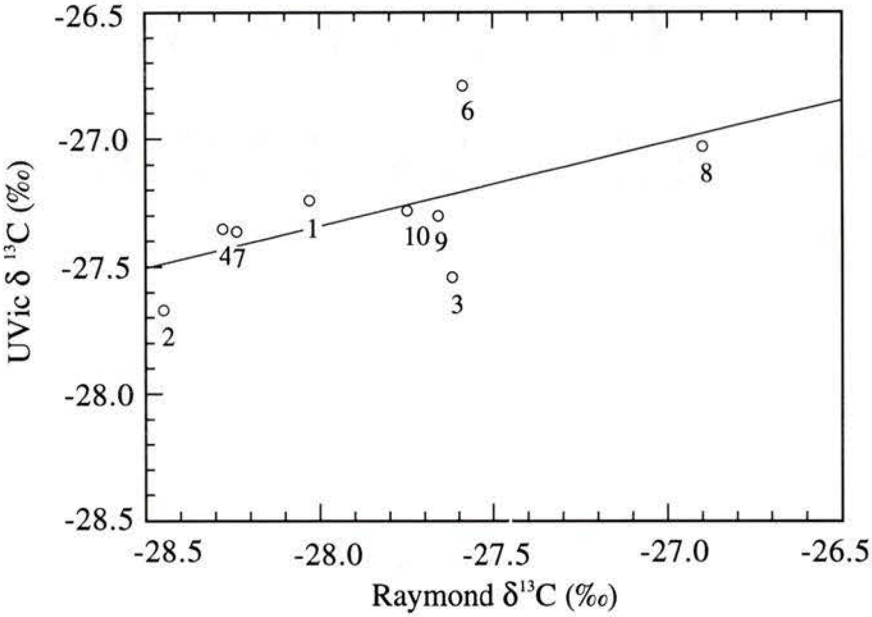

a)
Greenhouse
Irrigated
Droughted
δ 13C (‰)
-30.0
-29.0
-28.0
-27.0
-26.0
-25.0
-24.0
1
2
3
4
6
7
8
9
10
b)
δ 13C (‰)
c)
δ 13C(‰)
Family

Figure 3.8a The relation between the needle $\delta^{13}C$ of the needles from the irrigated seedlings and from the trees at Raymond. Regression line equation is: $y = -18.14 + 328.38X$. $r^2 = 0.36$. ($p<.05$). Numbers represent families.

Figure 3.8b The relation between the needle $\delta^{13}C$ of the needles from the irrigated and droughted seedlings. Regression line equation is $y = 1.22 + 0.99X$. $r^2 = 0.73$ ($p<.01$) with family 2 removed from the analysis. Numbers represent families.

a)


-26.5
-27.0
-27.5
-28.0
-28.5
UVic δ 13C (‰)
-28.5
-28.0
-27.5
-27.0
-26.5
Raymond δ13C (‰)
6
8
1
10
9
47
3
2

b)

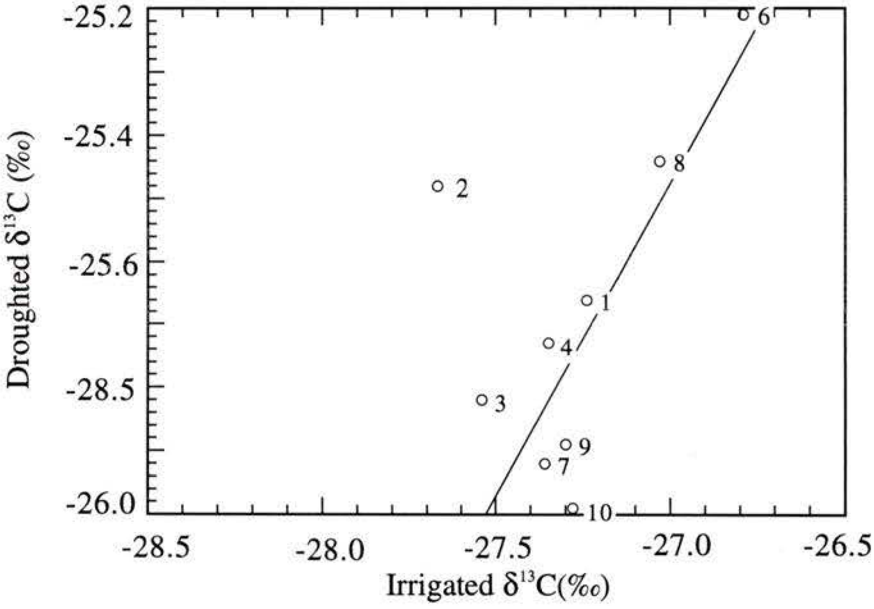
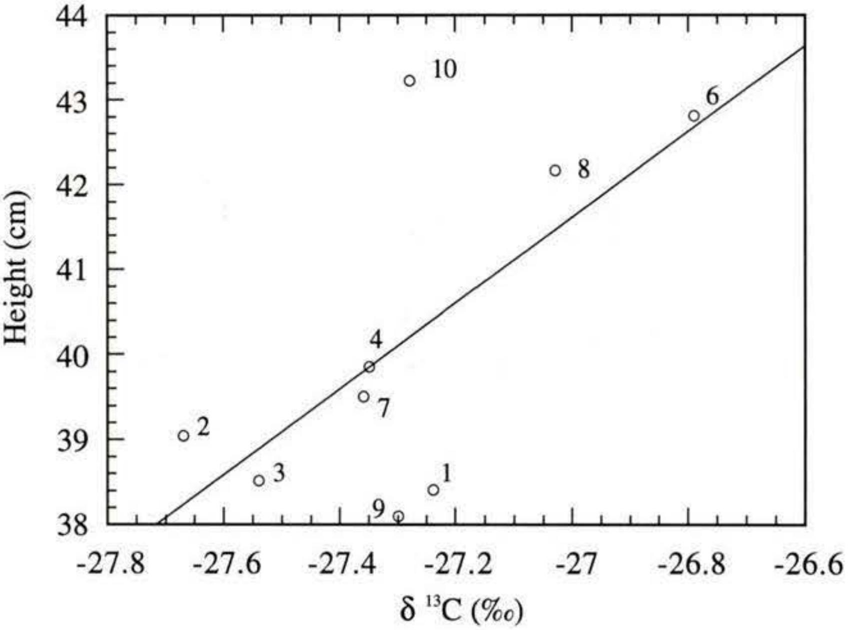

-25.2
-25.4
-25.6
-28.5
-26.0
Droughted δ13C (‰)
-28.5
-28.0
-27.5
-27.0
-26.5
Irrigated δ13C(‰)
6
8
2
1
4
3
9
7
10

Figure 3.9a The relation between the height (cm) and the needle $\delta^{13}C$ from the irrigated seedlings. Regression line is: $y = 178.05 + 5.03X$. $r^2 = 0.62$ ($p<.05$). Numbers represent families.

Figure 3.9b The relation between the height (cm) and the needle $\delta^{13}C$ from the droughted seedlings. Regression line is: $y = 113.91 + 3.17X$. $r^2 = 0.23$. ($p >.10$). Numbers represent families.

a)


Height (cm)
44
43
42
41
40
39
38
-27.8
-27.6
-27.4
-27.2
-27
-26.8
-26.6
δ 13C (‰)
10
6
8
4
7
2
3
1
9

b)

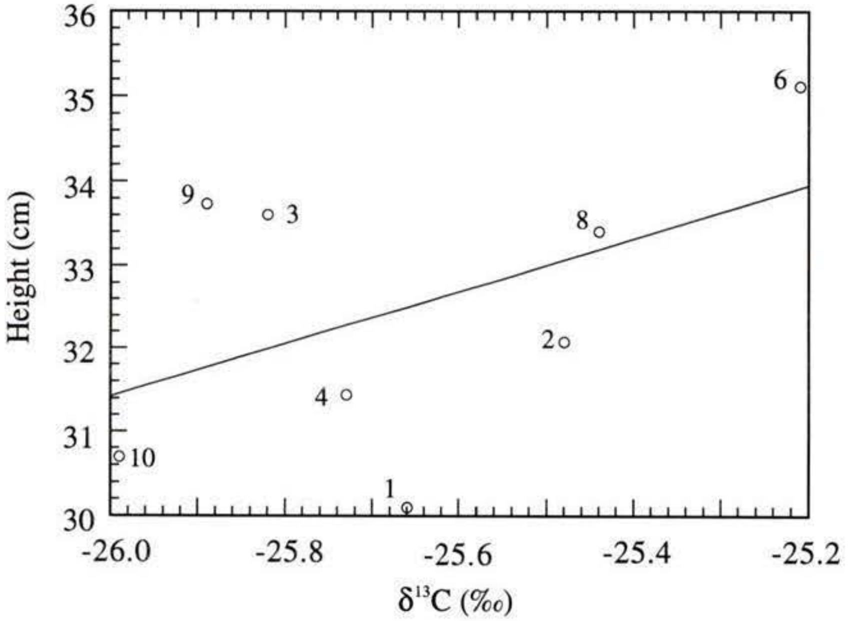

Height (cm)
36
35
34
33
32
31
30
-26.0
-25.8
-25.6
-25.4
-25.2
δ13C (‰)
6
9
3
8
2
4
10
1

Table 3.1 Parental identity numbers of the half-sib families at Raymond and Vail. Within each provenance, the same pollen mix was outcrossed with each female.

