TEMPERATURE ACCLIMATION OF ROOT RESPIRATION IN DOUGLAS-FIR AND WESTERN RED CEDAR SEEDLINGS

by

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Abstract

This study examined the ability of seedlings of coastal and interior provenances of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western red cedar (*Thuja plicata* Donn) to acclimate root respiration rates to growth temperature. A second objective was to determine whether acclimation involved an increase in activity and/or capacity of the alternative path of electron transport, a phenomenon observed in many crop species. Since western red cedar has less genetic variation than Douglas-fir, one might expect better acclimation in cedar.

Seedlings were grown hydroponically in computer-controlled mist boxes placed inside an environmental chamber (18/6 hr photoperiod, day/night). This allowed for precise manipulation of root temperature (11, 18, 25°C), while shoot temperature was unchanged between treatments (25/18°C, day/night). Respiration of excised root segments was measured with an oxygen electrode. Potassium cyanide and salicylhydroxamic acid were used to estimate the activity and capacity of the alternative path. Oxygen response curves were constructed, seedling water potentials were determined and shoot samples were analyzed for carbon isotope discrimination. Total plant weight, root to shoot biomass and root density were also measured.

The results showed that both the coastal and interior provenances of western red cedar and the coastal provenance of Douglas-fir were able to acclimate, while interior Douglas-fir was not. Acclimation did not seem to involve an increase in electron partitioning through the alternative path. It was suggested that the increase in alternative path respiration that is often seen at low temperatures is independent of compensatory respiration and sometimes occurs coincidentally with it to reduce the damage caused by active oxygen

species, which can be a problem at those temperatures. There is evidence that the cold-grown roots were able to increase the supply of O_2 to the mitochondria, thus compensating for the increased O_2 demand that occurs upon acclimation, although the manner in which they did this remains unclear. Seedlings appeared to be able to adjust stomatal conductance in order to avoid experiencing water stress. The optimal root growth temperature was between 18 and 25°C for both species. It seems that root signalling could be involved in rhodoxanthin accumulation in cedar shoots.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
A	carbon assimilation rate
ABA	abscisic acid
AO	alternative oxidase
AP	alternative path
APA	alternative path activity
APC	alternative path capacity
APR	alternative path respiration
C_i	$[CO_2]$ in leaf intercellular spaces
CP	cytochrome path
CPA	cytochrome path activity
CPC	cyrochrome path capacity
CPR	cytochrome path respiration
$\delta^{13}C$	measure of carbon isotopic composition (see Pg. 44)
E	transpiration rate
ETC	electron transport chain
FW	fresh weight
IAA	indole acetic acid
Km	O_2 concentration at which TDR is reduced to 50% max.
Q_{10}	factor of increase in TDR with a 10°C increase in temp.
Rubisco	ribulose-1,5-bisphosphate carboxylase oxygenase
SA	salicylic acid
SEM	standard error of the mean

SHAM .....salicylhydroxamic acid

TCA cycle .....tricarboxylic acid cycle

TDR .....total dark respiration

WUE .....photosynthetic water-use efficiency

Ψ_x .....shoot xylem water potential

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1.0 Introduction

Phenotypic plasticity is the ability of an organism to change its physiology and morphology in response to environmental stimuli. This is especially important for plant species since they are sessile and must deal with whatever conditions may occur. Genetically, plasticity is likely due to differences in allelic expression across environments along with changes in interactions among loci (Scheiner, 1993). Plasticity is often, but not always, greater in species with limited genetic heterozygosity. It has been hypothesized that there is a trade-off between phenotypic plasticity and heterozygosity for two reasons. Firstly, some propose that plasticity may be inversely related to heterozygosity due to the increase in developmental instability resulting from deleterious homozygous recessive genes (Pederson, 1968; Bradshaw, 1965). However, this assumes that unstable genotypes are more plastic and this has not been clearly shown (Schlichting, 1986). The second hypothesis suggests that plasticity should increase as heterozygosity decreases because they represent alternative ways of coping with changes in the environment (Marshall & Jain, 1968). This assumes that there is an extra cost to being both phenotypically plastic and heterozygous, something that has not really been proven (Schlichting, 1986).

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western red cedar (*Thuja plicata* Donn *ex* D. Don) are both commercially important tree species in British Columbia forests. They differ in their degree of genetic heterozygosity. Douglas-fir is one of the most genetically diverse of all tree species, both within and between populations (Haley, 1982). Western red cedar has a much less variable genotype. Cedar trees that thrive along the coast are genetically similar

to those found in the interior (Minore, 1990). This suggests that western red cedar may be more phenotypically plastic than Douglas-fir.

The difference in genetic variability between these two species is not the only reason one might expect a difference in their ability to acclimate to environmental changes. Western red cedar is a climax species in British Columbia, as is interior Douglas-fir. Coastal Douglas-fir, however, is subclimax (Watts, 1983). The environments of subclimax species are more variable in space than those of climax species, so one could argue that successional species might need more plasticity than climax species (Bradshaw, 1965). On the other hand, climax species that inhabit the same site for many hundreds of years will see more environmental changes with time, so perhaps they would require more plasticity (Bradshaw, 1965). Alternatively, we might expect interior species to be more plastic than coastal species due to increased exposure to environmental extremes. Clearly, it is difficult to predict precisely which situations should favour plasticity.

In this study I looked at the ability of seedlings of two provenances each (one coastal, one interior) of Douglas-fir and western red cedar to acclimate their rates of root respiration to growth temperature. If western red cedar is more phenotypically plastic than Douglas-fir, then its root respiration may acclimate better to temperature. However, it is important to note that plasticity of a character is specific to that character and not a general property of the whole genotype (Bradshaw, 1965).

Several researchers have examined the effects of growth temperature on respiratory capacity and some species have been found to acclimate very well (Collier & Cummins, 1990; Elthon et al., 1986; Fukai & Silsbury, 1977; Kiener & Bramlage, 1981; McNulty & Cummins, 1987; Pearcy, 1977; Rychter et al., 1988; Spencer & Wetzel, 1993). Fewer studies have looked specifically at root

respiration (Stewartet al., 1990a; Szaniawski & Kielkiewicz, 1982; Zimmerman et al., 1989) and rarer still are studies of temperature acclimation of root respiration in tree species (Rook, 1969; Weger & Guy, 1991). Thus, this research will contribute to a body of knowledge leading to a better understanding of the physiological role of plasticity and its distribution in nature.

The main objectives of my research project were as follows:

1. To discover whether western red cedar and/or Douglas-fir seedlings can acclimate their rates of root respiration to growth temperature.

2. To see if this acclimation process (if it occurs) involves an increase in activity and/or capacity of the alternative path of electron transport.

2.0 Literature Review

2.1 Douglas-fir

Douglas-fir is one of the most familiar and important tree species in western North America, inhabiting a north-south range of nearly 5000 kilometres, and extending from the Pacific coast to the eastern slope of the Rocky Mountains (Fowells, 1965). It exists as both a coastal variety (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) and an interior variety (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) (Little, 1953).

Coastal Douglas-fir occurs on the islands and mainland of the west coast (Hosie, 1979) up to approximately 850 metres above sea level (Watts, 1983). Although it is found on a variety of soils, it grows best on deep, rich, well-drained, sandy loams with plenty of moisture. Its climate is mild and humid with dry summers. Average annual rainfall is often more than 1.3 metres while temperatures range from approximately 20 to 27°C in July to -2 to 3°C in January (Hermann & Lavender, 1990). The frost-free period is usually between 195 and 260 days (Watts, 1983; Hermann & Lavender, 1990). Frost resistance is low in these trees which are unable to tolerate long cold winters (Krajina et al., 1982). Coastal Douglas-fir is a subclimax species (Watts, 1983) and is rather intolerant of shade and flooding (Klinka et al., 1990). Mature trees usually reach heights of 45 to 60 metres (Hosie, 1979).

Interior Douglas-fir grows from central British Columbia through southwestern Alberta, south through the western United States to Mexico (Hosie, 1979). It grows on a variety of soils and occurs at heights up to 1800 metres in the Rocky Mountains (Watts, 1983). The average annual rainfall is 350 to 1100 mm, with occasional summer water deficits. Average temperatures are 13 to 21°C in July and -9 to -2°C in January, while the frost-free period is usually

between 60 and 145 days (Watts, 1983). These trees are able to tolerate several weeks of very cold weather with adequate snow cover (Krajina et al., 1982). Interior Douglas-fir is a climax species with an intermediate tolerance to shade (Watts, 1983). It is shorter and stockier than the coastal variety, rarely growing taller than 43 metres (Hosie, 1979).

Douglas-fir is a very desirable tree species on the world market. Its hard, strong wood has numerous commercial uses, including structural lumber, ship-building, finishing, boxes, plywood, flooring and railway ties (Hosie, 1979). It is also a popular Christmas tree (Hermann, 1990).

2.2 Western Red Cedar

Western red cedar (*Thuja plicata* Donn) is another important timber producer in British Columbia forests. It grows across the coastal and interior wet belts with most of its volume occurring along the coast (Quenet & Magdanz, 1987). It extends from southern Alaska to Oregon, with its easternmost limit being the western slope of the Rocky Mountains (Gedney & Oswald, 1987). It can grow at heights up to 1370 metres above sea level, where it occurs as a shrub (Hosie, 1979). Western red cedar grows best on wet sites with nutrient rich soils, although it can grow on poor sites (Curran & Dunsworth, 1987). The average annual rainfall is 890 to 6600 mm along the coast and 710 to 1240 mm in the interior (Minore, 1990). Mean temperatures range from 8 to10°C on the coast to 6°C and up in the interior. The frost-free period is 60 to160 days (Watts, 1983). It is not very frost resistant and thus trees growing in the interior may only become established in areas where the ground is covered by snow prior to freezing (Krajina et al., 1982). Mature trees can grow to heights of 45-60 metres (Hosie, 1979).

Although western red cedar has been ubiquitous in coastal climax forests for over 3000 years, its stand dominance is being lost in some harvested areas. This is due to poor natural regeneration and a slow growth rate relative to forest rotation times (Curran & Dunsworth, 1987). However, to its credit, western red cedar is highly flood and shade tolerant, has few insect pests and is resistant to root rots (Curran & Dunsworth, 1987).

Western red cedar is a commercially important tree species in British Columbia as evidenced by both the level of harvest and its relative value (Quenet & Magdanz, 1987). Although the wood is neither hard or strong, it is attractive, has a pleasant odour and is quite resistant to decay (Hosie, 1979). It has many uses, such as posts, poles, shakes, shingles and siding (Graham et al., 1987).

2.3 Root Zone Temperature

Root zone temperature directly and indirectly affects many aspects of a plant's development. These include root and shoot growth and morphology, water relations and nutrient use. Although these characteristics are intricately interrelated, each will be discussed separately for convenience. It is important to remember that there are differences in the ways plants respond to root temperature, both between and within species (Bowen, 1991; Kaspar & Bland, 1992).

2.3 i. Effects of Root Temperature on Plant Growth and Morphology

Root temperature can affect root cell division and extension, root morphology, shoot growth and morphology, as well as plant carbon balance.

Tissue growth is a cyclical process of cell expansion and division. A cell grows, divides in two, and these new cells, in turn, expand and divide. The nucleus and all organelles replicate and divide within the cells (Barlow, 1987).

The time interval between one cell division and the next, including all the cellular events that occur, is called a cell cycle (Romberger et al., 1992). Although nuclear division can occur between approximately 0.5°C and 35°C, spindle production, and thus complete cell division, generally requires temperatures greater than about 5°C (Bowen, 1991). As the temperature increases, the cell cycle speeds up. In onion (*Allium cepa* L.) roots, cell division was more than twelve times faster at 25°C as compared to 5°C (Bowen, 1991).

It is particularly important to study the effects of temperature on cell expansion, since tissue extension is due to the expansion of existing cells (Green, 1976). The elongation of a cell occurs in two phases. First, there is a loosening and plastic stretching of the cell wall. Then there is a period of active cell wall formation (Burström, 1956). As in cell division, the rate of cell elongation increases with temperature (Burström, 1956). Despite this fact, Burström found that individual cells of cold-grown wheat (*Triticum aestivum* L.) grew bigger than warm-grown cells. He suggested this was because the cold-grown cells had more time to expand. Burström also recorded a decrease in cell wall plasticity with increasing temperatures. However, in roots of maize (*Zea mays* L.), cell size and cell wall plasticity increased with growth temperature (Pritchard et al., 1990). Cell elongation does not appear to be limited by decreased turgor pressure in maize roots (Pritchard et al., 1990).

Root growth occurs between approximately 5°C and 35 to 40°C (Bowen, 1991). Root dry weight follows a very typical temperature response curve (Kaspar & Bland, 1992), with an optimum around 20°C to 25°C (Bowen, 1991). The range and optimum vary between and within species (Bowen, 1991; Kaspar & Bland, 1992). Root length also increases as temperature approaches the optimum, and to a much greater extent than root weight (e.g., in canola (*Brassica napus* L.) and cotton (*Gossypium hirsutum* L.) (Cumbus & Nye, 1982; Loffroy et

al., 1983)). The difference in magnitude of the temperature effect is likely due to the increase in lateral root growth at optimal temperatures as observed in winter wheat (*Triticum aestivum* 'Centurk'), radiata pine (*Pinus radiata* Don.) and many other species (Bowen, 1969; Kaspar & Bland, 1992; Miyasaki & Grunes, 1990), since higher order roots tend to be long and thin (Kaspar & Bland, 1992). The low temperature limitation of root growth is more pronounced in taproots as compared to lateral roots in soybean (*Glycine max* (L.) Merr.) (Stone & Taylor, 1983). The inclination of lateral roots from the horizontal is also influenced by growth temperature (e.g., *Glycine max* (Kaspar et al., 1981) and *Zea mays* Onderdonk & Ketcheson, 1973). For conifer seedlings, the duration of cold storage may affect root growth in cold soils. Storage duration greater than 22 weeks in white spruce (*Picea glauca* (Moench) Voss) can be detrimental to root growth at low root temperatures (Harper et al., 1989; Harper & Camm, 1993). Roots grown at cold temperatures are often white while those grown at high temperatures are brown or discoloured (Kaspar & Bland, 1992). It appears that root suberization occurs further from the apex at optimal temperatures in many species (Bowen, 1991; Kaspar & Bland, 1992), including *Pinus radiata* (Nambiar et al., 1979).

It is not only the root system that is affected by root temperature. Plant shoot growth is also reduced at nonoptimal root temperatures. This is made particularly clear in studies where shoot temperature is held constant while the roots are exposed to different growth temperatures (Lyr & Garbe, 1995; Miyasaka & Grunes, 1990). Species from cold climates tend to have lower optimal root temperatures in terms of shoot growth than those from warmer regions (Kaspar & Bland, 1992). For example, maximum shoot growth occurred at 5-10°C for Scots pine (*Pinus sylvestris* L.), 20°C for European beech (*Fagus sylvatica* L.) and

basswood (*Tilia cordata* Mill. (Hungary)) and 25°C for English oak (*Quercus robur* L.) (Lyr & Garbe, 1995).

Root temperature appears to influence biomass allocation patterns in many species. It seems that a higher root/shoot ratio would be preferable when water uptake is restricted by temperature, since more resources would be allocated toward absorbing water. However, this does not always happen. Root to shoot ratios increase as root temperature approaches the optimum in perennial ryegrass (*Lolium perenne* L.) (Clarkson et al., 1986), *Brassica napus* (Cumbus & Nye, 1982) and the chaparral shrub *Ceanothus greggii* (Larigauderie et al., 1991). The opposite effect has been observed in white clover (*Trifolium repens* L.) (Gordon et al., 1989) and other species (Cooper, 1973). It appears that more dry matter may be allocated to roots at extreme root temperatures in mature plants while shoot growth is favoured in seedlings (Abbas Al-Ani & Hay, 1983; Bowen, 1991). A recent study suggests that tree species from cold climates may have minimum root/shoot ratios at optimal growth temperatures while species from warmer climates might have maximum root/shoot ratios when growth conditions are optimal (Lyr & Garbe, 1995). It has been proposed that sucrose is an important factor in carbon partitioning (Farrar & Williams, 1991).

Reduced root and shoot growth at extreme root temperatures is largely due to a lack of assimilate availability, since shoot photosynthesis is usually reduced at low and supraoptimal root temperatures (Foster et al., 1991; Lawrence & Oechel, 1983; Lyr & Garbe, 1995). However, photosynthesis of white spruce seedlings taken from frozen storage was not affected by soil temperature (Harper & Camm, 1993). Also, at supraoptimal temperatures, root respiration is greatly increased (Foster et al., 1991). But what is slowing photosynthesis when it is the roots, not necessarily the shoots, that are under temperature stress? Since extreme temperatures limit translocation from the

leaves, resulting in carbohydrate accumulation in the shoot, feedback inhibition might be acting to reduce carbon assimilation (Cumbus & Nye, 1982). However, this does not appear to be the case in winter wheat (Miyasaka & Grunes, 1990) and in general (Guinn & Mauney, 1980). Carbohydrates can also build up in the roots of plants, as observed in *Picea glauca* grown at low root temperatures (Weger & Guy, 1991). Plant water stress at extreme temperatures likely plays somewhat of a role (Bowen, 1991). Perhaps, however, plant growth regulators are the main cause.

Atkin et al. (1973) suggested that reduced *Zea mays* shoot growth at low root temperatures may result from an altered balance between growth promoters and inhibitors sent from the root to the shoot. They found that cytokinin and gibberellin content of the xylem exudate decreased with root growth temperature while inhibitor concentration increased (Atkin et al., 1973). Apple (*Malus*) trees respond to supraoptimal root temperatures by raising ethanol levels in the roots and leaves and lowering cytokinin content (Gur et al., 1972). In both these studies, a decrease in root and leaf cytokinins was accompanied by reduced leaf chlorophyll levels. In *Pinus sylvestris*, cooling of the root system resulted in slowed growth with a simultaneous decrease in gibberellin and IAA levels and increased ABA in the shoot (Menyailo et al., 1980). ABA has been shown to increase the permeability of carrot (*Daucus carota* L.) root tissue to water (Glinka & Reinhold, 1972). It can also stimulate root pressure which may be important in filling cavitated xylem vessels at night (Mansfield & McAinsh, 1995). A recent water flow model (Johnson et al., 1991) suggests that a hormone is generated as shoot or root pressure potential falls, inducing a shift of osmoticum from guard cells to surrounding mesophyll cells. This increases the osmotic potential of the guard cells which serves to reduce their pressure potential, and thus stomatal conductance is decreased. ABA is often cited as the hormonal message (Wilson,

1984; Johnson et al., 1991; Liang et al., 1996; Tardieu et al., 1996). Cytokinins may also play an important role in stomatal control (Incoll & Whitelam, 1977; Itai & Birnbaum, 1991), apparently by determining the extent of stomatal opening on leaves of different ages (Mansfield & McAinsh, 1995).

Root temperature did not appear to affect the time of bud break in *Pinus sylvestris*, *Fagus sylvatica*, *Tilia cordata* and *Quercus robur* L. Leaves of broad-leaved trees were smaller at low root temperatures (Lyr & Garbe, 1995).

2.3 ii. Effects of Root Temperature on Plant Water Relations

Water uptake is restricted at cold root temperatures for several reasons. Perhaps the two most important of these are the increased viscosity of water and the decrease in root permeability at low root temperatures. Water is twice as viscous at 0°C than at 25°C (Bowen, 1991). The decrease in root permeability seems to be due to changes in membrane composition (Kaufmann, 1977). In some cold-hardy species, fatty acid desaturation occurs in structural membrane phospholipids (Osmond & Wilson, 1982; Williams et al., 1996). Conifers such as Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and lodgepole pine (*Pinus contorta* Dougl. ex Loud.) appear to show minimal ability to acclimate membrane permeability to cold temperatures (Day et al., 1990; Running & Reid, 1980). Changes in microsomal fatty acid composition in *Pinus sylvestris* seedling roots are regulated primarily by temperature (Ryyppö et al., 1994).

Other reasons for restricted water uptake at low root temperatures include lowered metabolism, which results in less salt accumulation to fuel osmotic uptake, fewer unsuberized roots, and less available soil water (Kramer, 1983). There is a great deal of interspecific variability in the extent of temperature restriction on water uptake (Kramer, 1983). Species from cool climates are often better able to retain turgor and transpire at reasonable rates than species from

warm climates (Bowen, 1991). Western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings that previously received short-day treatments had less resistance to water movement at low root temperatures than those exposed to long days (Grossnickle, 1990). This indicates that photoperiod may play an important role in water relations of plants under temperature stress and thus may influence the acclimation of root respiration to temperature.

Since less water is absorbed by cold roots, the plant usually becomes water stressed. The water potential of *Pinus radiata* needles decreased with root temperature (Nambiar et al., 1979). Supraoptimal root temperatures can also result in plant water stress, as evidenced by the reduction in water potential in red maple (*Acer rubrum* L.) leaves at high root temperatures (Graves et al., 1989a). In response to the decrease in shoot water potential, plants can control stomatal conductance to minimize water loss (Teskey & Hinckley, 1986). Low soil temperatures reduced conductance in *Pinus contorta* (Running & Reid, 1980) and *Picea engelmannii* (Kaufmann, 1975) but not in *Pinus radiata* (Kaufmann, 1977). Alternatively, plants can adjust stomatal conductance to change or maintain leaf water potential instead of simply responding to it. Liang et al. (1996) found that soil drying resulted in reduced stomatal conductance long before leaf water potential declined in the tropical trees *Acacia confusa* and *Litsea glutinosa*. This was also the case for tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle) seedlings at supraoptimal root temperatures. Transpiration decreased when temperature was raised from 24 to 34°C with no change in leaf water potential (Graves et al., 1991; Graves et al., 1989b). Supraoptimal root temperatures reduced shoot water potential in *Acer rubrum* (Graves, 1989a). As mentioned in section 2.3 i., stomatal conductance appears to be under hormonal control.

The diffusive conductance for carbon dioxide in air is 0.625 times that of water vapor. However, a reduction in stomatal conductance decreases transpiration more than carbon dioxide uptake. This is because stomatal closure leads to a decrease in CO_2 concentration inside the leaf due to photosynthhthetic activity while humidity remains at approximately 100%. Thus, a moderate water deficit tends to increase photosynthetic water-use efficiency (assimilation / transpiration) by inducing partial closure of the stomates (Elsik et al., 1993). The direct relationship between water-use efficiency and the CO_2 diffusion gradient (C_a-C_i) is described in equation 1, below.

It is important to remember that the reduction in photosynthesis caused by low root temperature is not always due simply to reduced stomatal conductance. The decrease in photosynthesis in *Picea engelmannii* seedlings that occurred when root growth temperature was lowered was found to be due primarily to nonstomatal limitations such as decreased photosynthetic utilization of internal CO_2, carboxylation efficiency and apparent quantum yield (Delucia, 1986).

$$WUE = A/E = \frac{(C_a-C_i) \times g_sCO_2}{(P_i-P_a) \times g_sH_2O} = 0.625 \times \frac{(C_a-C_i)}{(P_i-P_a)} \qquad (1)$$

WUE = photosynthetic water-use efficiency
A = carbon assimilation rate
E = transpiration rate
C_a = [CO_2] outside leaf
C_i = [CO_2] in leaf intercellular spaces
P_i = vapor pressure inside leaf
P_a = vapor pressure outside leaf
g_s = stomatal conductance

2.3 iii. Effects of Root Temperature on Nutrient Uptake and Use

Nutrient uptake rate depends on the availability of nutrients in the soil and the plant's uptake potential (Chapin, 1991). Root temperature can affect both of these. Since water uptake is often reduced in cold roots, so is the supply of nutrients (e.g., nitrogen) moving to the root by mass water flow (Bowen, 1991). It also follows that ion diffusion into roots is slower at low temperatures (e.g., phosphate and potassium). Nutrient uptake potential is lower in cold roots because metabolism, and thus ion absorption, is slower at low temperatures (Bowen, 1991).

Although the rate of ion uptake by plant roots decreases with temperature, the various Q_{10}s are often lower than those for most other metabolic reactions (Bowen, 1991). That is to say that nutrient uptake rates are not as dependent on temperature as other metabolic processes. This is because uptake is subject to feedback controls which are governed by nutrient demand, relative growth rate, and root absorbing area (Bowen, 1991; Rufty et al., 1981). For example, Cumbus and Nye (1982) found that nitrogen uptake in *Brassica napus* roots was relatively temperature insensitive between 10 and 30°C but very sensitive at 35°C. This was likely in response to the reduced root to shoot ratio that occurred at the high root temperature (Cumbus & Nye, 1982). Low root temperatures in *Lolium perenne* resulted in small root to shoot ratios and thus a high demand for many nutrients (Clarkson et al., 1986). However, increasing the root temperature resulted in marked root growth and more area with which to absorb nutrients, and thus a reduction in nutrient demand. Apparently, feedback inhibition occurred at higher root temperatures so that nutrient uptake was much less influenced by temperature (Clarkson et al., 1986). Engels et al. (1992) found that although uptake and translocation of potassium, nitrogen and calcium decreased in the short term in *Zea mays* when root temperature decreased, in the long term both

uptake and translocation increased. This, they suggested, was due to an increased demand per unit fresh weight in the shoots of plants with cold roots.

Phosphate uptake can be a problem at low root temperatures. Not only is root surface area reduced but phosphate is absorbed primarily by diffusion, which is slower than mass flow and greatly limited by cold. Some species, such as *Pinus radiata*, compensate for this somewhat with a sustained ability for phosphate uptake along the root instead of just in the apical centimeter or two (Bowen, 1969).

Many plant species preferentially absorb nitrogen in the form of NH_4^+ at low temperatures and NO_3^- at higher temperatures. This seems to be an adaptive mechanism since nitrification is usually reduced in cold soils and this leads to the increasing dominance of NH_4^+ over NO_3^- ions as soil temperature decreases (Rorison et al., 1983). Nitrogen is a particularly important nutrient since deficiency can lead to a decreased biochemical capacity to photosynthesize. This is largely because Rubisco is limited by nitrogen supply. This was found to be the case in cold-grown spruce (*Picea abies* Karst.) seedlings in hydroponic culture (Vapaavuori et al., 1992). In jack pine (*Pinus banksiana* Lamb.), carboxylation capacity seemed to be impaired more than electron transport and photophosphorylation, while stomatal conductance was minimally affected by low nitrogen levels (Tan, & Hogan, 1995). Rufty et al. (1981) found that not only is nitrogen absorbed more slowly in cold *Glycine max* roots, but also that relatively less is translocated to the shoots. This was also observed in *Picea abies* whereas in *Pinus sylvestris*, more nitrogen was sent to the shoots (Vapaavuori et al., 1992).

2.3 iv. Soil Temperature and Forest Regeneration

It is apparent that soil temperature plays a crucial role in a seedling's success in the field. Slow root growth in cold soil has contributed to the failure of *Picea glauca* plantations in B.C. (Butt, 1986; Harper et al., 1989). Chilling was found to decrease starch and increase glucose concentration in *Picea engelmannii* roots, which may allow root growth to continue at low soil temperatures (Delucia, 1986). It seems that different forest species may have distinct acclimation strategies for dealing with planting stress (Lamhamedi et al., 1992). Because of the interspecific variation in strategy and ability to deal with root temperature stress, developing a successful reforestation program for a particular species will involve careful planning of planting conditions. These include site preparation (which helps control soil nutrition, moisture and temperature) and planting date. In addition, tree species which are able to acclimate to various climatic conditions (i.e., they are phenotypically plastic) may be a good choice in the face of global climate change.

2.4 Respiration

2.4 i. Introduction to Respiration

Respiration is the process by which carbohydrates, lipids and proteins are broken down to release energy via mitochondrial oxidation. This energy is used to make ATP, which fuels active cellular processes. Carbohydrates are by far the most important substrate for plant respiration. For the sake of convenience, respiration is divided into three stages: glycolysis, the citric acid cycle, and the electron transport chain.

Glycolysis occurs in the mitochondrial matrix in the presence or absence of oxygen. Carbohydrates, which are usually stored as sucrose and starch, are

converted to glucose-6-phosphate in the first step of glycolysis. Glycolysis produces two 3-carbon molecules of pyruvate for each glucose molecule and there is a net gain of two ATP molecules. In anaerobic conditions, respiration can go no further than glycolysis and pyruvate must be reduced to lactate or ethanol in order to replenish NAD^+. In aerobic respiration, however, pyruvate is oxidized to acetyl-CoA, which enters the citric acid cycle to become further oxidized.

The citric acid cycle (TCA) also takes place in the mitochondrial matrix. Acetyl-CoA combines with oxaloacetate to produce citrate. Citrate then undergoes a series of reactions, becoming oxidized to oxaloacetate which can reenter the cycle. Ultimately, the pyruvate molecule is completely broken down to CO_2. The oxidation of pyruvate to acetyl-CoA and the oxidative degradation of citrate do not directly produce ATP. However, these oxidation reactions are paired with the reduction of electron carriers (NADH and $FADH_2$). These carriers release the electrons to the electron transport chain on the inner mitochondrial membrane.