Families of Trees

	FAMILY		
Wet Provenance Source	Female	Male	NUMBER
	1014	9131	1
	1004	9131	2
	1087	9131	3
	1055	9131	4
	1026	9131	5
Dry Provenance Source	635	9152	6
	622	9152	7
	667	9152	8
	643	9152	9
	616	9152	10

Table 3.2 1973-1994 growing season weather variable averages for Raymond and Vail.

1973 - 1994 Growing Season Weather Variable Averages for Raymond and Vail

	Rainfall (mm)		Water Deficit (mm)		Vapour Pressure Deficit (kPa)	
	Raymond	Vail	Raymond	Vail	Raymond	Vail
Average	604	546	284a**	420a**	1b**	1.6b**
SD	131	100	51	71	0.08	0.17
COV	0.22	0.18	0.18	0.17	0.06	0.11

NOTE : **p < .001
: same letter denotes that variables are significantly different
: SD is standard deviation
: COV is coefficient of variation

Table 3.3 Average height and diameter of families of trees at Raymond and Vail. Numbers in parentheses indicate one standard deviation.

	HEIGHT (m)		DBH (cm)	
Family	Raymond	Vail	Raymond	Vail
1	7.45(.89)	2.91(.82)	8.08(1.6)	2.38(1.13)
2	7.68(.67)	2.51(.80)	9.06(1.26)	2.15(.96)
3	7.31(.84)	2.25(.45)	8.07(2.30)	1.61(.82)
4	7.42(.65)	2.29(.76)	8.39(1.47)	1.76(1.02)
5	7.6(1.23)	2.12(.61)	8.89(2.14)	1.69(.99)
6	7.04(.67)	2.9(.72)	7.56(1.30)	2.43(.99)
7	7.16(.86)	3.1(.73)	8.25(1.29)	2.78(1.16)
8	7.26(.53)	2.66(.89)	8.47(1.18)	2.66(.89)
9	7.09(.84)	2.76(1.09)	7.60(1.51)	2.72(1.08
10	7.08(.78)	2.86(1.34)	8.10(1.16)	2.86(1.34)
Total mean	7.31a**	2.64a**	8.25b**	2.31b**
SD	0.19	0.25	0.39	0.48
COV	2.46	9.46	4.73	20.78

Table 3.4 Needle Nitrogen Content (% N gm^{-1}) for needles harvested from all sites in 1994. T-test applied to Raymond and Vail data only.

Needle Nitrogen Content (%N /gm)

	Raymond	Vail	University Irrigated	University Droughted
Mean	1.5**	1.0**	2.21	2.03
SD	0.12	0.11	0.16	0.11

NOTE: :**p<.001

:SD means total standard deviation

Table 3.5 Average needle $\delta^{13}C$ for families growing at all sites. Numbers in parentheses indicate one standard deviation. Total site averages are also shown.

Stable Carbon Isotope Composition
Family and Total Site Means

Family	Raymond		Vail		Green house	University	
	1993	1994	1993	1994		Irrigated	Droughted
1	-28.06(0.62)	-28.03(0.79)	-29.83(0.68)	-29.56(0.91)	-27.28(0.50)	-27.24(0.56)	-25.66(0.62)
2	-27.69 (0.66)	-28.45(0.78)	-29.93(1.05)	-29.03(0.75)	-27.59(0.55)	-27.67(0.90)	-25.48(0.89)
3	-27.47(0.48)	-27.62(0.81)	-30.26(0.77)	-29.50(1.32)	-27.07(0.94)	-27.54(0.55)	-25.82(0.86)
4	-27.57(0.40)	-28.28(0.96)	-30.07(0.72)	-28.93(1.51)	-27.96(1.29)	-27.38(0.48)	-25.73(0.86)
5	-27.85(0.51)	-28.50(1.66)	-30.12(0.71)	-29.57(1.30)			
6	-27.58(0.52)	-27.59(0.77)	-29.76(0.98)	-29.63(1.03)	-27.42(0.60)	-26.79(0.50)*	-25.21(0.51)
7	-27.66(0.64)	-28.24(1.01)	-29.17(0.56)	-29.10(0.88)	-27.44(0.45)	-27.36(0.54)	-25.92(0.78)
8	-27.83(1.04)	-26.90(0.38)	-29.37(0.82)	-28.62(0.93)	-26.85(1.05)	-27.03(0.45)*	-25.44(0.44)
9	-27.65(0.53)	-27.66(0.68)	-29.73(0.77)	-28.12(0.76)	-27.65(0.94)	-27.30(0.60)	-25.89(0.60)
10	-27.85(0.62)	-27.75(0.78)	-29.80(0.47)	-28.96(0.90)	-27.25(0.99)	-27.28(0.36)	-25.99(0.98)
Total Mean	-27.72ad**	-27.9be**	-29.8a**	-29.1b**	-27.28d**	-27.39ce**	-25.68c**
SD	0.175	0.494	0.332	0.485	0.259	0.33	0.26
COV	0.63	1.77	1.15	1.67	0.95	1.21	1.01

NOTE: : *p<.05
: ** p<.001
: same letters denote significantly different
: numbers in parentheses are standard deviation
: SD is total standard deviation
: COV is coefficient of variation
: family 5 not available for university site

Table 3.6 Morphological traits measured on the seedlings grown at the field facilities at the University of Victoria.Family averages and total averages for all seedlings within a treatment are shown. Numbers in parentheses are one standard deviation.

Morphological Traits University Seedlings
Irrigated and Droughted Treatment
Family and Total Treatment Means

Family	Root:shoot ratio		Dry Weight (gm)		Height (cm)		Diameter (cm)	
	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted	Irrigated	Droughted
1	0.70(0.19)	0.65(0.14)*	38.48(6.2)*	15.86(2.4)	38.41(4.52)	30.10(3.86)	0.10(0.11)	0.73(0.05)
2	0.69(0.19)	0.88(0.17)	31.22(6.2)	16.20(6.1)	39.05(4.78)	32.08(2.42)	0.10(0.07)	0.68(0.20)
3	0.68(0.11)	0.88(0.23)	20.93(11.9)	12.37(3.0)	38.52(5.97)	33.61(4.14)	0.90(0.19)	0.69(0.09)
4	0.68(0.12)	0.75(0.07)	30.88(5.2)	16.65(2.9)	39.86(5.33)	31.44(4.26)	0.10(0.15)	0.67(0.08)
6	0.67(0.13)	0.83(0.05)	30.94(6.4)	13.94(3.9)	42.81(7.90)	35.12(4.75)	0.10(0.13)	0.73(0.08)
7	0.68(0.12)	0.80(0.09)	29.88(6.1)	14.10(2.1)	39.51(5.20)	32.50(4.30)	0.10(0.12)	0.73(0.08)
8	0.71(0.12)	0.99(0.15)	28.00(7.8)	15.48(1.3)	42.17(5.72)	33.41(3.92)	0.10(0.13)	0.75(0.05)
9	0.64(0.09)	0.66(0.07)*	27.83(8.2)	15.02(3.0)	38.10(3.63)	33.74(4.81)	0.96(0.13)	0.70(0.09)
10	0.66(0.14)	0.88(0.29)	28.54(6.7)	13.63(3.1)	43.23(7.15)	30.70(4.49)	0.96(0.10)	0.73(0.08)
Total mean	0.68a**	0.81a**	29.63b**	14.81b**	40.18c**	32.52d**	0.98e**	0.71e**
SD	0.02	0.11	4.57	1.39	2.01	1.61	0.45	0.03
COV	2.94	13.83	15.42	9.41	4.99	4.95	93.7	4.01

NOTE: : * p<.05
: **p<.001
: same letter denotes significantly different
: SD means total standard deviation
: COV means coefficient of variation
: numbers in parentheses are one standard deviation

CHAPTER 4
VARIATION IN STABLE CARBON ISOTOPE COMPOSITION OF ANNUAL TREE RINGS AND ITS RELATION TO WATER USE AND PRODUCTIVITY

4.1 INTRODUCTION

Annual tree rings provide information about the environmental conditions prevalent during a tree's growth. Numerous studies have established relationships between seasonal and annual climatic factors, soil water availability, and tree radial growth (Fritts, 1976; Francey and Farquhar, 1982; Spittlehouse et.al,1985; Giles *et al.*, 1985; Robertson, 1990). Water stress, resulting from a lack of soil moisture, has been reported to be the major factor limiting summer tree radial growth in the Pacific Northwest (Waring and Franklin, 1979). Knowledge of the relationships between climate and tree growth should aid foresters in assessing future forest productivity. In particular, tree ring parameters might be used as an index of growth under varying climatic conditions.

There has been increasing interest in defining the causes of variation in the $\delta^{13}C$ of tree rings in recent years. Many studies have shown that the $\delta^{13}C$ of tree rings is related to climatic variables such as air temperature, solar radiation, and precipitation (Tans and Mook, 1980; Francey and Farquhar, 1982; Leavitt and Long, 1983; 1989; 1991). Others have shown that the variation of $\delta^{13}C$ is primarily due to soil water availability and seasonal cumulative transpiration (Leavitt and Long, 1991; Dupouey, 1993; Livingston and Spittlehouse, 1993; 1996). These studies suggest that tree ring $\delta^{13}C$ could be used as a sensitive indicator of long-term tree water use. Recently, some work has shown that annual tree ring $\delta^{13}C$ and tree productivity are positively correlated (Dupouey *et al.*, 1993; Livingston and Spittlehouse, 1993; 1996). Therefore, the $\delta^{13}C$ of tree rings may potentially be a surrogate for integrated, long-term water use efficiency.

Measurements of $\delta^{13}C$ in tree rings have also been used in studies of the global carbon balance and to assess the effects of air pollution on tree growth (Peng *et al.*,1983; Martin and Sutherland, 1990). Most studies have found large temporal variations in tree ring $\delta^{13}C$ with an overall downward trend starting in the late 1800's. The downward trend is likely due to the input of ^{13}C-depleted air, derived from the burning of fossil fuels into the atmosphere (Keeling *et al.*, 1979).

The objectives of the work described in this chapter are to establish: 1) the variability in terms of $\delta^{13}C$ of whole annual tree rings in 10 half-sib families of Douglas fir 2) the relationship between annual ring $\delta^{13}C$ and tree growth and 3) the relationship between annual tree ring $\delta^{13}C$ and tree water use.

4.2 MATERIALS AND METHODS

TREE SOURCE

Refer to Chapter 3, Section 3.2 for the tree's sources.

WASHINGTON FIELD SITES

Location of Site

Tree cores were collected only from the trees growing at the Raymond site. For the location of this site, refer to Chapter 3, Section 3.2.

Weather Stations

An automated weather station was installed at Raymond for the 1993 and 1994 growing seasons. The station is described in detail in Chapter 3, Section 3.2.

Water Balance Model

The water balance model is described in detail in Chapter 3, Section 3.2.

3A.2d Soil Analysis

The soil analysis is described in detail in Chapter 3, Section 3.2.

EXPERIMENTAL DESIGN

Raymond Site

The experimental design for Raymond is described in Chapter 3, Section 3.2. Families growing at Vail and at the University of Victoria were not included in this study.

WOOD ISOTOPE COMPOSITION

Wood Collection

Three wood cores were taken from all 240 trees at Raymond in December 1993. One core was extracted at breast height; the other two cores were extracted from the same side of the tree in a vertical line from the first core. Core extractions were never more than 10 cm from the first core taken. Cores were taken from the bark to the pith. All cores were tagged and stored within plastic straws until the spring of 1994.

Soluble Organics Extraction

To prepare the wood for carbon isotope analysis, soluble organic extractives (i.e. hemicellulose, resins, tannic acids, oils) were removed from the cores, since these materials can migrate from ring to ring over time (Tans and Mook, 1980). Organic extractives are typically 1-2 ‰ lighter than cellulose $\delta^{13}C$ (Freyer, 1979; Tans and Mook, 1980). The procedure was performed by technicians at Weyerhaeuser's research facilities in Tacoma, Washington. Each core was thin-sawed to a uniform thickness of 2.0 mm, and mounted on a cedar mounting stick. The samples were subsequently placed within a Soxhlet apparatus and refluxed for approximately 24 hours with a benzene/alcohol solution as described in Tappi, (1987). After extraction, the samples were air-dried for several days to obtain a minimum, constant wood moisture content.

X-Ray Densitometry

To precisely separate the tree cores into annual rings, an x-ray beam was passed through the cores, and the resulting transmitted radiation was measured and calibrated to the specific wood density. Generally, the radiation beam is attenuated as it passes through the core. The degree of attenuation is a function of the density, thickness, and the absorptivity of the wood core and is calculated using:

$$I_o = I_i e^{-ult} \qquad [18]$$

where

I_o = the transmitted radiation

I_i = the initial radiation at the surface

u = wood absorption coefficient

l = the wood specific density in gm cm^{-3}

t = the wood thickness (mm)

The procedure was performed by technicians at the Weyerhaueser research facility in Tacoma, Washington. The specific wood density (i.e. oven dry weight/green volume) of 10 cores was empirically calibrated to a cellulose polyacrylamide wedge. The wood density of each core was graphically displayed by plotting the wood density along the length of the core against the distance from the bark to the pith. The highest peaks in the graph corresponded to the most dense portions of the core. (i.e. the latewood of each year). The annual ring widths were discerned by measuring the distance between adjacent maximum peaks. Early and latewood components were also separated by measuring the distance between the highest and lowest peak within an annual ring. Early and latewood components were separated for one family only. It was estimated that ring widths were accurate to within 1/100 of an inch.

Wood Grinding

Before carbon isotope analysis, the 1988-1993 whole rings of 8 families and the early and latewood of 1 family were ground to a fine powder (i.e. 40 mesh) using a diamond-head Dremel drill bit mounted on a conventional drill press. Rings were individually wedged into a custom-made stainless steel thimble that fit into a wooden holder on the stage of the drill press. While grinding, contact between the drill bit and sides of the thimble was prevented to ensure that there was no metal contamination of the samples.

Carbon isotope analysis

Carbon isotope composition was determined on composite samples of 3 ground whole rings/tree of 8 families and the early and latewood of 1 family. Analysis of $\delta^{13}C$ was similar to the procedure outlined in Chapter 3, Section 3.2.

MORPHOLOGICAL MEASUREMENTS

Total ring width and basal area increment were used as indices of productivity for these trees. Total ring width was determined from x-ray densitometric analysis (See Section 4.2). The sum of all ring widths within a core were used to calculate the basal area increment (BAI) for one tree. Growth trends were removed by dividing the BAI for a year by the BAI value predicted for that year from the respective trend curve. This results in values that have a mean of 1 and a relatively homogeneous variance over time (Fritts, 1976). The deviation of the index from 1 indicates that the trees grew more or less in that year than might be expected under "average" conditions.

STATISTICAL ANALYSIS

Water Balance Analysis

The water balance was analyzed for the growing seasons (i.e. April 1 - October 31) of 1973-1994 (See Chapter 3, Section 3.2). Total soil water deficits, and vapor pressure deficits for the 1988-1993 growing seasons are shown in this chapter. The cumulative growing season transpiration was evaluated by summing daily transpiration for April through October.