It is the electron transport chain (ETC) where most of the ATP from respiration is produced. The electron carriers NADH and $FADH_2$ are oxidized in the ETC, which consists of a series of coupled oxidation-reduction reactions. The electrons are passed from carrier to carrier to the terminal electron acceptor, oxygen, which becomes reduced to water. Plants have two pathways for electron transport: the cytochrome path (CP) and the alternative or cyanide resistant path (AP). The energy released during some of the ETC reactions is used to make ATP in the cytochrome path. There appears to be no phosphorylation site on the AP of electron transport, although in the past this was a matter of some debate (Wilson, 1970; Wilson, 1980). See Figure 1 for an overall view of respiration and Figure 2 for the path of electron flow through the

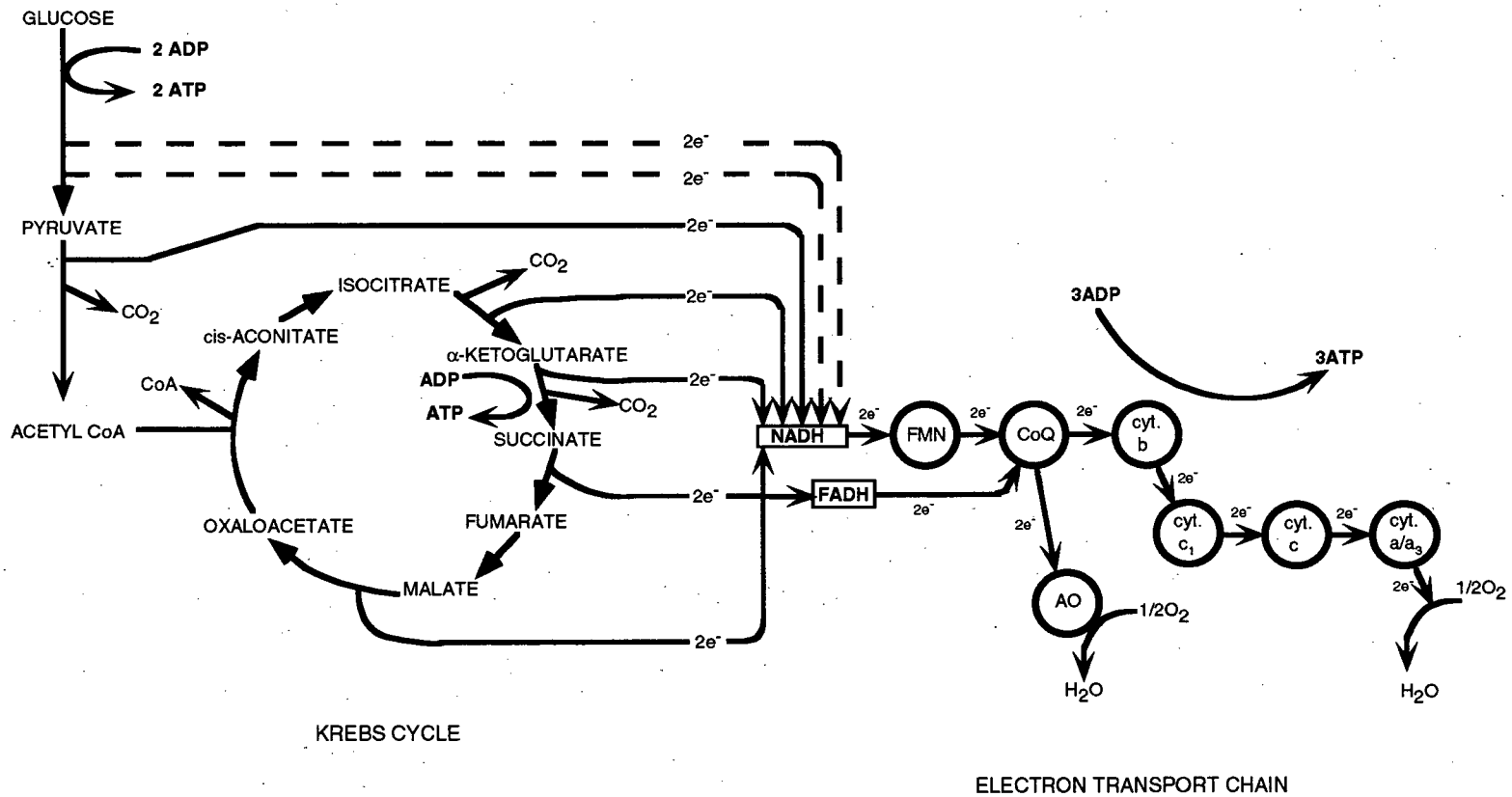

Figure 1: Summary of plant respiration (based on Raven et al., 1986)

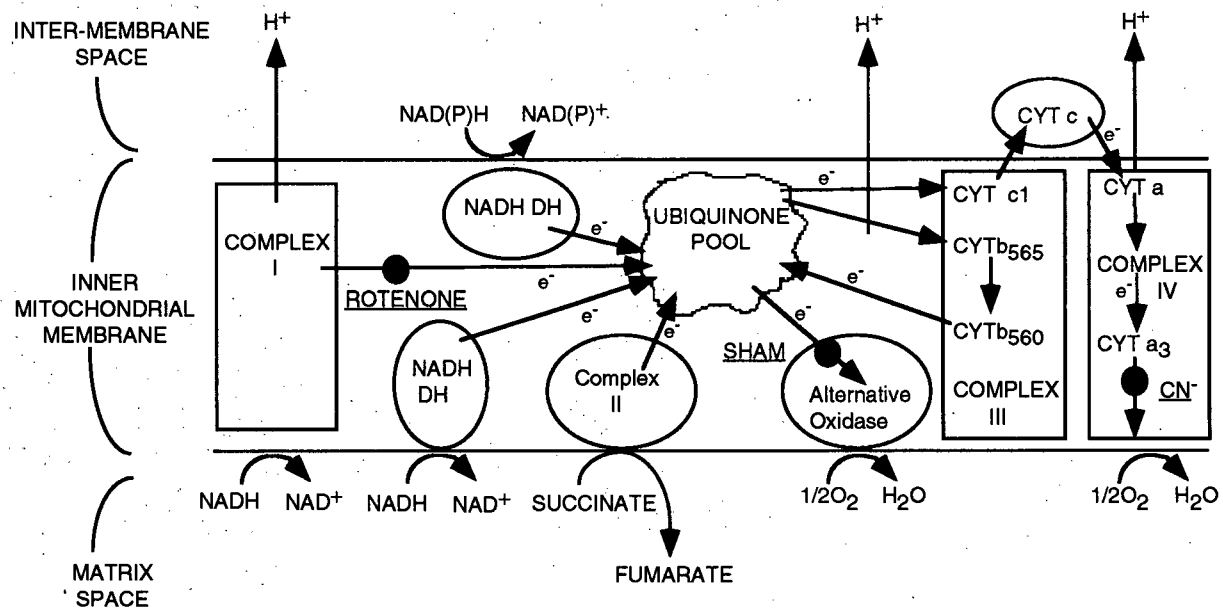

Figure 2: Diagram of the plant inner mitochondrial membrane showing the generalized location and path of electron flow through the electron transport components (complexes I-IV, two additional NADH dehydrogenases (DH), the alternative oxidase and the ubiquinone pool). The three sites of energy conservation are recorded as vertical arrows indicating H^+ extrusion. The action sites of several electron transfer inhibitors (underlined) are shown (based on Siedow & Berthold, 1986).

electron transfer components. Note that the branch point for the AP is ubiquinone (Henry & Nyns, 1975; Storey, 1976).

2.4 ii. Alternative Path Respiration

The alternative oxidase (AO) is the enzyme that catalyzes AP respiration (APR). It was first isolated in voodoo lily (*Sauromatum guttatum* Schott) (Elthon & McIntosh, 1987). Based on this and subsequent studies, we now know that it consists of one to three polypeptides between 29 and 39 kD (Hiser & McIntosh, 1990; Kumar & Söll, 1992; Berthold & Siedow, 1993; Vanlerberghe & McIntosh, 1994) and exists as a dimer in both a disulfide-linked oxidized and reduced form (Umbach & Siedow, 1993; Umbach et al., 1994). The AO has been reported to be encoded by a single nuclear gene in *Sauromatum guttatum* and *Arabidopsis thaliana* Heynh. (Raskin, 1992; Rhoads & McIntosh, 1993) and by a multigene family of at least three genes in *Glycine max* (Whelan et al., 1996). It appears to be an integral membrane protein (Moore & Siedow, 1991) but the nature of its active site remains unclear. AO has a lower affinity for oxygen than cytochrome oxidase (CO) (K_m < 0.1 μM O_2 for CO and K_m = 1-2 μM for AO) (Siedow & Berthold, 1986). However, since both oxidases have a high oxygen affinity, APR should be independent of O_2 concentration in the range employed in most respiration studies (Solomos, 1977).

If the AP is nonphosphorylating, then why does it exist in the roots, leaves and storage organs of most plant species? Lambers (1982) calls it 'wasteful' in terms of energy conservation. However, its usefulness in thermogenic species is well established. When electrons are channeled through the AP, the energy produced is not used to make ATP; instead it is lost as heat. This heat allows eastern skunk cabbage (*Symplocarpus foetidus* L.) to successfully pollinate very early in the spring (Bahr & Bonner, 1973a; Knutson, 1974) and lets *Sauromatum*

guttatum volatilize amines to attract insects that aid in pollination (Meeuse, 1975). Nonthermogenic species also possess the AP throughout their tissues, but it is usually engaged to a far lesser extent (Elthon et al., 1986). Some have suggested that the slight increase in temperature that results from APR in nonthermogenic cold-tolerant species may raise tissue temperature enough to maintain metabolic rates at low air temperatures (Knutson, 1974; Kiener & Bramlage, 1981; Moynihan et al., 1995). However, McNulty and Cummins (1987) have used energy models to show that this is highly unlikely, since it seems that less than 0.02°C can be gained by leaves with very high rates of APR. The AP may, however, play a role in the ripening of some fruits such as mango (*Magnifera indica* L.) (Kumar et al., 1990) and banana (*Musa paradisiaca* var. *Mysore Kadali*) (Kumar & Sinha, 1992), seed germination (McCaig & Hill, 1977), and tissue differentiation (Liang & Lü, 1984). It has also been suggested that the AP might contribute to homeostasis and plasticity of a plant in situations of environmentally induced stress such as osmotic and water stress (Lambers, 1982; Weger & Day, 1993), nutritient limitation (Weger & Dasgupta, 1993; Lambers et al., 1981) and disease (Molinari, 1991). Other theories as to the purpose of the AP propose that it may have helped cyanogenic plants survive periodic bursts of cyanide or that it might be a vestige of an inefficient mode of electron transport (Lambers, 1982; Lambers, 1985).

Bahr and Bonner (1973a) speculated that a nonphosphorylating electron path might be advantageous when mitochondria play a role in the metabolism of carbon compounds and not just ATP production. Palmer (1976) hypothesized that it may allow for the interconversion of organic acids of the TCA cycle when the energy charge is high. Similarly, Horn and Mertz (1982) suggested that the CP and AP may have evolved together for the purpose of removing excess reducing equivalents (e.g., replenishing NAD^+) without producing as much ATP. This would be useful when intermediates of the TCA cycle are being removed for

amino acid biosynthesis and must be quickly replaced via glycolysis (Horn & Mertz, 1982), and might also prevent the accumulation of fermentation products (Lambers, 1985) and metabolites that could reach toxic levels (Stewart, 1990). However, Lambers (1985) reasoned that a high rate of production of intermediates for biosynthesis usually coincides with a high ATP requirement. Moreover, extensive organic acid synthesis occurs in many species without increased engagement of the AP (Lambers, 1985). Weger et al. (1990) found that the AP did not play a role in maintaining carbon flow for amino acid biosynthesis in *Chlamydomonas reinhardtii* Dangeard. Alternatively, we must remember that electrons travelling through the AP have already produced one ATP molecule at phosphorylation site I, so if the CP is restricted for some reason, total ATP production might increase upon engagement of the AP. This is discussed below.

Lambers (1980) proposed that the AP in higher plant roots is of significance in the oxidation of sugars translocated from the shoots that are in excess of those needed for energy production, structural growth, storage and osmoregulation. He suggested that the AP may help prevent the accumulation of large pools of sugars in the roots (Lambers, 1980) and might contribute to homeostasis and plasticity of the plant (Lambers, 1982). Van der Plas and Wagner (1983) observed a positive correlation between sucrose concentration in the medium and APR in callus-forming potato (*Solanum tuberosum* L.) tuber discs. Laties (1982) concluded that the AP became engaged only when the CP was saturated with electrons (but see section 2.4 iv). AP capacity (APC) was greatly increased in Belgium endive (*Cichorum intybus* L.) roots upon transfer to continuous light and thus a higher carbohydrate content (Atkin et al., 1993). These and similar observations led to the most widely accepted theory of AP function, which states that it acts as an energy overflow conduit (Lambers, 1978),

becoming engaged when the rate of substrate supply exceeds the capacity of the CP (Lambers, 1985). The overflow hypothesis seems to hold for more than just roots. Azcón-Bieto and Osmond (1983) and Azcon-Bieto et al. (1983a) observed that AP respiration (APR) in spinach (*Spinacia oleracea* L.) and *Triticum aestivum* leaves only occurred after extensive photosynthesis and thus a build-up of carbohydrates in the leaves. Applying sugars to *Triticum aestivum* leaves harvested at the end of the night stimulated respiration through the AP (Azcón-Bieto et al, 1983c). APR was higher after prolonged periods of photosynthesis in leaves of five temperate species (Collier & Cummins, 1990). Increasing the sucrose supply to rice (*Oryza sativa* L.) callus cells increased APR without affecting the CP (Furuhashi et al., 1989). The AP may not always be functioning just to burn up excess substrates. De Visser et al. (1986) suggested that the AP may function as an 'overflow overcharge', contributing to ATP production when extra energy is needed. Bingham and Farrar (1988) had a similar theory, postulating that the AP may help produce extra ATP by allowing a greater flux through glycolysis and perhaps site I of oxidative phosphorylation. Bryce et al. (1990) suggested that adenylate control of electron transport could be further reduced by combining the operation of the rotenone-insensitive bypass with APR. This idea was previously investigated by Wiskich and Day (1982), who concluded that there was insufficient evidence that a direct relationship exists between the rotenone-resistant dehydrogenase and the AO.

Since excess sugar supply tends to increase APR, it seems that the control of glycolysis is not so tight that it prevents the overproduction of substrates (Day & Lambers, 1983; Lambers, 1985). Theologis and Laties (1978a,b) used an uncoupler to show that substrate mobilization and oxidation occurred to such a degree that the electron transport capacity of the CP was exceeded in sweet potato (*Ipomoea batatas* Lam.) slices. However, if the AP

becomes engaged when there is an imbalance between substrate supply and CP capacity (CPC), then factors affecting CPC will regulate the AP along with those controlling glycolysis (Azcón-Bieto et al., 1983b). Cytochrome path saturation is regulated by the amount of cytochromes and the concentration of ADP and Pi, while glycolysis depends on the substrate supply and adenylates (Hoefnagel et al., 1993). Bingham and Farrar (1988) showed that the partitioning of electrons between pathways in barley (*Hordeum distichum* (L.) Lam) depended on ATP turnover and not sugar availability. Similarly, leaf respiration in poplar (*Populus tremuloides*) was found to be restricted by adenylates (Collier et al., 1992). Lambers (1985) suggested that respiration rate is controlled primarily by substrate supply at low sugar levels and by adenylates when sugar levels are high. Similarly, others have proposed that respiration is under fine control by adenylates and course control by carbohydrates (Williams & Farrar, 1990; Bingham & Stevenson, 1993). Hoefnagel et al. (1993) observed that the alternative path was engaged in *Cathoranthus roseus* (L.) G. Don cells only upon phosphate or nitrogen starvation in combination with excess sugar. This was because nitrogen deprivation decreased the capacity of the cytochrome path (thus resulting in an overflow) and phosphate starvation lowered the adenylate content (Hoefnagel et al., 1993; Hoefnagel et al., 1994).