Analysis of $\delta^{13}C$ and Productivity

Data was analyzed as a completely randomized block design with six replicates/family. ANOVA was used to establish differences between families in terms of carbon isotope composition and to determine any differences between years at the site. Linear regressions were performed to evaluate the relationships between $\delta^{13}C$ transpiration, and water deficits. Regression analysis was used to determine the relationships between basal area increment index and total tree ring width.

4.3 RESULTS AND DISCUSSION

Variability in Annual Ring $\delta^{13}C$

There were no significant differences in $\delta^{13}C$ of the total annual rings for the 10 families of Douglas fir, despite consistent ranking between some families from year to year (See Figure 4.1). However, there were highly significant differences between years. These results suggest that $\delta^{13}C$ of annual tree rings is more strongly influenced by environmental than genetic factors. Over a 6 year period and 10 families, the range in $\delta^{13}C$ of individual trees was 3.24 ‰ and the variability between families was small.(See Figure 4.1). These results suggest that genetic variability in annual ring $\delta^{13}C$ is limited.

Other studies have also shown low genetic variability in annual ring $\delta^{13}C$ of Douglas fir. Aitken, (1993) found that genetic differences between open-pollinated populations of coastal Douglas fir growing in a common garden environment were weak ($0.05 < p < 0.10$). However, the average $\delta^{13}C$ of populations was closely related to mean annual precipitation in the source environment. ($r^2 = 0.99$; $p < .05$). Livingston and Spittlehouse (1993) found that over a 17 year period, ranking of annual ring $\delta^{13}C$ between 3 individual trees at the same site was generally maintained, despite high intertree variability (i.e. range in $\delta^{13}C$was 4.0 ‰). This suggests that there were genetic differences between trees. Nevertheless, it was found that cumulative transpiration explained most of the variability in tree ring $\delta^{13}C$. Generally, it seems that in Douglas-fir, the variability in $\delta^{13}C$

of annual tree rings is influenced more by tree water use rather than genetic factors.

Productivity and Annual Ring $\delta^{13}C$

Total ring width and a basal area increment index were used as indices of growth for the 1988-1993 growing seasons at Raymond. There were no significant relationships between annual ring $\delta^{13}C$ and total ring width or BAI index (data not shown). This suggests that $\delta^{13}C$ of whole ring tissue in Douglas fir does not reflect seasonal diameter growth, even though BAI and total ring width varied genetically between families (Tables 4.1a and 4.1b). It is possible that ring growth is related to the amount of stored carbohydrates produced in the previous year. Robertson *et al.* (1990) found that approximately half of the annual variation in earlywood width is determined by the previous year's growth. Fritts (1976) has also suggested that in conifers, a large portion of the previous year's stored carbohydrates is used in earlywood growth in the spring. However, no relationship was found between the previous year's $\delta^{13}C$ and the current year growth parameters (data not shown). Further, 59% of the variability in family mean annual ring $\delta^{13}C$ can be explained by the corresponding needle $\delta^{13}C$ for 1993.(See Figure 4.2). It is probable that the expansion of the tracheids during early spring substantially contributes to the width of the annual ring relative to the amount of structural carbon present. It has been shown that earlywood width is significantly negatively correlated with earlywood density (Robertson *et al.*,1990; Aloni, 1991). Since the ratio of earlywood to total width is usually large in mesic sites (Brix, 1972; Robertson, 1990), a wide annual ring would correspond to low density and depressed secondary cell wall thickness overall. Thus, $\delta^{13}C$ would not be expected to correlate well with ring width.

The $\delta^{13}C$ of the earlywood and latewood of one family of Douglas fir was determined. The results are shown in Figure 4.3. The $\delta^{13}C$ of the earlywood was more negative than the $\delta^{13}C$ of the latewood for each year. This shows that the photosynthate for earlywood growth was formed when the intercellular partial pressure of CO_2 (P_i) was high. High P_i can result from increased stomatal conductance or from a lowered photosynthetic capacity when stomatal conductance is low (Farquhar *et al.*, 1989). It is likely that a relatively high stomatal conductance occurred throughout the early and late months of the growing seasons at Raymond because of the moderate air temperatures and relatively wet soils. Thus, photosynthate formed in the spring, early summer and fall would have a more negative $\delta^{13}C$. Assuming that the $\delta^{13}C$ does not change during carbohydrate translocation and storage, the current and reserve photosynthate that contributes to earlywood growth would lower $\delta^{13}C$.

The less negative $\delta^{13}C$ of the latewood shows that latewood was formed when P_i was lower. Many studies have documented that the initiation of latewood growth and the width and density of latewood is positively related to the onset and duration of soil water deficits and high air temperatures (Brix, 1972; Robertson et al., 1990; Aloni, 1991). At Raymond, soil water deficits and vapor pressure deficits generally increased by July and declined by early October from 1988-1993 (See Figure 4.5a and Figure 4.5b). Thus, stomatal conductance would likely have been reduced during the summer months influencing latewood's $\delta\ ^{13}C$. Others have found that the $\delta^{13}C$ of latewood is isotopically heavier than earlywood. Freyer (1979) found that the mean $\delta^{13}C$ for 50 individual trees was lower in earlywood than latewood. Leavitt (1993) found that earlywood had more negative $\delta^{13}C$ than latewood in pine and maple trees, with the largest difference occurring in the driest years. Interestedly, Livingston and Spittlehouse (1996) found that at a relatively mesic site, in years with little or no soil water deficits in the summer, latewood in coastal Douglas fir had appreciably more negative $\delta^{13}C$ than earlywood. They predicted that the isotopic composition in latewood only becomes heavier than earlywood when there is more than a 55 mm difference between spring and summer cumulative transpiration. Their study involved 9 individual trees and $\delta^{13}C$ was evaluated from tree rings from 1962 to 1981 inclusive. Thus, the 6 years examined on one family in this study may not sufficiently express the difference in $\delta^{13}C$ between earlywood and latewood over the long-term. Nevertheless, the results are consistent with Livingston and Spittlehouse, in that $\delta^{13}C$ of latewood was isotopically heavier than earlywood in years with higher soil water deficits.

Latewood $\delta^{13}C$ was positively correlated (i.e. $r^2 = 0.42$) with latewood width as shown in Figure 4.5. This is consistent with the supposition that current photosynthate is used to produce latewood and that latewood exhibits high density characteristics (Robertson *et al.*,1990).

Environmental Influences on Annual Ring $\delta^{13}C$

Growing season cumulative transpiration and seasonally accumulated water deficits were compared to the whole annual ring $\delta^{13}C$ to determine the extent of the environmental influence on $\delta^{13}C$. Generally, there was no significant relationship between $\delta^{13}C$ and seasonal cumulative transpiration or water deficits for any time period (See Table 4.2). Figure 4.6 shows that trends between April-August accumulated transpiration, water deficits and $\delta^{13}C$ were inconsistent. Plots of other time periods and $\delta^{13}C$ were also variable (data not shown). Correspondence of the early and latewood $\delta^{13}C$ of one family and the cumulative transpiration and water deficits for any time period were also not significant (data not shown). These results are contrary to other studies. In 1989, Leavitt and Long found that the $\delta^{13}C$ of annual rings of several species of pine were related to regional drought indices. Further work on several other conifer and hardwood species, showed that the $\delta^{13}C$ of tree rings were significantly correlated to precipitation and soil moisture content (Leavitt and Long, 1991). Dupouey *et al.* (1993) reported that the relative extractable soil water of July was responsible for the majority of annual ring $\delta^{13}C$ variation in beech trees. Livingston and Spittlehouse (1993) found good correspondence between $\delta^{13}C$ of whole tree rings and modelled cumulative transpiration and water deficits of young, coastal Douglas fir growing on a wet site on Vancouver Island over a 17-year period. They found a dramatic improvement in the relationship between cumulative transpiration and tree ring $\delta^{13}C$ when the whole ring was separated into early and latewood components. Some transpiration estimates accounted for almost 93% of the variability in latewood $\delta^{13}C$ (Livingston and Spittlehouse, 1996). Each of these studies used data that covered a period of at least 17 years. It may be that in this study, 6 years of data were not sufficient to express the true relationship between tree water use and annual ring $\delta^{13}C$. More likely, however, limitations of the water balance model used to assess cumulative transpiration and water deficits accounts for the apparent lack of a relationship.

The model was originally designed for established stands (Spittlehouse and Black, 1981; Spittlehouse, 1985). Coefficients used in the model were obtained from studies completed on Douglas fir stands that were 15 years old or greater (Giles *et al.*,1985; Spittlehouse, 1989; Livingston and Spittlehouse, 1993) where root zone depth and canopy closure would have been relatively constant. In this study, the trees were outplanted in 1986 as saplings. Fullness of the canopy, tree size and rooting depth would have changed considerably from 1986-1994. These changes would have affected tree water use that is not necessarily reflected by the model. For example, Figure 4.6 shows that from 1988 to 1991, a decrease in cumulative transpiration corresponded to an decrease in annual ring $\delta^{13}C$. On the other hand, in 1992 and 1993, a decrease in cumulative transpiration resulted in a increase in annual ring $\delta^{13}C$. In 1992, the average family annual ring $\delta^{13}C$ was the least negative (-24.83 ‰) during the measurement period. However, even though 1992 had the relatively driest summer (236.5 mm cumulative transpiration for June, July, August), 1989 also had a dry summer (257.9 mm cumulative transpiration), and the average annual ring $\delta^{13}C$was relatively negative (-25.65 ‰). Similarly, 1993 had the wettest summer (363.3 mm cumulative transpiration) and the least negative average annual ring $\delta^{13}C$ (-25.88 ‰) whereas the wet summer in 1988 (339.81 mm cumulative transpiration) coincided with a higher average annual ring $\delta^{13}C$ (-25.13 ‰). All years experienced a wet spring and fall. It is probable that the trees had a deeper root zone and a fuller canopy in 1992 and 1993 relative to earlier years. It may be that the annual ring $\delta^{13}C$ may have been more accurately reflected by the modelled cumulative transpiration in these years. Therefore, a water balance model that took into account the changing root zone depth and/or canopy growth would have been more appropriate to use in this study.

4.4 CONCLUSIONS

Three conclusions can be drawn from this study:

First, the variability in the $\delta^{13}C$ of annual tree rings is influenced more by environmental than genetic factors. Variability in $\delta^{13}C$ between families was low, whereas variability between years differed significantly. However, there is genetic variability in needle $\delta^{13}C$ (See Chapter 3). Thus, despite little genetic variability in annual tree rings, foliage $\delta^{13}C$ may be a good surrogate for long-term water use efficiency in Douglas fir.

Second, it may be that the $\delta^{13}C$ of tree rings is a good indicator of tree water use over the long-term. To clearly establish the nature of the relationship between $\delta^{13}C$ and water use however, it is important that an appropriate model be used to assess tree water use.

Finally, the $\delta^{13}C$ of tree rings is not reflected within the basal area increment index or the total ring width of Douglas fir. However, when the $\delta^{13}C$ of the annual ring is analyzed separately for the early and latewood components, a positive correspondence results between latewood width and $\delta^{13}C$. This suggests that the whole annual ring should be delineated into the early and latewood components to evaluate any further relationships between $\delta\ ^{13}C$ and other parameters.

Figure 4.1 The year-to-year variability in annual ring $\delta^{13}C$ for two families growing at Raymond. Each point represents the growing season average $\delta^{13}C$.

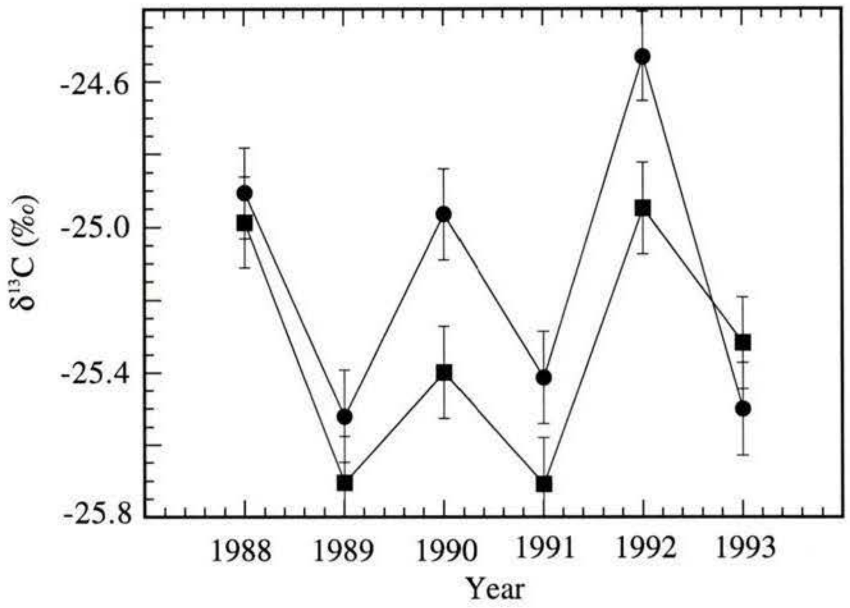

-24.6
-25.0
-25.4
-25.8
$\delta^{13}C$ (‰)
1988
1989
1990
1991
1992
1993
Year

Figure 4.2 The relation between the needle $\delta^{13}C$ and the annual ring $\delta^{13}C$ for 1993 from the trees at Raymond. Regression line is: $y = 2.76 + 1.03X$. $r^2 = 0.59$. ($p<.05$)

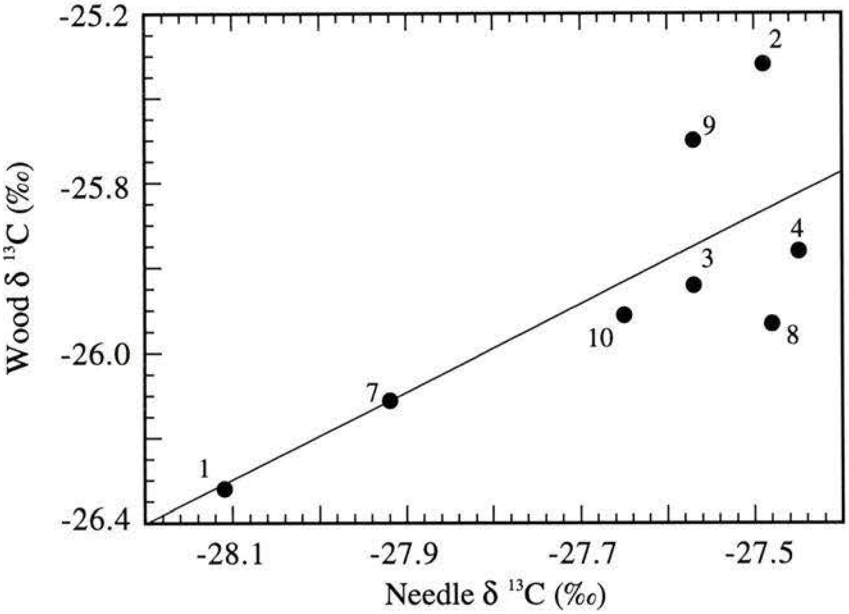

-25.2
-25.8
-26.0
-26.4
-28.1
-27.9
-27.7
-27.5
Wood δ 13C (‰)
Needle δ 13C (‰)
1
2
3
4
7
8
9
10

Figure 4.3 The $\delta^{13}C$ of the early and latewood of Family 635 for 1988-1993.