It seems that plants can regulate the capacity, and not just the activity, of the AP as needed. Upon inhibition of the CP in tobacco (*Nicotiana tabacum* L.) cells, AP activity (APA) and AP capacity (APC) increased, with the latter effect apparently due to *de novo* synthesis of the AO protein (Vanlerberghe, 1992a). It has been suggested that mitochondria can produce a limited but significant amount of ATP even when the ATP/ADP ratio is very high by a cycling of ADP and ATP, involving the action of the adenylate carrier with adenylate kinase (Fricaud et al., 1992; Moore, 1992).

2.4 iii. Temperature Acclimation of Respiration

The capacity and activity of the AP seem to be determined by various factors, including tissue specificity (Kearns et al., 1992), developmental stage (Obenland et al., 1990; Sesay et al., 1986; Blaquière & de Visser, 1984), substrate type (Wagner et al., 1995; Day et al., 1994), genotype (Collier & Cummins, 1989), mineral nutrition (Weger et al., 1993; Rychter & Mikulska, 1990; de Visser et al., 1986) and other environmental conditions, particularly temperature (Stewart et al., 1990a). Although these factors may act partly through regulation of AO content, it has been shown that while the amount of AO limits the capacity of the AP in young *Glycine max* cotyledons, other factors control capacity in older ones (Obenland et al., 1990). This section will focus on the effects of temperature on respiration and the role of the AP in the temperature acclimation of respiration.

Obviously, temperature has a major effect on respiration. Generally, enzymes become less active and thus reaction rates decrease as temperature is lowered. The Q_{10} of respiration is generally around 2.0 and is sometimes found to decrease as temperature rises, although the explanation for this is unclear (Lambers, 1985). Respiration has a Q_{10} of ca. 2-2.5 between 5 and 10°C in English plantain (*Plantago lanceolata* L.) (Smakman & Hofstra, 1982). If respiration is so temperature-restricted, how do plants from cold climates manage to respire enough to keep up with the demands of growth and maintenance? Interestingly, plants native to both warm and cold regions often exhibit similar dark respiration rates in their respective environments (McNulty & Cummins, 1987). Apparently, respiration is enhanced in such species so that arctic and alpine plants respire more at a given temperature (Körner & Larcher, 1988), although compensation is not always 100%. Mitochondrial respiration

Nevada showed marked variation with latitude, with greater rates in plants from higher elevations (Klikoff, 1968). When comparing respiration rates of populations of the herbaceous perennial *Polygonum bistortoides* Pursh, Mooney (1963) found that subalpine and coastal plants had lower metabolic rates at a given temperature than arctic and alpine plants. Several other (but not all (Atkin & Day, 1990)) studies have led to similar conclusions (Gent, 1992; Wager, 1941).

Since particular species and even populations of the same species are so well adjusted to their temperature environment, it leads one to wonder if some plants are able to acclimate their respiration rates to changing temperatures. It is well established that many plants can acclimate photosynthetically to temperature changes (Berry & Björkman, 1980; Mooney et al., 1978; Mawson et al., 1986) and there is strong evidence that this may also be true for respiration. *Hordeum distichum* roots transferred from 20 to 25 or 30°C have lower respiration rates per unit dry mass (Abebe, 1990). Nodding saxifrage (*Saxifraga cernua* L.) grown at 10°C respired faster than plants grown at 20°C over a range of measurement temperatures (McNulty & Cummins, 1987). A typical temperature response is shown in Figure 3. Temperature acclimation of respiration was also observed in *Zea mays* (Stewart, 1990a,b), *Spinacia oleracea* (Holaday et al., 1992), *Brassica napus* (Rychter et al., 1988), *Nicotiana tabacum* (Vanlerberghe & McIntosh, 1992b), subterranean clover (*Trifolium subterraneum* L.) (Fukai & Silsbury, 1977), holly (*Ilex crenata* (Thunb.)) (Ruter & Ingram, 1991), eelgrass (*Zostera marina* L.) (Zimmerman et al., 1989), five temperate ruderal species (Collier & Cummins, 1990), many alpine and lowland grasses, herb and cushion plants (Larigauderie & Körner, 1995), hornwort (*Ceratophyllum demersum* L.) (Spencer & Wetzel, 1993) and the evergreen shrub *Atriplex lentiformis* (Torr.) Wats. (Pearcy, 1977). However, acclimation

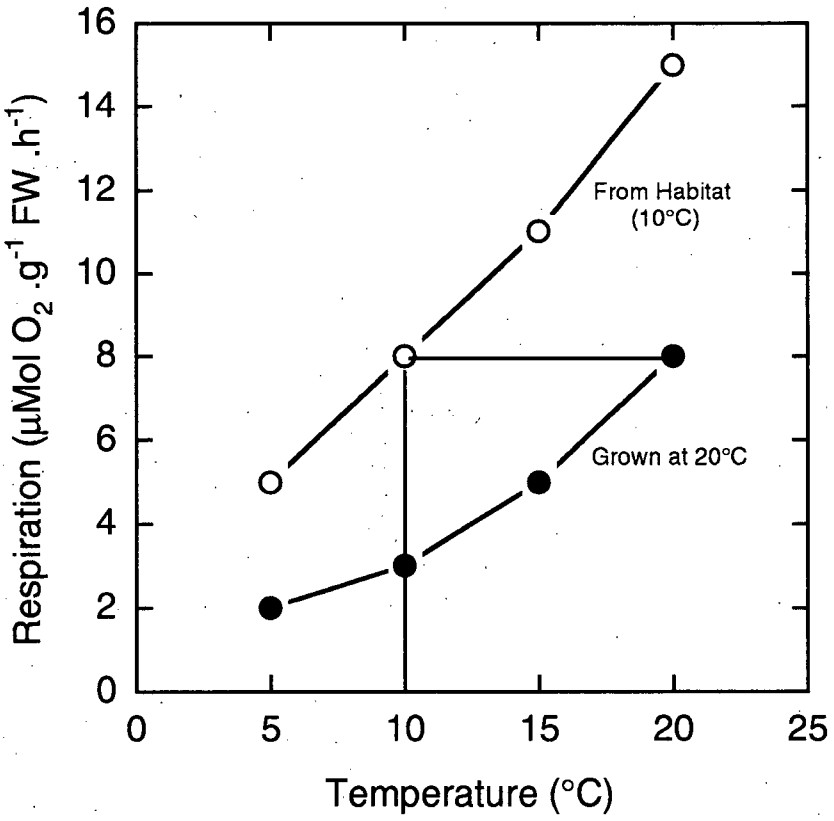

Figure 3: Example temperature response of respiration for a hypothetical species from a cool climate before and after acclimation to a warm temperature

was not apparent in *Trifolium repens* (Ryle et al., 1992), *Saxifraga biflora* (Larigauderie & Körner, 1995), slash pine (*Pinus elliottii* Engelm. var. *elliottii*) (Cropper & Gholz, 1991), or *Picea glauca* (Weger & Guy, 1991). Respiration rates of several abalone (*Haliotis*) species mitochondria were able to acclimate to laboratory temperatures, but only those spanning the extremes of each species' known temperature range (Dahlhoff & Somero, 1993). Thermal acclimation of respiration occurs more slowly in response to lowered temperatures than for increased temperatures (Dahlhoff & Somero, 1993).

There is much evidence that the acclimation of respiration to cold temperatures involves an increase in APA and APC. The AP seems to become engaged as an overflow conduit when the CP is saturated (Lambers, 1978). Such a mechanism would be useful not only in increasing respiration rate but also in helping 'burn up' excess carbohydrates (Lambers, 1980). Rychter et al. (1988) found that enhanced oxygen uptake in *Brassica napus* leaves at cold growth temperatures involved higher APR. Moreover, they concluded that the engagement was due to an increased sugar supply. Carbohydrates accumulate in the cold because growth is more severely impaired by low temperature than is photosynthesis (Körner & Larcher, 1988). Others have shown that the AP is only active when there is an imbalance between substrate supply and demand (Smakman & Hofstra, 1982; Lambers, 1985). This is especially the case in early spring when the roots are very cold but the shoots are warm and actively photosynthesizing under the bright sun. The AP was found to be preferentially engaged in cold-enhanced respiration in many other species including *Zea mays* (Van de Venter, 1985; Stewart et al., 1990a; Elthon et al., 1986; Stewart et al., 1990b), *Solanum tuberosum* callus (Hemrika-Wagner et al., 1983), the arum lily *Arum maculatum* (Cook & Cammack, 1985), five temperate ruderal species (Collier & Cummins, 1990), *Plantago lanceolata* (Smakman & Hofstra, 1982)

Nicotiana tabacum (Vanlerberghe, 1992b) cucumber *Cucumus sativus* L. (Kiener & Bramlage, 1981), and *Saxifraga cernua* (McNulty & Cummins, 1987). An increase in APC often accompanies the rise in APA.

Kiener and Bramlage (1981) proposed that the AP may protect respiration from damage by acting as a reserve against failure of the CP at low and high temperatures. However, other studies have shown that APR is not enhanced at high temperatures (Ruter & Ingram, 1991). In fact, it seems that the AP is more sensitive to high temperature stress than the CP (Chauveau et al., 1978; Lin & Markhart, 1990). However, it may play a protective role during cold temperature stress (Van de Venter, 1985). At low temperatures the AP is much less temperature-sensitive than the CP (Hemrika-Wagner et al., 1983; McNulty & Cummins, 1987; Collier & Cummins, 1990). In *Saxifraga cernua*, the Q_{10} values for respiration between 5 and 25°C for TDR and APR were 3.37 and 0.97, respectively, for plants grown at 20°C, and 2.55 and 0.79 for those grown at 10°C (McNulty & Cummins, 1987). Collier and Cummins (1990) found that the Q_{10} for CP respiration (CPR) in five temperate ruderal species between 10 and 20°C was 1.2-3.6 while the Q_{10} for APR was 0.81-1.63. This means that the AP varies little with temperature over these ranges while the CP is more active at higher temperatures. Leopold and Musgrave (1979) suggested that respiratory damage following chilling stress in *Glycine max* seed tissues was the result of deterioration of the CP. This decay was accompanied by AP engagement.

Yoshida and Tagawa (1979) observed that no alteration in electron partitioning occurred in chill-resistant red-osier dogwood (*Cornus stolonifera*) callus upon exposure to low temperature stress while in the chill-sensitive variety, the alternative path was preferentially engaged. Chaveau et al. (1978) observed that the CP was more resistant to cold inactivation in species with an AP than those without one. This is another indication that the AP might act to protect the

CP. Perhaps a different proteo-lipid composition of the mitochondrial membrane associated with the AP has a protective side-effect on the CP (Chauveau et al., 1978).

In conjunction with the idea that the AP plays a protective role during cold stress, it has been suggested that this pathway may assist in the avoidance of oxidative stress at low temperatures (Purvis & Shewfelt, 1993). Oxidative stress contributes to chilling injury in plants (Burdon et al., 1994), as evidenced by increases in lipid peroxidation breakdown products such as malonaldehyde and 4-hydroxyalkenals and higher levels of H_2O_2 and superoxide in chilled tissues (O'Kane et al., 1996). It seems to be a combination of increased degradative reactions and compromised defence and repair mechanisms that leads to the disruption in membrane integrity that typifies chilling injury (Davies et al., 1990). In other words, stressful conditions such as chilling increase the production of active oxygen species (Purvis & Shewfelt, 1993) while low temperatures are known to inhibit scavenging enzymes like superoxide dismutase and glutathione reductase (Öquist & Huner, 1993).

Many researchers have reported an increased capacity of the scavenging system in cold-hardened evergreens (Esterbauer & Grill, 1978; Anderson et al., 1991; Wingsle & Hällgren, 1993). Plants are generally more sensitive to low temperature stress in the light than the dark (Pomeroy & Mudd, 1987). The combination of low temperature and high light intensity experienced by high alpine plant species induces higher antioxidant contents in their tissues (Wildi & Lüts, 1996). Three-year-old field-grown *Pinus sylvestris* seedlings under photoxidative stress exhibited enhanced protection against free radicals (Karpinski et al., 1993). Exposure of *Arabidopsis thaliana* callus to ABA led to increased activity of ascorbate peroxidase and glutathione reductase (O'Kane et al., 1996), suggesting that this enzyme may play a role in stress-resistance.

Increasing the capacity of scavengers is one way to deal with the increase in active oxygen species, but it seems that the AP may act to reduce the level of superoxide generated by mitochondria in the first place (Purvis & Shewfelt, 1993). Two sites of superoxide generation have been identified in the mitochondrion. These are the flavoprotein region of the NADH dehydrogenase segment of the respiratory chain (Rich & Bonner, 1978), and ubiquinone (Fisher, 1988). More H_2O_2 is produced when the absence of ADP results in a slow respiratory rate and a high reduction of the ETC components (Boveris & Cadenas, 1982). Adding an uncoupler or ADP reduces H_2O_2 production. The AO seems to be induced by superoxide in the yeast *Hansenula anomala* (Minagawa et al., 1992). Thus, Purvis and Shewfelt (1993) suggested that mitochondria produce superoxide and H_2O_2 when the CP is impaired due to a high energy charge or stress-induced physical changes in the membrane components. Allowing these electrons to flow through the AP would keep the production of active oxygen species in stressed tissues to a minimum and more in balance with scavenging systems. Similarly, Wagner and Krab (1995) proposed that APR may keep ubiquinone reduction levels low to prevent high levels of free radical production.

2.4 iv. Models and Theories for Electron Partitioning

APA in some tissues is controlled primarily through *de novo* synthesis of AO protein (Hiser et al., 1996) while another strategy is to regulate the AP by activating the AO that is already there (Wagner & Krab, 1995). The exact mechanism of electron distribution out of the ubiquinone pool is not certain, although recent advances have been made. Bahr and Bonner (1973a,b) proposed that the AP is only engaged when the redox state of the ubiquinone pool is sufficiently low (i.e., when enough of it is reduced) to allow its oxidation by

the AO to become thermodynamically favourable. In other words, electrons spill over into the AP when the CP is saturated. This model contributed to the idea that the AP functions as an overflow conduit (Lambers, 1982; Laities, 1982). In 1978, de Troomstembergh and Nyns suggested that electron partitioning depends on the relative rate constants for the reactions between reduced ubiquinone and the two terminal oxidases, and that the pathways compete for electrons from a single ubiquinone pool. This would result in a constant ratio of partitioning with the AP always being engaged to some extent, while in the Bahr and Bonner model, partitioning varies with the extent of reduction of the ubiquinone pool.

Most studies have found that electrons only flow through the AP after the ubiquinone pool is sufficiently reduced, which is consistent with Bahr and Bonner. However, after becoming engaged, the AP often deviates from the standard linear response of activity versus ubiquinone reduction level (Reed & Ragan, 1987; Dry et al., 1989; Moore, 1992). This led Siedow and Moore (1992) to develop a two-stage reduction model which more effectively accounts for the relationship between APR and the state of ubiquinone reduction. Their model is based on the idea that the AP is regulated by ubiquinone pool behaviour and is consistent with that of Bahr and Bonner. It indicates that the variations in the relation between ubiquinone reduction and APA among different plant mitochondria is associated with differences in reaction rates between the reduced oxidase and oxidized ubiquinone, and suggests that oxygen reduction by the AO proceeds via the initial formation of a four-electron reduced enzyme (Siedow & Moore, 1993).

There are some observations that do not comply with the above models. It seems that the AP can sometimes accept electrons before the CP is saturated. Inhibition of the AO in soybean cotyledon mitochondria increased electron flow

through the CP (Hoefnagel et al., 1995). Atkin et al. (1995) concluded that inhibiting the AP in the presence of pyruvate resulted in the diversion of electrons from the AP to the unsaturated CP in fescue (*Festuca ovina* ssp. *ovina* L.), bean (*Phaseolus vulgaris* L.) and six species of bluegrass (*Poa*) seedling roots. Others have observed a coordinate regulation of the AP and CP (Wilson, 1988; Vanlerberghe & McIntosh, 1992a). Recently, it has been shown that other factors besides ubiquinone reduction level and the amount of AO protein can affect AP engagement. The AO exists as an oxidized or reduced disulfide-linked dimer in the mitochondrial membrane, with the reduced form being more active (Umbach & Siedow, 1993; Umbach et al., 1994). Organic acids such as succinate, malate (Wagneret al., 1989; Wagner et al., 1995) and particularly pyruvate (Hoefnagel & Wiskich, 1996; Hoefnagel et al., 1995; Day et al., 1994) can further activate the AO by increasing its affinity for ubiquinol, although succinate and malate may do so indirectly by generating pyruvate via malic enzyme (Millar et al., 1996). Thus, some substrates are oxidized more through the AP than others (Day et al., 1991; van den Bergen et al., 1994). All this means that there will be times when APA is significant although ubiquinone reduction levels are low (Wagner & Krab, 1995).

The model simulations of van den Bergen et al. (1994) are based on Michaelis-Menten kinetics, but do not assume that the AO interacts with two ubiquinone molecules like Siedow and Moore's model. Another difference is that they model reducing pathways along with oxidizing pathways. They show two kinds of dehydrogenase kinetics: 'low affinity' wherein the rate of the dehydrogenase proportionally increases with ubiquinone oxidation levels, and 'high affinity' wherein changes in oxidation levels have little effect on the rate at a high ubiquinone oxidation level. Their modelling demonstrates that differences

in dehydrogenase kinetics can cause an apparent 'preferential access' of some substrates over others to the AP (Wagner & Krab, 1995).

Membrane conditions may play a role in the activity of the AO (Maeshima & Asahi, 1984; Nakamura & Asahi, 1976). Wagner et al. (1995) suggested a role for membrane fluidity in the stimulation of the AP by carboxylic acids. Perhaps changes in lipids alter the accessibility of the AO for reduced ubiquinone, or maybe there is a stabilizing effect of lipid packing on enzyme configuration (Wagner et al, 1995). The alternative path is induced naturally in *Sauromatum guttatum* by salicylic acid (SA) (Raskin et al., 1987). The addition of SA to tobacco cell-suspension culture increases both the capacity and activity of the AP by redirecting electrons from the CP to the AP, regardless of CP saturation (Kapulnik et al., 1992). SA increased APA and APC in dormancy-breaking potato slices (Wen & Huo-Guo, 1994). It may act by changing the redox potential of the ubiquinone pool or through another mechanism, such as the higher expression of the AO gene (Kapulnik et al., 1992). Chen et al. (1993) suggested that SA binds to a catalase, thus inhibiting it and leading to increased levels of H_2O_2 in the cell, which stimulates gene expression by activating a transcription factor. H_2O_2 elicitation in abraded cucumber hypocotyls is enhanced by SA (Fauth et al., 1996).

2.4 v. Methods and Assumptions for Determining APA and APC

The most popular method of determining the capacity and activity of the AP has been through the use of inhibitors. Such methods are based on the electron partitioning model of Bahr and Bonner (1973a) and assume that the AP is engaged as an overflow conduit when the CP is saturated. To estimate APA, one can add an inhibitor of the AP, such as salicylhydroxamic acid (SHAM), and observe the extent to which respiration is inhibited. Schonbaum et al. (1971)

were the first to find that hydroxamic acids were specific inhibitors of the AP. APC is determined by measuring respiration rate in the presence of an inhibitor of the CP, like potassium cyanide (KCN). However, one must subtract the residual respiration rate from the rate with cyanide so as not to overestimate APC. Residual respiration is defined as the rate of respiration in the presence of inhibitors of both pathways. Although its biochemical nature is still unclear, residual respiration may result from one or more extra-mitochondrial oxidases since it is very small or absent in studies of isolated mitochondria (van der Plas & Wagner, 1983).

Lipoxygenase is an enzyme that catalyzes the dioxygenation of certain unsaturated fatty acids (Christopher et al., 1970). Like the AO, it is sensitive to SHAM and insensitive to KCN. Because of this, some researchers have expressed concern that measurements of the AP might be confounded by lipoxygenase activity (Parrish & Leopold, 1978; Siedow & Girvin, 1980; Goldstein et al., 1981). However, it has been shown that lipoxygenase does not significantly contribute to the cyanide resistance of substrate oxidation (Rustin, 1987).

Many problems have been identified in the measurement of APC and APA in plant tissues. Low concentrations of hydroxamic acids can stimulate O_2 uptake in some tissues (Sesay et al., 1986), apparently by activating a peroxidase (Spreen Brouwer et al., 1986; van der Werf et al., 1991; Bingham & Stevenson, 1995). Peroxidases are widespread and varied in plant tissues and while their role is not always clear, they may act in cell wall formation and lignification (van der Plas et al., 1987). Peroxidase stimulation can usually be avoided by choosing a high SHAM concentration (=25 mM) (Spreen Brouwer et al., 1986; de Visser et al., 1986), although Möller and Bérczi (1986) found that 1 mM and 20 mM SHAM resulted in the same amount of stimulation in *Triticum aestivum* roots.

High SHAM concentrations can sometimes result in inhibition of the cytochrome path (Bingham & Farrar, 1987). Thus, it is clear that one must be very cautious when using inhibitors to determine APA and APC (Møller et al., 1988; Day, 1992; Bingham & Stevenson, 1995).

Perhaps the greatest drawback of using inhibitors to estimate APA and APC is the fact that the Bahr and Bonner model of electron partitioning on which it is based may not be totally accurate, as previously discussed. Another method for determining electron partitioning to the AP is through analysis of fractionation of stable oxygen isotopes during respiration (Guy et al., 1989; Guy et al., 1992; Robinson et al., 1992). This method is based on the fact that the AO discriminates against ^{18}O more than CO does. It avoids many of the problems and side effects that accompany inhibitor experiments but it is very time-consuming and expensive. Microcalorimetry is another way to estimate APA while avoiding the problems of inhibitors. It has been shown that the detection of heat generated by the AP can be used to effectively measure APA in intact tissues, as determined by a high correlation with respiration measurements performed using an O_2 electrode (Ordentlich et al., 1991).

Great care must also be taken in monitoring and controlling the conditions in which the roots are respiring, as these can affect respiration rates. Weger and Guy (1991) determined that respiration of *Picea glauca* roots was saturated by O_2 concentrations in equilibrium with air. While there was no stimulation at high O_2 levels, respiration was rapidly limited below 250 μM. The AP in particular may be limited by oxygen availability (Purvis, 1988). O_2 uptake can also become inhibited by high CO_2 concentrations, as has been observed with the use of O_2 electrodes (Reuveni et al., 1993). However, it seems that the AP is not specifically suppressed by high CO_2 (Reuveni et al., 1995).

3.0 Materials and Methods

Respiration rates were determined for roots grown over a range of temperatures. Q_{10}s were calculated for respiration, oxygen response curves were constructed, seedling water potentials were measured and samples were analyzed for carbon isotopic composition. Total plant weight, root to shoot biomass and root density were measured and visible structural differences were recorded.

3.1 Seed Lots and Growth Conditions

All four seed lots were from British Columbia. Western red cedar seed lots #27153 (interior provenance) and #8633 (coastal provenance) along with lots #33124 (interior Douglas-fir) and #1276 (coastal Douglas-fir) were used in these experiments. They came from the Kelowna area, Queen Charlotte Islands, Revelstoke and Squamish, respectively. All seeds were obtained from the Surrey Seed Centre, Ministry of Forests. Douglas-fir seeds were stratified for three weeks prior to sowing.

Seeds were germinated every four weeks in plastic Cone-tainers (Stuewe and Sons, Inc.) filled with a peat mixture and topped with forestry gravel (Target Forestry Sand). To prepare this mixture, one bale (4 ft^3) of peat was broken up with a shovel and mixed 3 : 1 with perlite. 1000 g Nutracote 16:10:10 controlled release fertilizer (Chisso-Asahi Fertilizer Co.), 550 g dolomite and 90 g Stem Micronutrients (Plant Products Co.) were then mixed in. Seedlings were grown for eight weeks in a Conviron PGV36 environmental chamber at 25/18°C, 16/8 hrs (day/night), while receiving water and 20:8:20 fertilizer (Plant Prod Forestry Seedling Special) every two days. After this period, roots were washed and seedlings were randomly transferred to six aeroponic mist boxes (Hubick et al.,

1982), each capable of holding 48 seedlings. The misting apparatus consisted of Elite 801 and 802 aquarium pumps that drew nutrient solution through a nylon filter and air-lifted it up a tube into a beaker. A funnel attached to a perforated petri plate turned via a Dayton 3M558B motor to centrifugally lift and distribute nutrient solution from the beaker to the roots (Figure 4). A computer data acquisition and control system (WB-820, Omega Engineering, Inc) was used to continuously monitor root zone temperatures within the boxes in combination with several copper-constantan thermocouples. Temperatures were automatically adjusted to set-point with stainless steel cooling coils circulating cold alcohol solutions with AC-2CP-MD centrifugal pumps (March MFG Inc.), and with Askoll IP68 aquarium heaters. Roots were held at 11, 18 or 25°C while stem temperature and photoperiod remained as stated above. Light levels were maintained at approximately 300 $\mu mol/m^2/sec$. The nutrient solution inside the mistboxes was replenished regularly; nutrient concentrations are presented in Table 1. See Figure 5 for a photograph of seedlings inside mistboxes.

Seedlings were allowed to grow in the boxes for four weeks before sampling over the following four week period, except for 11°C seedlings, which were grown in the boxes for eight weeks prior to sampling. This was because seedlings at this temperature were very slow to grow new roots. In order to keep a continuous supply of seedlings for measurement, half of each box was available for measurement while the other half contained samples for the subsequent month. An equipment failure part-way through 1995 forced the transfer of the experiment to a second growth chamber where respiration rates were uniformly (but inexplicably) enhanced. To account for this in analyzing the data, rates were expressed as a percentage of the average rate for samples grown and measured at 25°C, both before ($Control_1$) and after the move ($Control_2$).

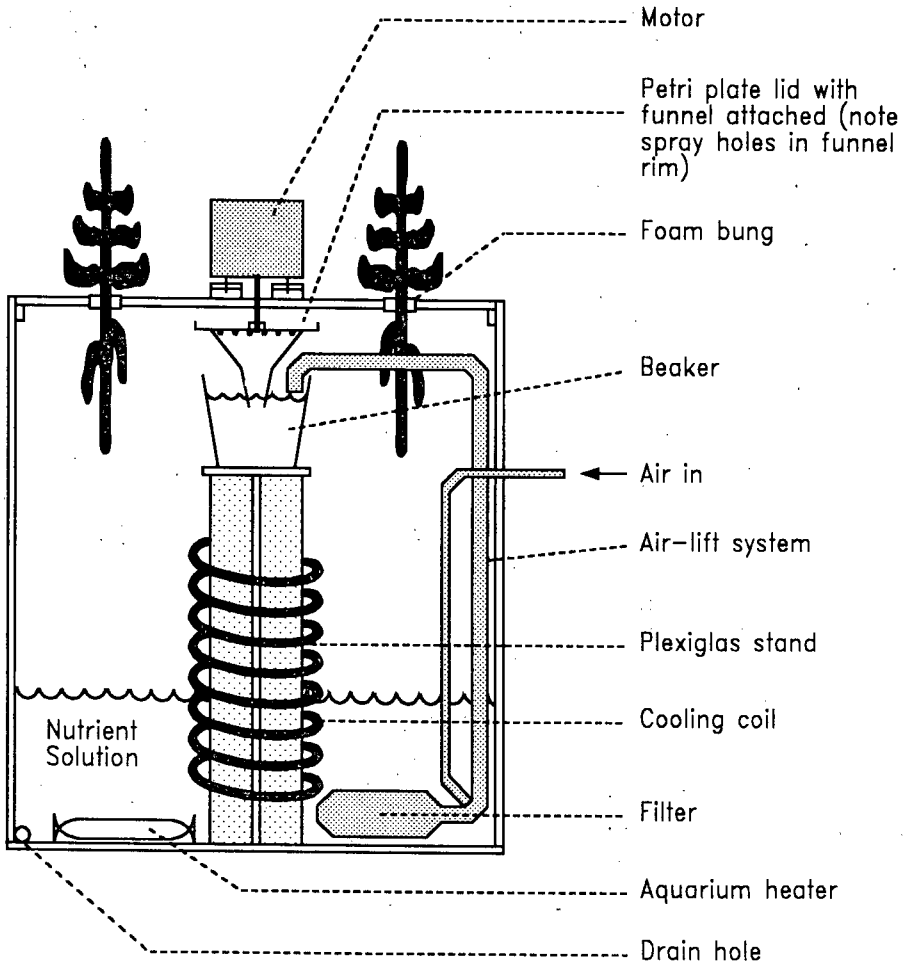

Figure 4: Diagram of mistbox

Table 1: Nutrient concentrations in mistbox solution

Nutrient	Concentration (‰)
nitrogen (N)	40
phosphorus (P)	16
potassium (K)	40
calcium (Ca)	26
magnesium (Mg)	13
sulfur (S)	34
iron (Fe)	3

* Nutrient solution formula per 18 litres was as follows: 3.600 g 20:8:20 fertilizer (Plant Prod), 2.287 g $MgSO_4.7H_20$, 1.550 g $CaSO_4.2H_2O$, 0.300 g $CaCO_3$, 0.180 g $FeSO_4$, 0.068g Stem Micronutrients (Plant Products Co.)