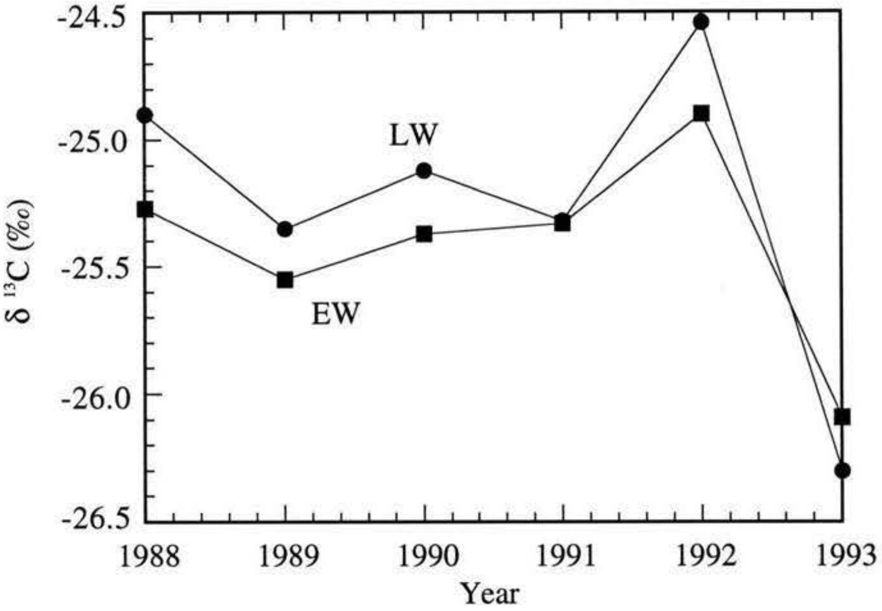
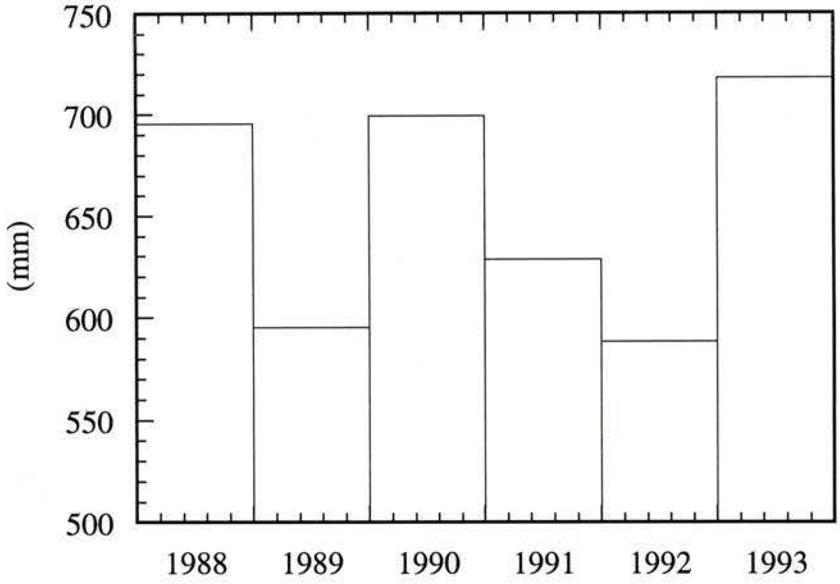

-24.5
-25.0
-25.5
-26.0
-26.5
δ ¹³C (‰)
LW
EW
1988
1989
1990
1991
1992
1993
Year

Figure 4.4a Cumulative transpiration at Raymond from April 1 - October 31 for 1988 to 1993.

Figure 4.4b Cumulative water deficits at Raymond from April 1-October 31 for 1988 to 1993.

a)


750
700
650
600
550
500
(mm)
1988
1989
1990
1991
1992
1993

b)

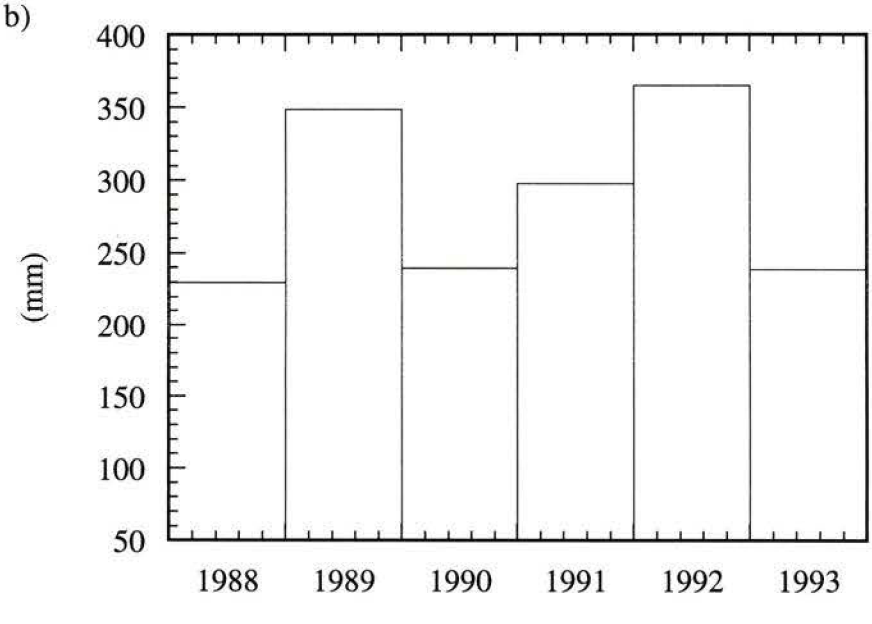

400
350
300
250
200
150
100
50
(mm)
1988
1989
1990
1991
1992
1993

Figure 4.5 Latewood $\delta^{13}C$ vs latewood width for Family 635. Regression line equation is: $y = 1.23 + 0.46X$. $r^2 = 0.42$ ($p<.05$).

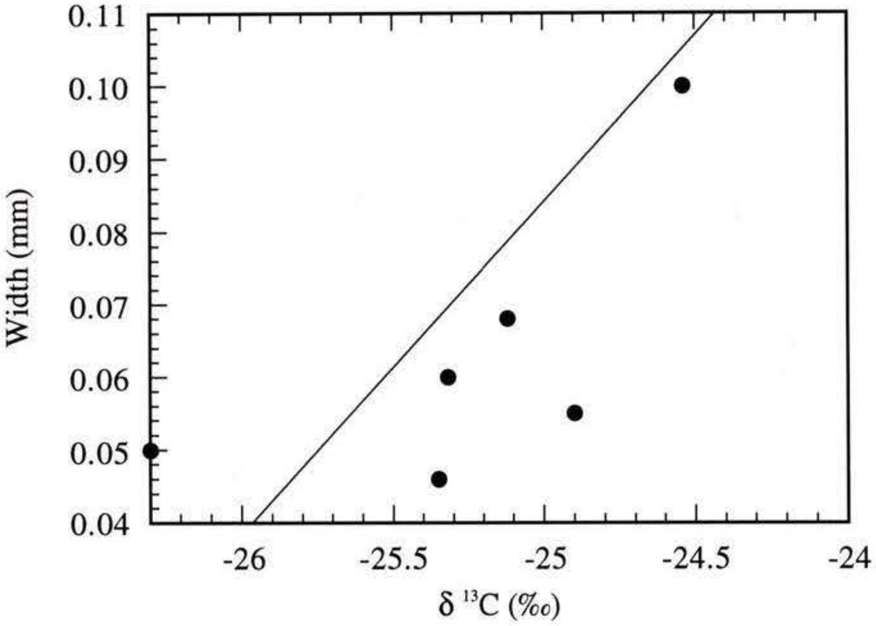

Width (mm)
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
-26
-25.5
-25
-24.5
-24
δ 13C (‰)

Figure 4.6 Cumulative transpiration and water deficits for April 1 - July 31 for 1988-1993 at Raymond compared to the annual ring $\delta^{13}C$ for one family.

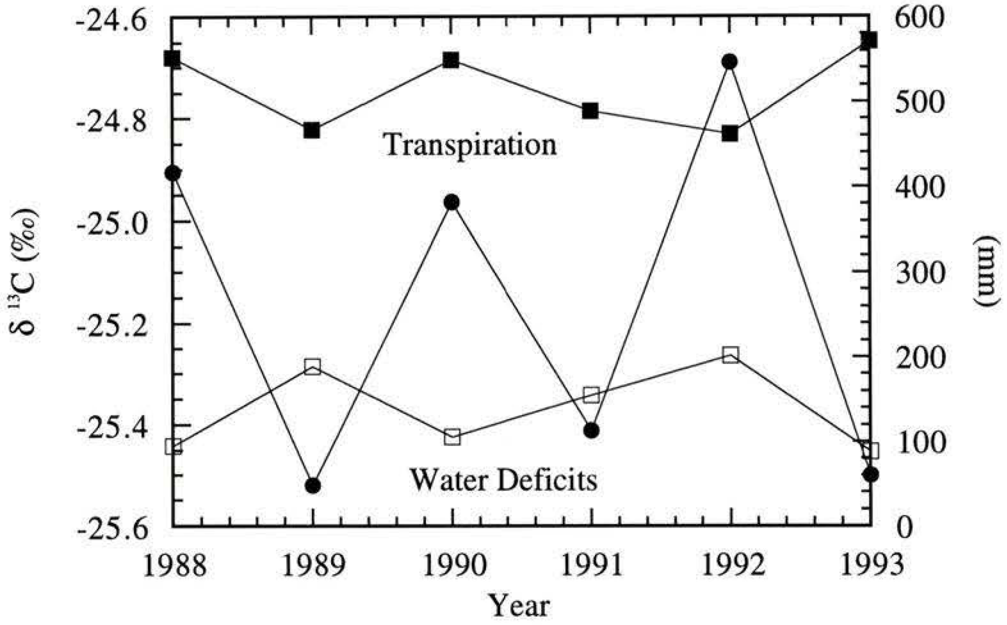

-24.6
-24.8
-25.0
-25.2
-25.4
-25.6
δ ^{13}C (‰)
600
500
400
300
200
100
0
(mm)
Transpiration
Water Deficits
1988
1989
1990
1991
1992
1993
Year

Table 4.1a One factor ANOVA results for differences between families at Raymond in terms of basal area increment index.

Table 4.1b One factor ANOVA results for differences between families at Raymond in terms of annual ring width.

a) ANOVA Basal Area Increment Index

Source	DF	SS	MS	F	p<.001
Families	9	0.78	0.09	13.98**	Fcrit=<2.89
Error	50	0.31	0.01		
Total	59	1.08			

b) ANOVA Annual Ring Width

Source	DF	SS	MS	F	p<.001
Families	9	0.04	0.004	7.33**	Fcrit<2.89
Error	50	0.03	0.001		
Total	59	0.07			

Table 4.2 Coefficient of determination (r^2) for relationships between annual ring $\delta^{13}C$ and cumulative transpiration and annual ring $\delta^{13}C$ and cumulative water deficits. Time periods over which transpiration and water deficits are cumulative are shown.

	Coefficient of Determination (r^2)			
	April-June	April-July	April-August	April-October
Transpiration Total Ring	0.07	0.11	0.13	0.12
Water Deficits Total Ring	0.07	0.13	0.14	0.08

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APPENDIX A

Total daily solar radiation (Rg) is needed to evaluate daily net radiation. Because of the lack of available solar radiation data for Vail and Raymond from 1973-1992, daily solar radiation was modelled using known solar geometrical relationships. Solar radiation equals:

$$Rg = Rg_{max}(\text{actual daylength/potential daylength}) \qquad [1]$$

where Rg_{max} equals the maximum amount of irradiance that can occur at a given latitude on a given day, actual daylength equals the daily sunshine hours, and the potential daylength equals the daily sunshine hours that would occur on a clear, sunny day. Rg_{max} is evaluated by:

$$Rg_{max} = 2(\text{potential daylength}/3.14) \times s_t \qquad [2]$$

where s_t is the total irradiance on a horizonal surface. It is equivalent to the sum of the direct irradiance on a horizontal surface (S_b) and the diffuse sky irradiance (S_d). S_b equals:

$$S_b = S_p \times \sin \phi \qquad [3]$$

where ϕ is the sun elevation angle from the horizon and S_p is a function of the distance travelled by the solar beam through the atmosphere, the transmissivity of the atmosphere, and the incident flux density. A simple expression for S_p is:

$$S_p = a^m S_{po} \qquad [4]$$

where S_{po} is the extraterrestrial flux density normal to the solar beam (1.36 kW m^{-2}), a is the atmospheric transmission coefficient and m is the ratio of the slant pathlength through the atmosphere to the zenith pathlength. (i.e. the optical air mass number). m is given by:

$$m = (P/P_o)/\sin\phi \qquad [5]$$

The ratio P/P_o is the atmospheric pressure at the site divided by sea level atmospheric pressure and was assumed to be 1 at both sites.

The transmission coefficient (a) varies from around 0.9 for a very clear atmosphere to around 0.6 for a hazy atmosphere (Campbell, 1977). A value of 0.7 was used here as a rough approximation as suggested by Jones, (1992).

Diffuse sky irradiance (S_d) can be roughly estimated using:

$$S_d = 0.5 \times S_{po}(1 - a^m)\sin\phi \qquad [6]$$

Campbell (1977) points out that S_d is a constant fraction of S_{po} over most of the range of elevation angles. Thus, the clear sky diffuse irradiance was evaluated at $\phi = 90$ degrees.

The solar elevation angle was given by:

$$\sin\phi = [\sin(\text{latitude})\sin(\text{solar declination})] \qquad [7]$$

$$+$$

$$[\cos(\text{latitude})\cos(\text{solar declination})\cos(h)]$$

The solar declination angle is the angle between the sun's rays and the equatorial plane of the earth and is a function of the time of year. It was evaluated daily from:

$$\text{solar declination angle} = -23.4\cos[360(td + 10/365] \quad [8]$$

where td is the Julian date (Jones, 1992).

h is the angular distance from the meridian of the site. By solving $\sin \phi = 0$, h is given as:

$$\cos h = -\tan(\text{latitude})\tan(\text{solar declination}) \quad [9]$$

The potential daylength in hours then equals:

$$\text{potential daylength} = 2h/15. \quad [10]$$

This solar radiation model was derived from equations reported in Jones, (1992) and Campbell, (1977). The latitude was 46 degrees for both sites. Because it was impossible to estimate cloud cover for any given day during 1973-1992, the actual daylength was expressed as the potential daylength.

Irradiance was measured using a quantum sensor (Licor Inc. at both sites during the 1993-1994 growing seasons and compared to modelled irradiance for 1994. (see Figure IA). The modelled irradiance for 1973 - 1992 was then adjusted by 27 % to account for the observed offset between the measured and modelled radiation. The offset was primarily due to an underestimation of the potential clear sky irradiance.

APPENDIX B

To determine the extent that the weather data from the state weather stations were applicable to each site, precipitation and air temperature data collected from the small weather stations installed at each site in 1994 was compared to precipitation and air temperature from the state weather stations for 1994. Figures 1B and 2B show that daily average air temperatures from the state and site weather stations were 81% and 85% correlated for Raymond and Vail, respectively. Figures 3B and 4B show that daily precipitation totals from both weather stations were 75% correlated for Raymond and 88% correlated for Vail. Because of the high correlations associated with the air temperature and precipitation data from both weather stations for each site, it was concluded that the weather data collected from 1973 to 1994 from the state weather stations was appropriate for Raymond and Vail. Therefore, the state weather data applied to applied to each site was not adjusted.

Figure 1A Measured and modelled daily radiation (MJ m^{-2} day^{-1}) for June 1 to October 31, 1994. Top line represents measured radiation.

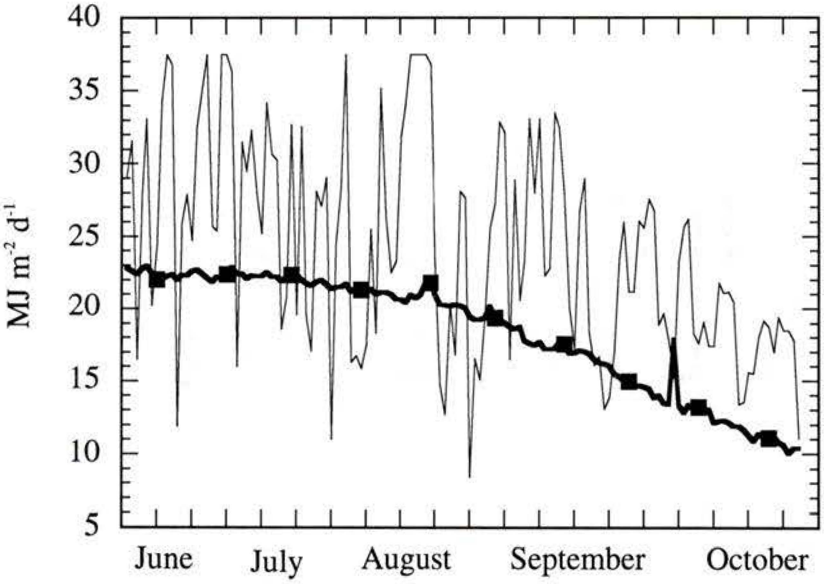
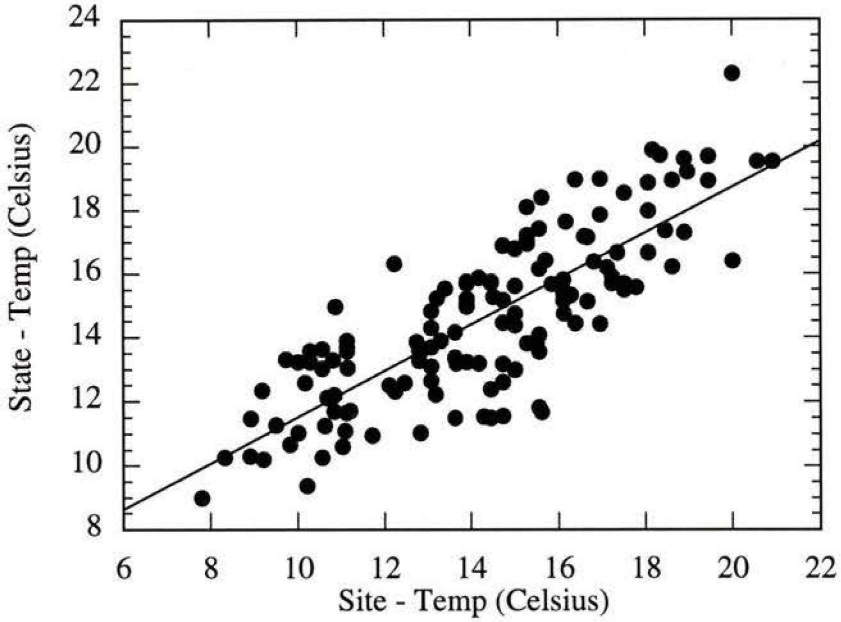