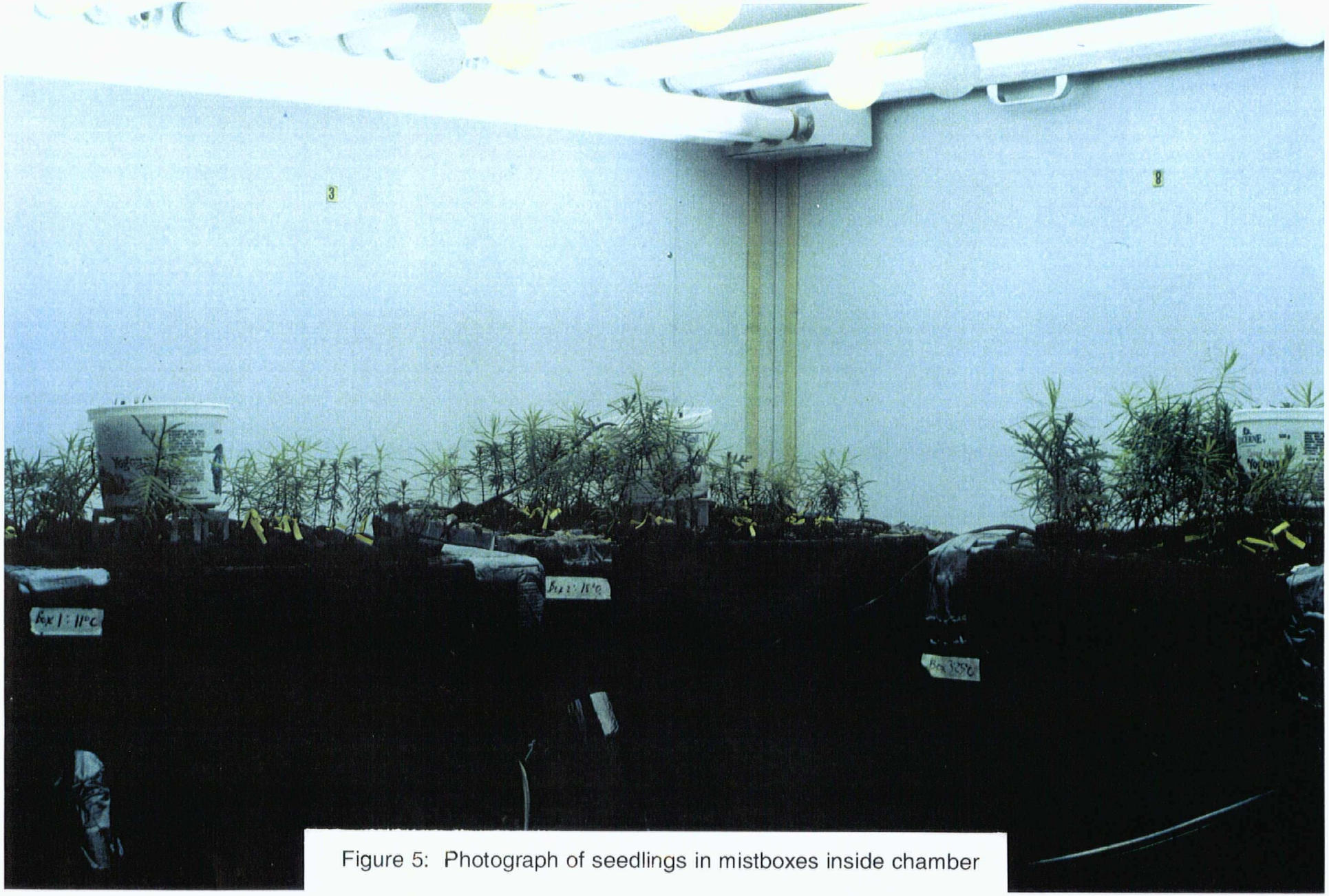

Figure 5: Photograph of seedlings in mistboxes inside chamber

3.2 Respiration Measurements

Respiration measurements were performed on excised root segments. New white roots were cut with a sharp blade, rinsed with distilled water and divided into ca. 7 mm sections. Root tips were discarded. Segments were rinsed again with distilled water and gently blotted on paper towels prior to weighing. Great care was taken to quickly and gently handle roots to minimize trauma. 25-50 mg were then placed in a temperature-controlled electrode cuvette containing 1 mL of 20 m*M* 2-(N-morpholino) ethanesulfonic acid (MES) buffer, pH 6.0, containing 0.5 m*M* $CaSO_4$. Pure O_2 was bubbled through the buffer to increase its concentration in solution, since initial O_2 response curves showed that the O_2 level should be kept above air saturation to avoid any diffusional limitations. The cuvette was then sealed and covered with a dark cloth to block out light. Roots were left to respire for at least 25 minutes to allow for temperature equilibration and the achievement of a steady rate of O_2 uptake. Respiration rate was measured with a Hansatech CB1-D Clarke oxygen electrode at temperatures of 11, 18, 25 or 32°C, as maintained with a Hakke D8 W13 water bath and a Hakke EK12 cooler. Respiration curves were recorded on a chart recorder. The electrode was calibrated at least once a day for a particular cuvette temperature by measuring air saturated buffer and calculating the equilibrium O_2 concentration using the tables of Green and Carritt (1967). Zero O_2 was acquired with the addition of sodium dithionite to the buffer.

Inhibitors were used to assess the capacity and activity of the AP as described in section 2.4 v. Preliminary titration curves determined that 5 m*M* SHAM and 0.3 m*M* KCN were sufficient to block the AP and CP, respectively. Residual respiration was measured for every sample. SHAM was added from a 1 *M* stock 2-methoxy-ethanol solution while KCN was supplied as a 0.1 *M*

solution in distilled water. Full inhibition took approximately 25 minutes for SHAM and 10 minutes for KCN. Inhibitors were obtained from Sigma Chemical Co.

Q_{10}s were calculated from the maximum respiration data. Oxygen response curves for total dark respiration (TDR) rates were done (in addition to initial curves mentioned above) to check for possible differences in diffusional limitations between seedlings grown at the various temperatures. Respiration rates were measured as explained above while O_2 concentration was carefully manipulated and monitored between zero and near saturation.

3.3 Other Measurements

Mid-day shoot xylem water potential (Ψ_x) was measured for a single set of seedlings with a Soil Moisture Equipment pressure bomb. After four weeks in the boxes, seedlings were randomly sampled to account for any time-of-day effects on Ψ_x.. A microscope was used to aid in the detection of xylem sap at the cut end.

Seedling shoots were analyzed for stable carbon isotopic composition. Shoots from 11 and 18°C were harvested in October while 25°C samples were taken in November of 1995. This difference was due to equipment failures and the implications will be discussed later. After four weeks in the boxes, seedlings were divided into roots and shoots and frozen in liquid nitrogen. They were then freeze dried for several days and weighed. The dry shoots were then ground in liquid nitrogen with a mortar and pestle and transferred to scintillation vials. These shoot samples were sent to the Department of Oceanography at UBC for combustion on a Carlo Erba elemental analyzer and analysis of $\delta^{13}C$ via a VG Prism triple-collecting ratio mass spectrometer. $\delta^{13}C$ is a measure of $^{13}CO_2/^{12}CO_2$ as compared to a recognized primary standard VPDB (Vienna Peedee belemnite):

$$\delta^{13}C = [(^{13}CO_2/^{12}CO_{2\ (sample)} / {}^{13}CO_2/^{12}CO_{2\ (standard)}) - 1] \times 1000 \qquad (2)$$

Negative $\delta^{13}C$ values indicate less ^{13}C and therefore more discrimination against the heavier isotope in carbon uptake. C_3 plants discriminate against ^{13}C because it is heavier than ^{12}C and diffuses more slowly, and more importantly because the carboxylation of ribulose bisphosphate is slower with ^{13}C (O'Leary, 1993). When stomates are closed or partially closed, the partial pressure of CO_2 inside the leaf (C_i) decreases. Thus, as ^{12}C is depleted, the relative amount of ^{13}C increases so that the plant discriminates less against ^{13}C. In this way, carbon isotopic composition is an indicator of the assimilation-averaged C_i. In as much as stomatal closure leads to a lowering of C_i (and a concommittant increase in WUE), $\delta^{13}C$ values are also sensitive stress indicators.

Root density was measured on a set of seedlings after four weeks in the mistboxes. New white root segments were cut with a sharp blade, rinsed with distilled water, blotted and weighed. Approximately 100 mg were placed in a small vial of distilled water on a balance. The roots were submerged and the weight of the displaced water was recorded and converted to a volume measure, assuming 1 g H_2O/cm^3. Dividing the mass by the volume rendered root density. Structural differences between the seedlings at different root temperatures were noted and recorded.

3.4 Statistical Analysis

Two-way ANOVAs were used to test the effects of growth and measurement temperature on TDR, APA and APC. There was a 3 x 4 factorial arrangement of treatments (3 growth temperatures x 4 measurement temperatures) for each provenance. The analyses were run as for a completely

randomized design in which a single measurement represents one experimental unit. Measurements were independent of one another since a seedling was only measured at one temperature. Sample size was four at any combination of growth and measurement temperatures for TDR, APA and APC at any combination of growth and measurement temperatures. Each of the four plants was measured twice for TDR. It was assumed that growth conditions were similar in the two boxes held at the same temperature. When treatment differences were found to exist, linear contrasts were performed on the data to determine which growth or measurement temperatures were different from one another. Each contrast was was tested using an F-test with one degree of freedom. Linear contrasts allowed testing for differences between one pair of growth temperatures for a particular provenance while factoring out the effects of measurement temperature (or vice versa). However, since the probability of Type I error for each F-test was $\alpha = 0.05$, the probability that at least one Type I error occurred was much greater than this. One-way ANOVAs were employed to determine the effect of growth temperature on the response to O_2 concentration (Km), water potential, $\delta^{13}C$, biomass allocation, total plant dry weight and root density. Tukey's multiple comparison procedure was used to as a followup to the one-way ANOVAs to find which means were different. This is a conservative test that compares all possible pairs of means based on a statistic that uses the largest and smallest sample means. Tukey's test was run at a significance level of $\alpha = 0.05$. All data analyses were done on SYSTAT.

4.0 Results

4.1 Effect of Root Temperature on Respiration

4.1 i. Effect of Root Temperature on TDR

The effects of growth and measurement temperature on TDR for roots of coastal and interior western red cedar and Douglas-fir seedlings are shown in Figures 6-9. A two-way ANOVA was done for each provenance. An example ANOVA table showing components of variance and F-test equations is presented in Appendix A. TDR increased significantly with measurement temperature for every provenance ($P<0.0005$). Linear contrasts were performed to compare each possible pair of measurement temperatures. For coastal cedar, increasing measurement temperature from 11 to 18°C did not significantly raise respiration rate ($P=0.303$), while significant differences were noted between 18 and 25°C ($P=0.001$) as well as between 25 and 32°C ($P<0.0005$). For the other three provenances, each measurement temperature increase resulted in significantly higher rates of TDR ($P<0.0005$).

Decreasing growth temperature significantly increased TDR for coastal and interior cedar provenances ($P<0.0005$) and coastal Douglas-fir ($P<0.0005$), but not for interior Douglas-fir ($P=0.146$). There was a significant interaction effect between growth and measurement temperature in interior cedar ($P<0.0005$) and coastal Douglas-fir ($P=0.023$). This can be observed in Figures 7 and 8, where the rate of increase of TDR with measurement temperature varies somewhat with growth temperature. However, it is apparent from these figures that the trends with growth and measurement temperature are real. A series of linear contrasts was performed on pairs of growth temperatures for coastal and interior cedar and coastal Douglas-fir. Increasing growth temperature decreased

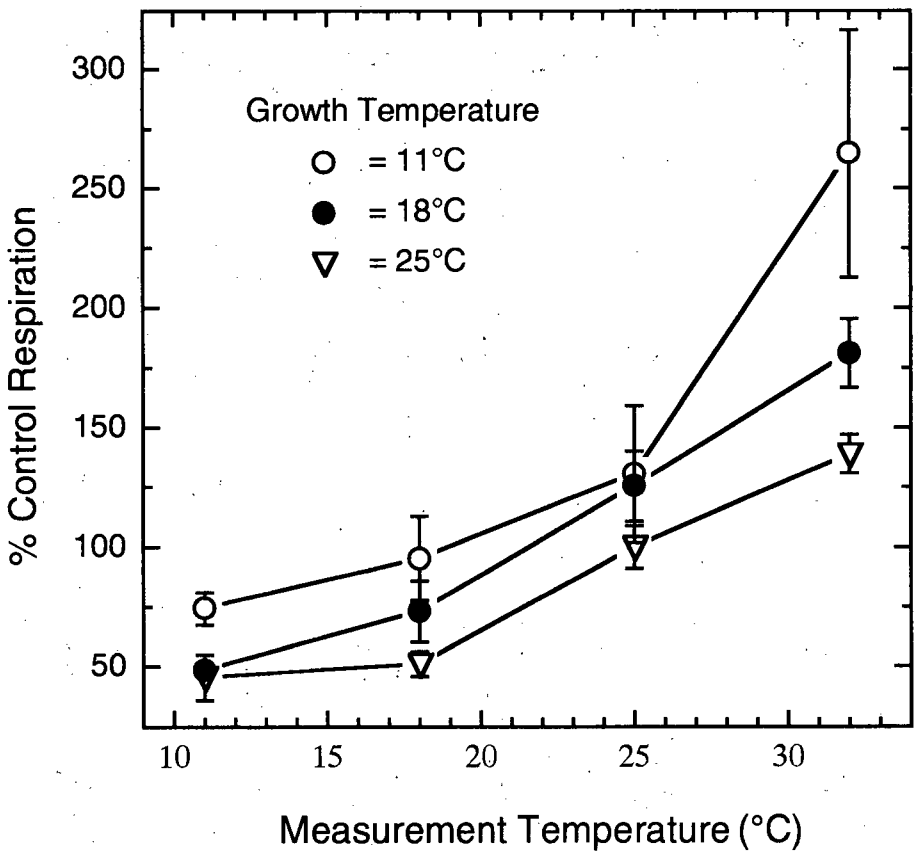

Figure 6: Root TDR rates for coastal western red cedar seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of control, which is defined as the mean rate for seedlings grown and measured at 25°C. (n = 4 samples, where each sample is a mean of 2 rates; $Control_1$ = 10.594 μmol $O_2.g^{-1}FW.h^{-1}$, $Control_2$ = 38.526 μmol $O_2.g^{-1}FW.h^{-1}$; error bars = SEM)

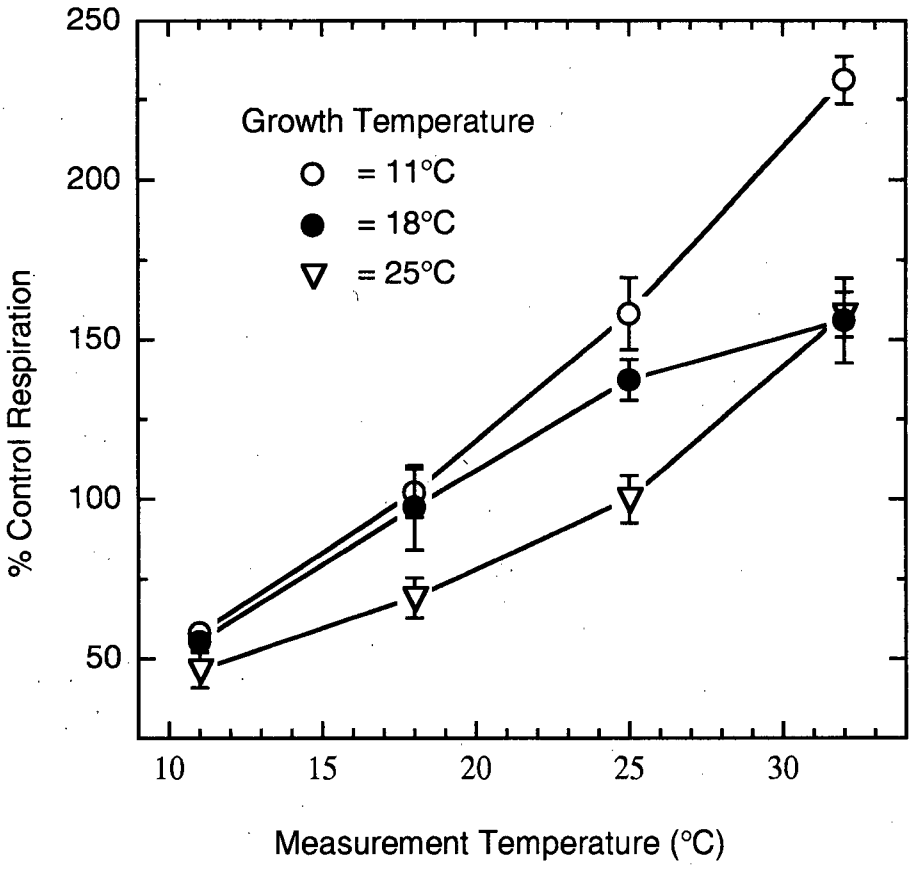

Figure 7: Root TDR rates for interior western red cedar seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of control, which is defined as the mean rate for seedlings grown and measured at 25°C. (n = 4 samples, where each sample is a mean of 2 rates; $Control_1$ = 10.946 µmol $O_2.g^{-1}FW.h^{-1}$, $Control_2$ = 24.978 µmol $O_2.g^{-1}FW.h^{-1}$; error bars = SEM)

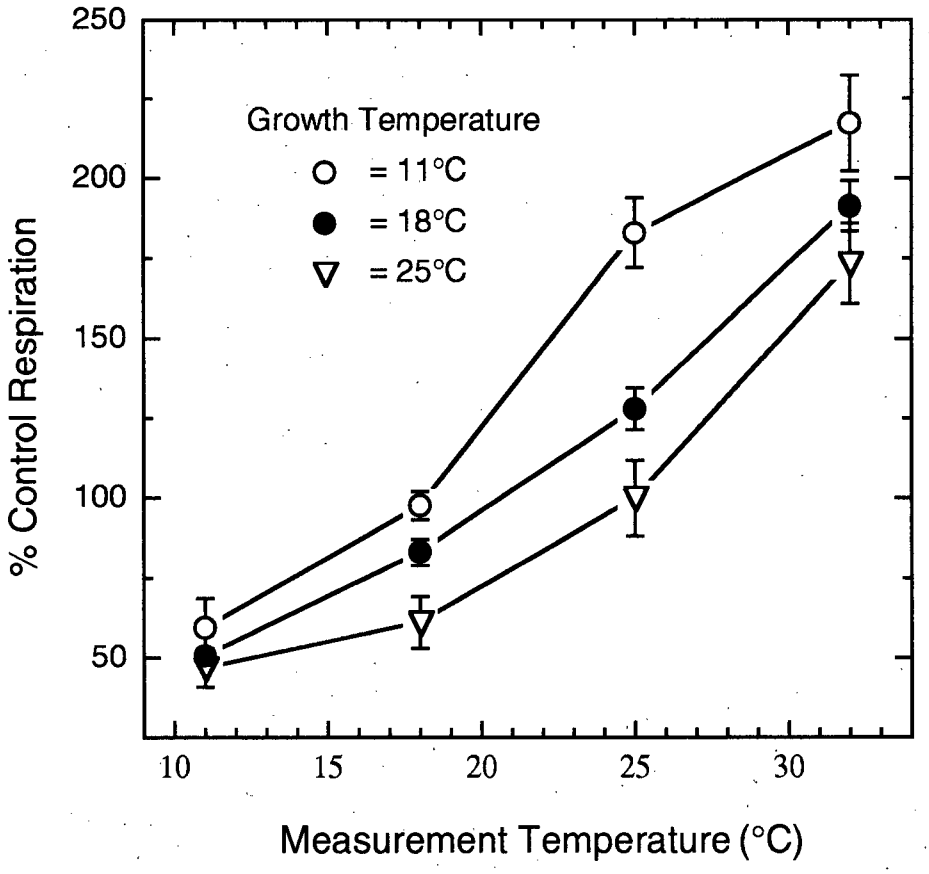

Figure 8: Root TDR rates for coastal Douglas-fir seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of control, which is defined as the mean rate for seedlings grown and measured at 25°C. (n = 4 samples, where each sample is a mean of 2 rates; $Control_1$ = 17.717 µmol $O_2.g^{-1}FW.h^{-1}$, $Control_2$ = 42.625 µmol $O_2.g^{-1}FW.h^{-1}$; error bars = SEM)

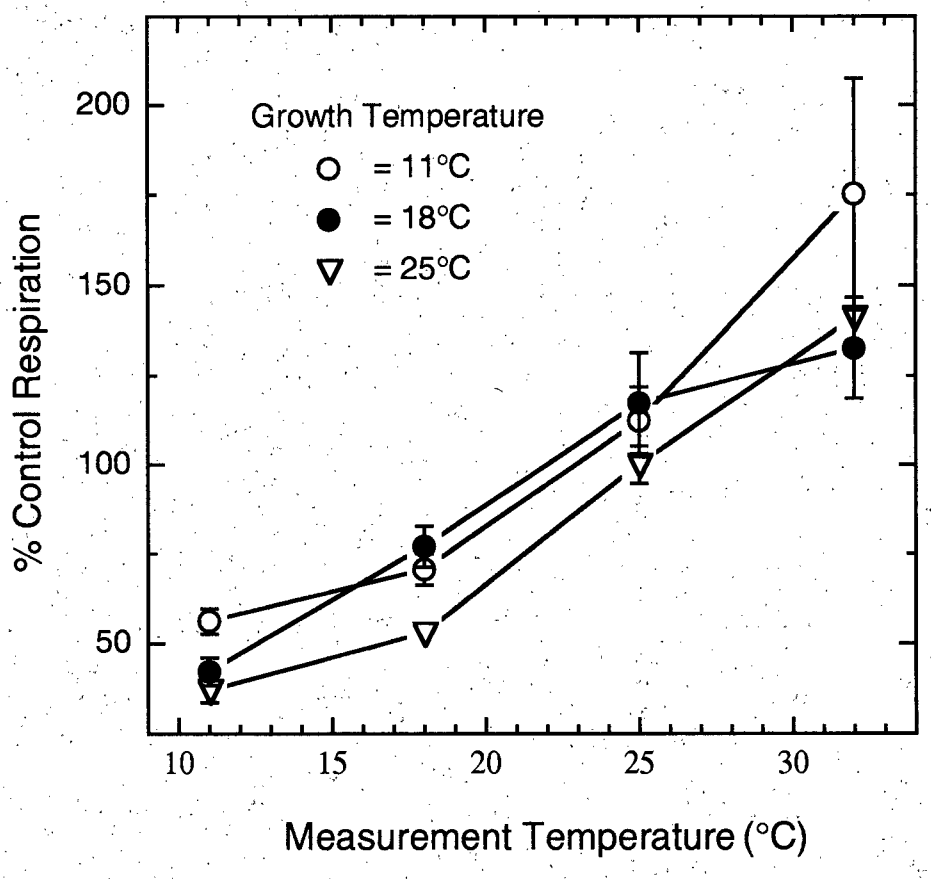

Figure 9: Root TDR rates for interior Douglas-fir seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of control, which is defined as the mean rate for seedlings grown and measured at 25°C. (n = 4 samples, where each sample is a mean of 2 rates; $Control_1$ = 20.316 µmol $O_2.g^{-1}FW.h^{-1}$, $Control_2$ =51.923 µmol $O_2.g^{-1}FW.h^{-1}$; error bars = SEM)

TDR except between 18 and 25°C in the coastal provenance of western red cedar (P=0.107).

Q_{10}s were calculated for TDR for each provenance. Mean values were between 1.6 and 1.8 for 11 - 32°C (Table 2). No differences existed between provenances or growth temperatures. Q_{10}s between 11 and 25°C were similar to those from 18 to 32°C (not presented).

4.1 ii. Effect of Root Temperature on APA and APC

The effects of growth and measurement temperature on APA and APC for roots of coastal and interior western red cedar and Douglas-fir seedlings are presented in Figures 10-13. No trends in activity or capacity of the AP are apparent from these data. Two-way ANOVAs found no significant effects of growth or measurement temperature on the AP for any provenance ($0.086 \leq P \leq 0.995$).

4.1 iii. Effect of Root Temperature on O_2 Response

Oxygen response curves were constructed for TDR of seedling roots for each provenance at measurement temperatures of 11 and 25°C. These data are presented in Figures 14-21. The points were fitted to Michaelis-Menton kinetics and three Km values (one for each sample) were calculated for every growth temperature (also shown in Figures 14-21). Here, Km refers to the O_2 concentration at which TDR is one-half that of the maximum O_2-saturated rate. One-way ANOVAs were run on the Km values to detect any differences in response to O_2 concentration between growth temperatures at both measurement temperatures for each provenance. There were no significant differences in O_2 response between root growth temperatures ($0.113 \leq P \leq 0.645$) except between 11 and 25°C for the coastal provenance of western red cedar

Table 2: Q_{10}s for root TDR between 11 and 32°C

Provenance	Growth Temp.	Q_{10}
Western red cedar (Coastal)	11°C	1.698
	18°C	1.773
	25°C	1.457
		mean=1.643
Western red cedar (Interior)	11°C	1.906
	18°C	1.343
	25°C	1.618
		mean=1.622
Coastal Douglas-fir	11°C	1.748
	18°C	1.799
	25°C	1.765
		mean=1.771
Interior Douglas-fir	11°C	1.486
	18°C	1.499
	25°C	1.817
		mean=1.601

* Q_{10}s for particular growth temperatures were calculated from mean rates of 4 measurements.

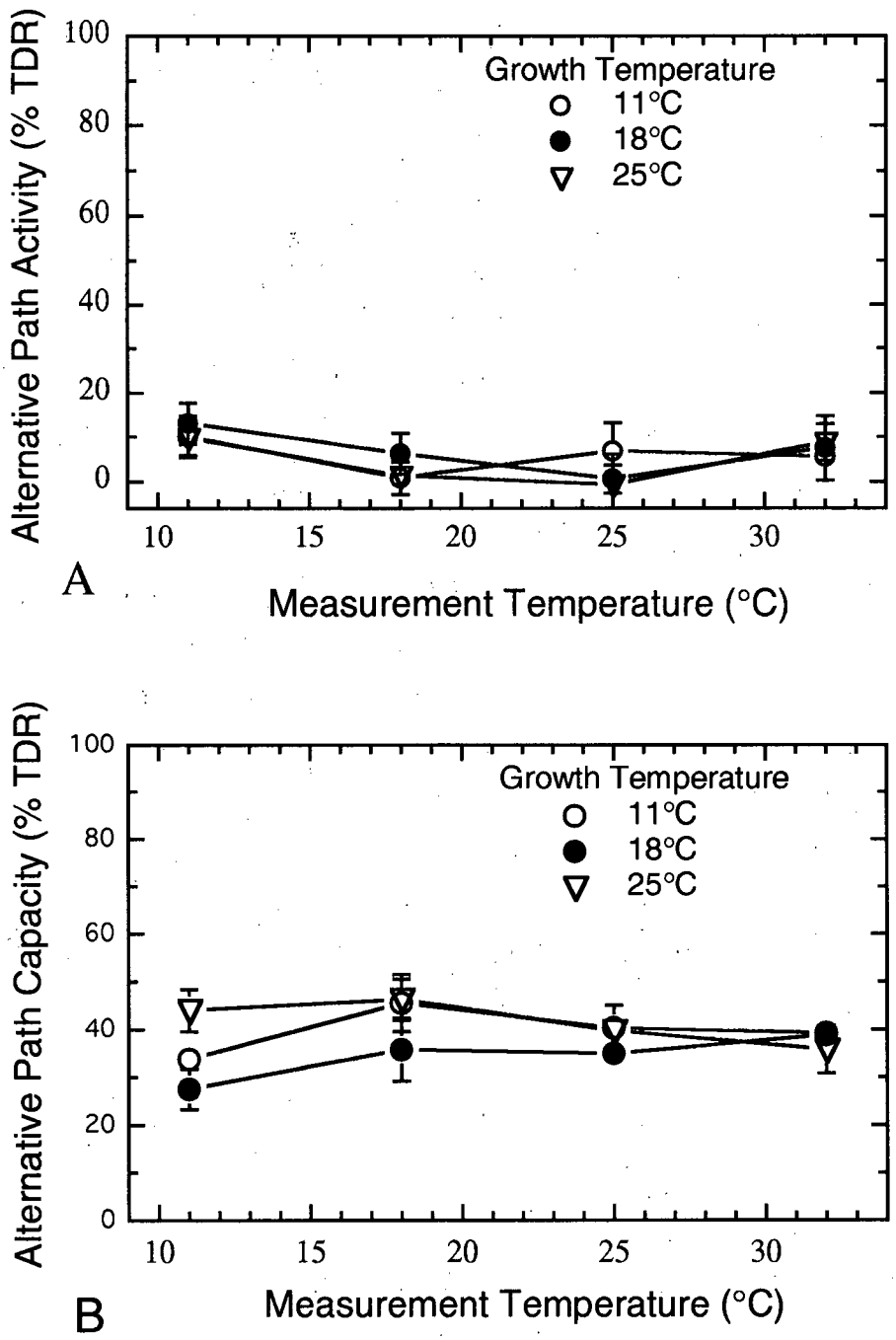

Figure 10: Root APA (A) and APC (B) for coastal western red cedar seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of TDR. (n = 4; error bars = SEM)