40
35
30
25
20
15
10
5
MJ m^{-2} d^{-1}
June
July
August
September
October

Figure 1B State versus Raymond site weather station data for daily average air temperature in Celsius from April 1 to October 31, 1994. Regression line is $y = 4.29 + 0.72X$. $R = 0.81$ ($p<.01$).

Figure 2B State versus Vail site weather station data for daily average air temperature in Celsius from April 1 to October 16, 1994. Regression line is $y = 2.8 + 0.77X$. $R = 0.85$ ($p <.01$).

1B


State - Temp (Celsius)
8
10
12
14
16
18
20
22
24
6
8
10
12
14
16
18
20
22
Site - Temp (Celsius)

2B

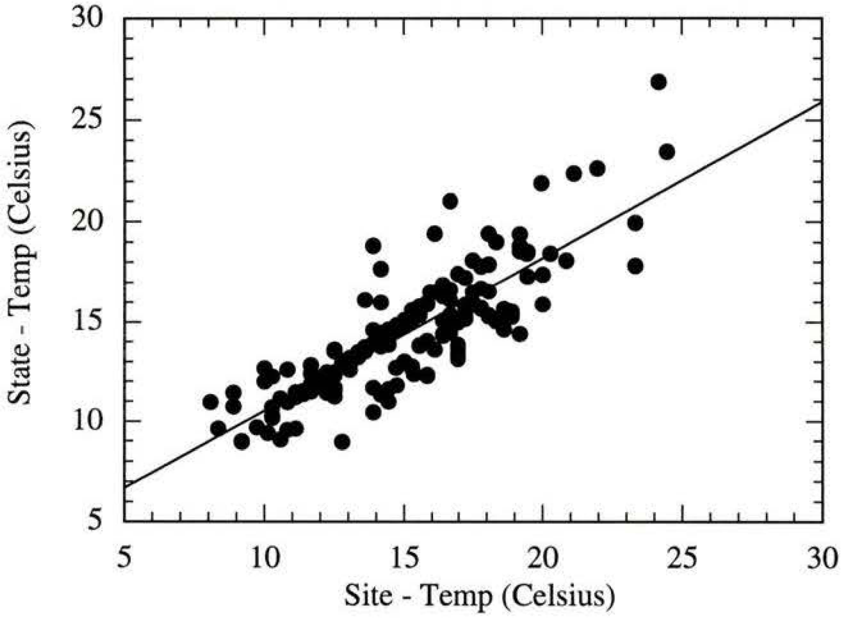
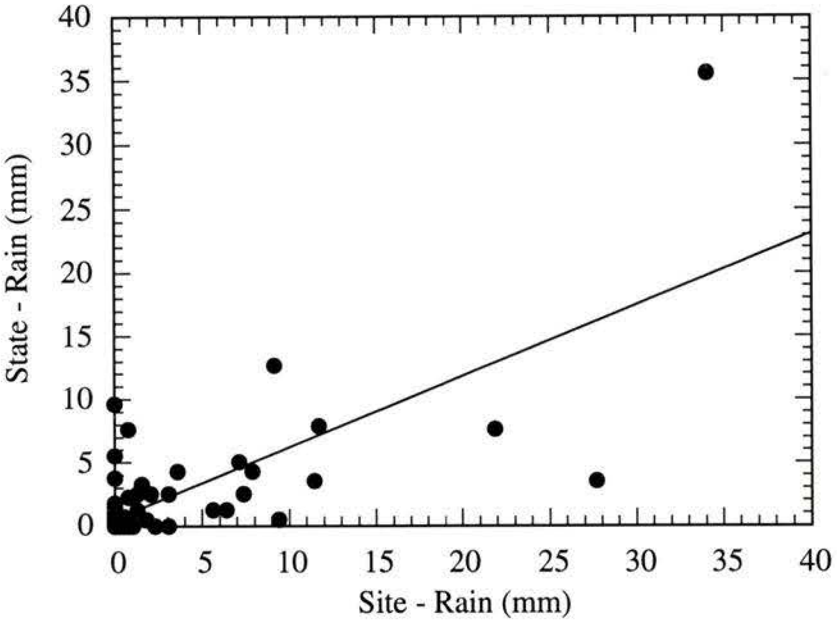

State - Temp (Celsius)
5
10
15
20
25
30
5
10
15
20
25
30
Site - Temp (Celsius)

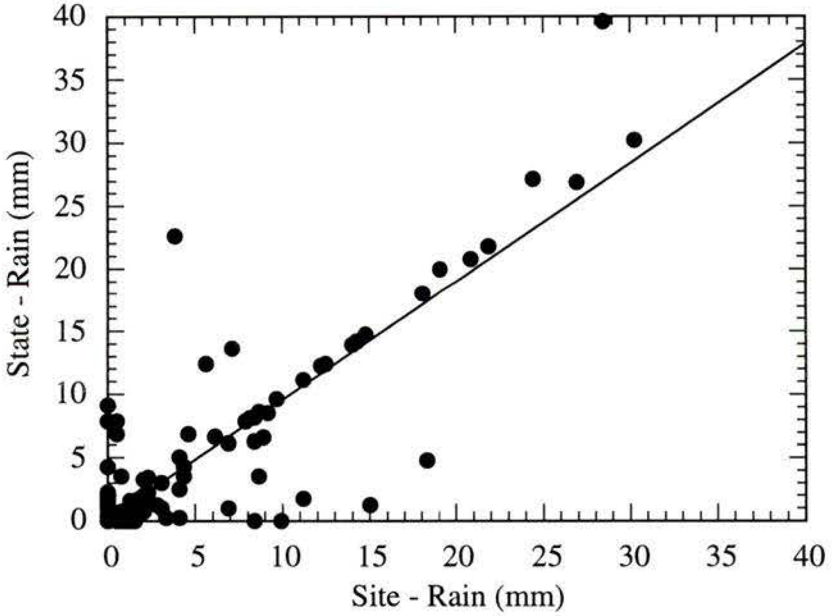
Figure 3B State versus Raymond site weather station data for daily rainfall in (mm) from April 1 to October 31, 1994. Regression line is $y = 0.29 + 0.58X$. $R = 0.75$ ($p < .05$)

Figure 4B State versus Vail site weather station data for daily rainfall in (mm) from April 1 to October 31, 1994. Regression line is $y = 0.17 + 0.94X$. $R = 0.88$ ($p < .05$).

3B


State - Rain (mm)
Site - Rain (mm)
0
5
10
15
20
25
30
35
40

4B


State - Rain (mm)
Site - Rain (mm)
0
5
10
15
20
25
30
35
40

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Title of Thesis:

Variation in the Stable Carbon Isotope Composition of Needle and Annual Rings in *Pseudotsuga menziessi* and its Relation to Water Use, Needle Nitrogen Content and Productivity

Author [redacted]

Wendy Lynne Allan
March 30,1996