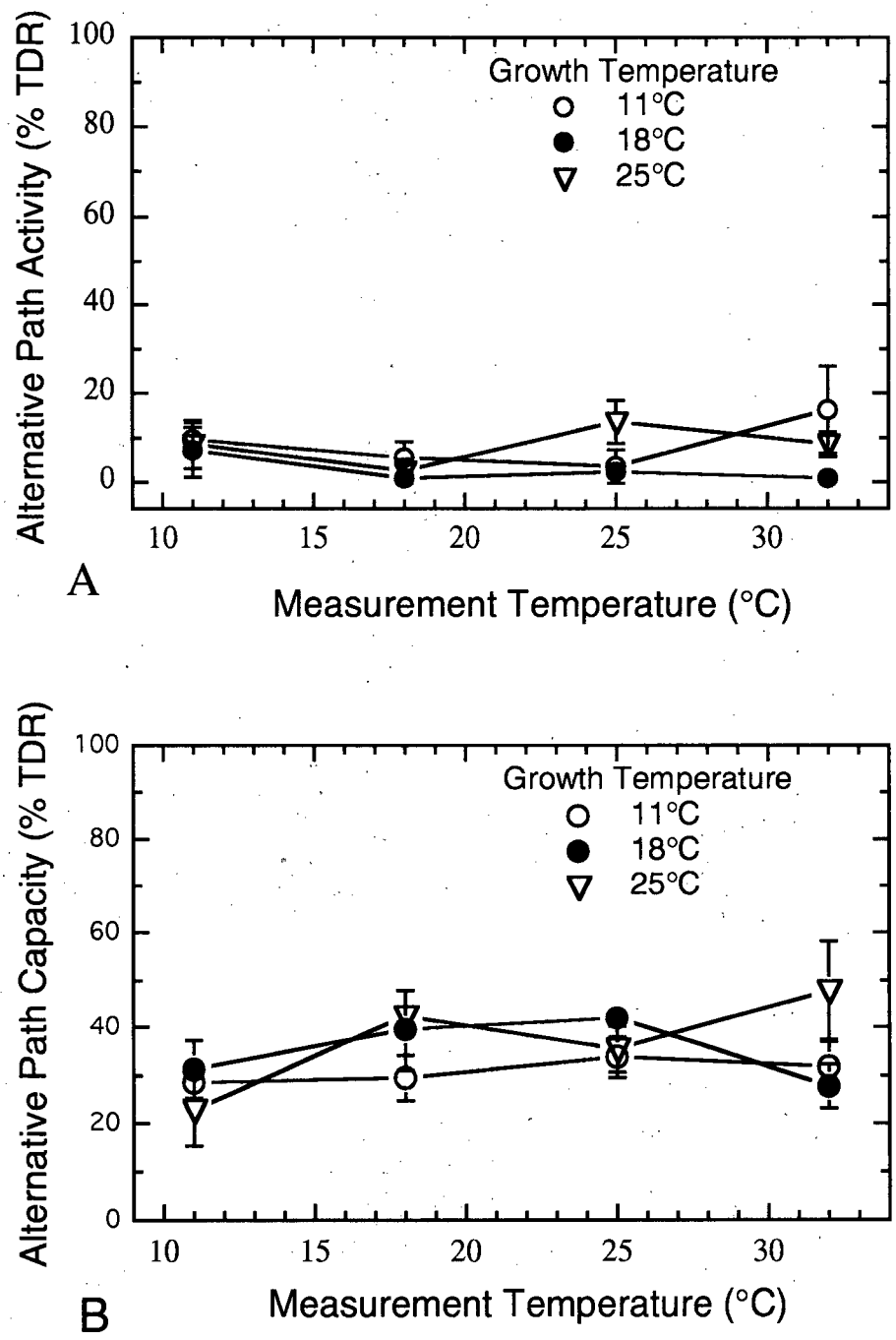

Figure 11: Root APA (A) and APC (B) for interior western red cedar seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of TDR. (n = 4; error bars = SEM)

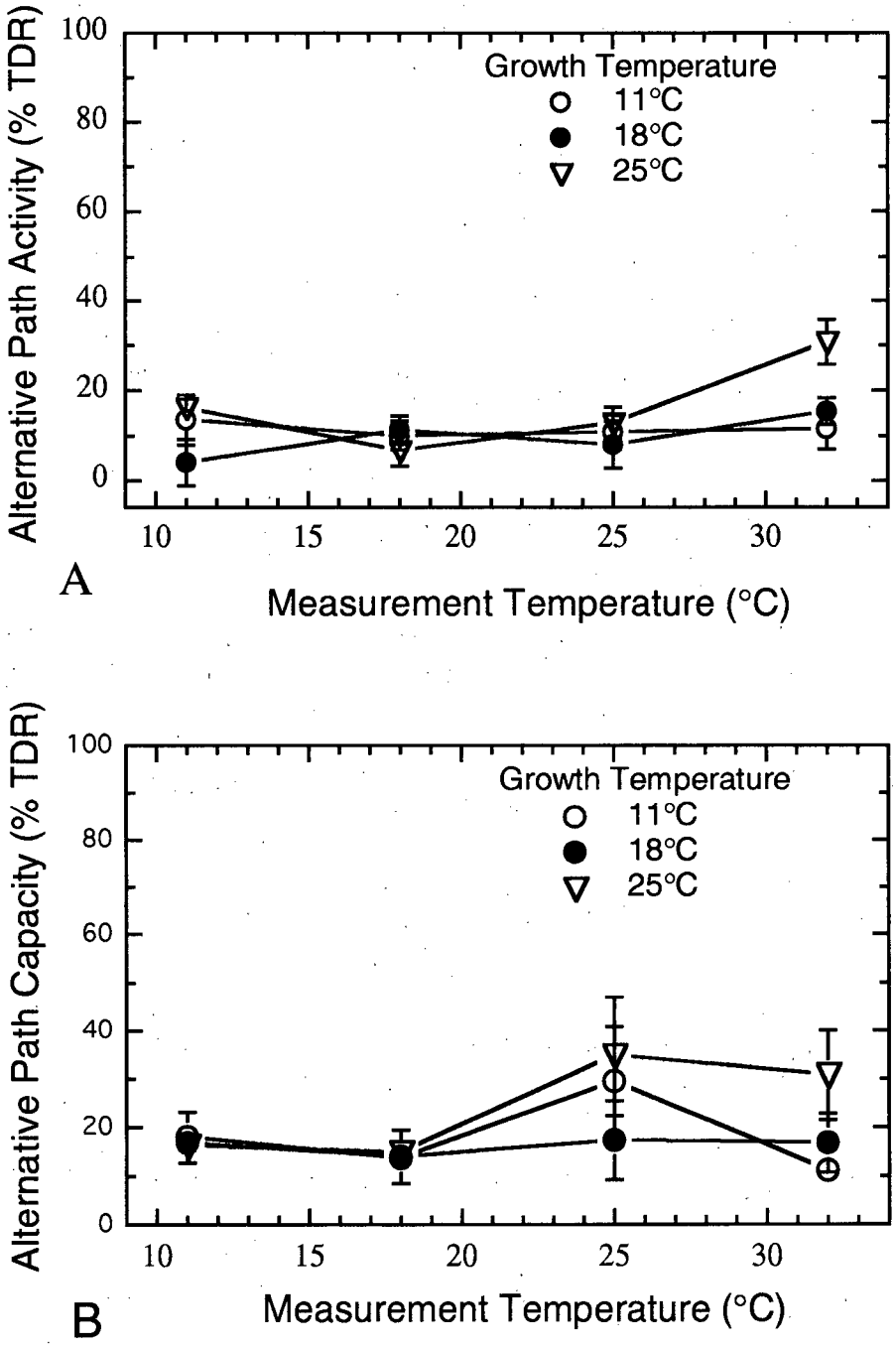

Figure 12: Root APA (A) and APC (B) for coastal Douglas-fir seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of TDR. (n = 4; error bars = SEM)

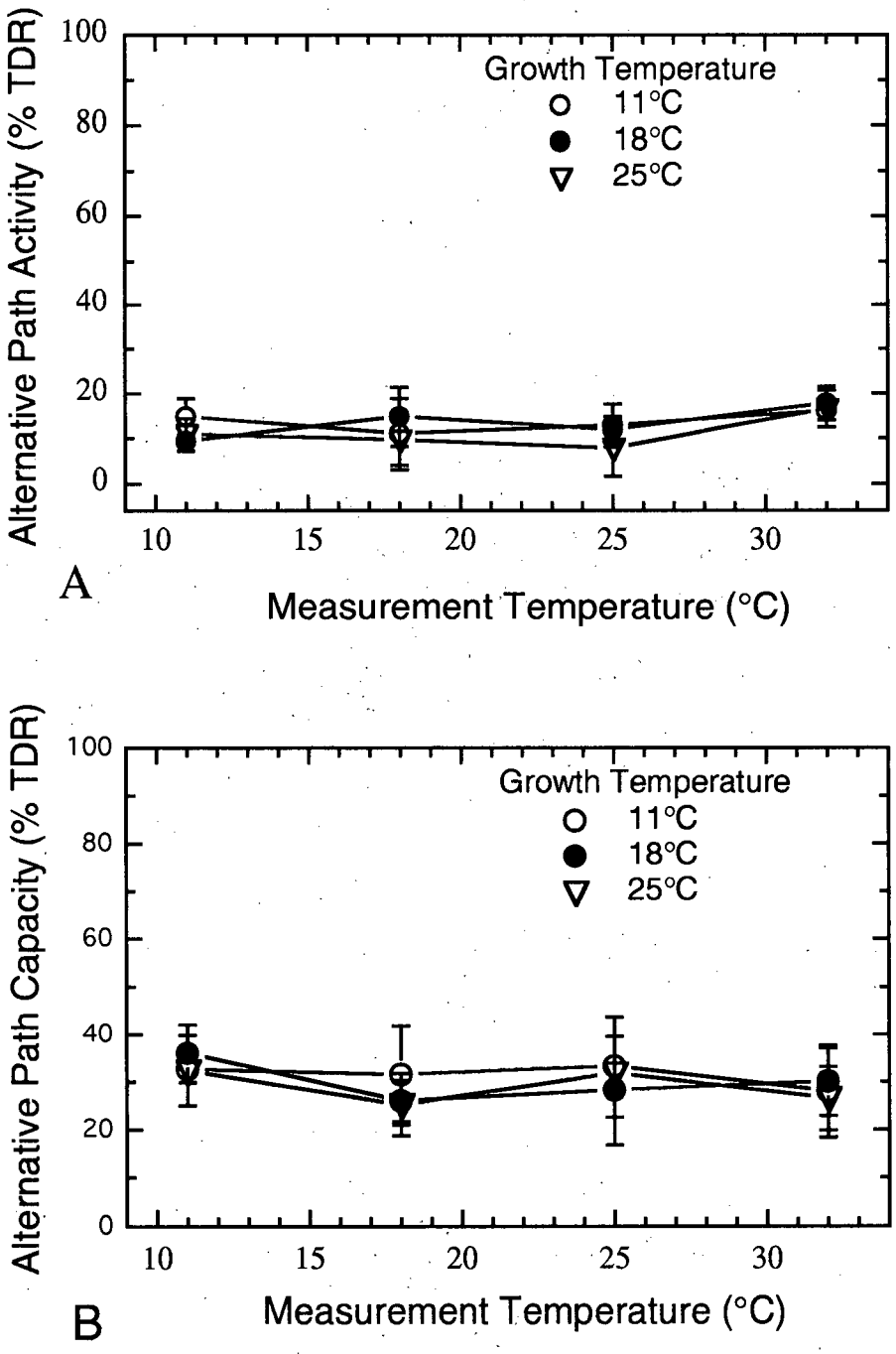

Figure 13: Root APA (A) and APC (B) for interior Douglas-fir seedlings over a range of growth and measurement temperatures. Rates are expressed as a percentage of TDR. (n = 4; error bars = SEM)

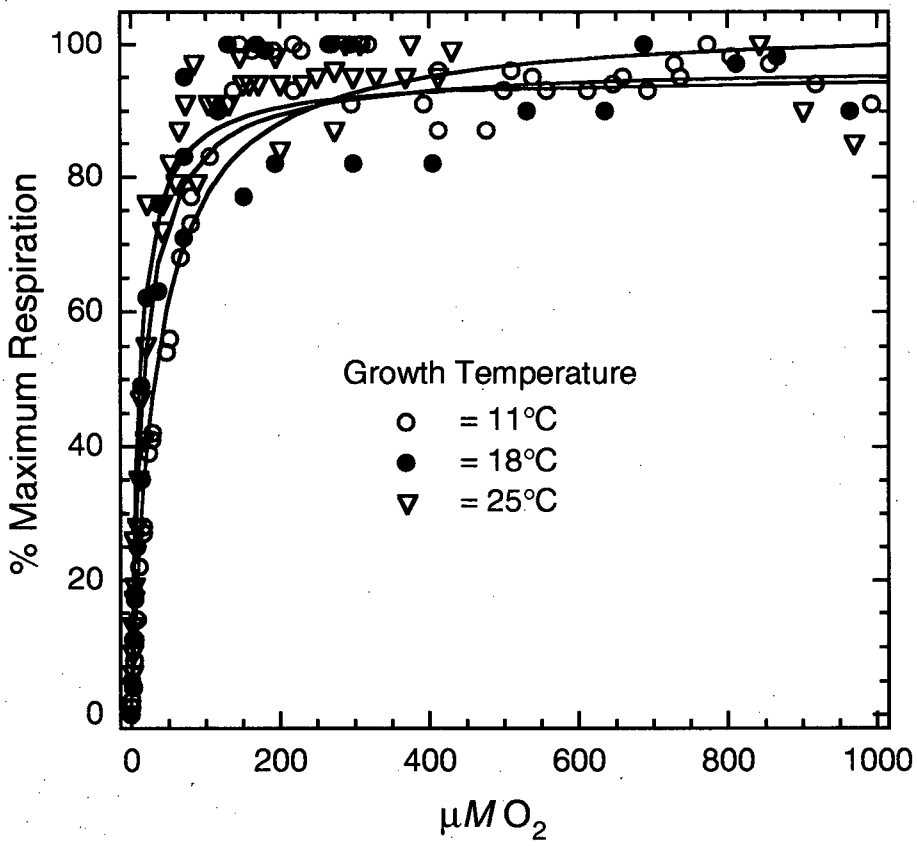

Figure 14: Oxygen response curves for root TDR in the coastal provenance of western red cedar, measured at 11°C. Mean Km values for 11, 18 and 25°C growth temperatures are 34.3, 16.3 and 10.6 $\mu M\ O_2$, respectively. Three seedlings were sampled from each growth temperature.

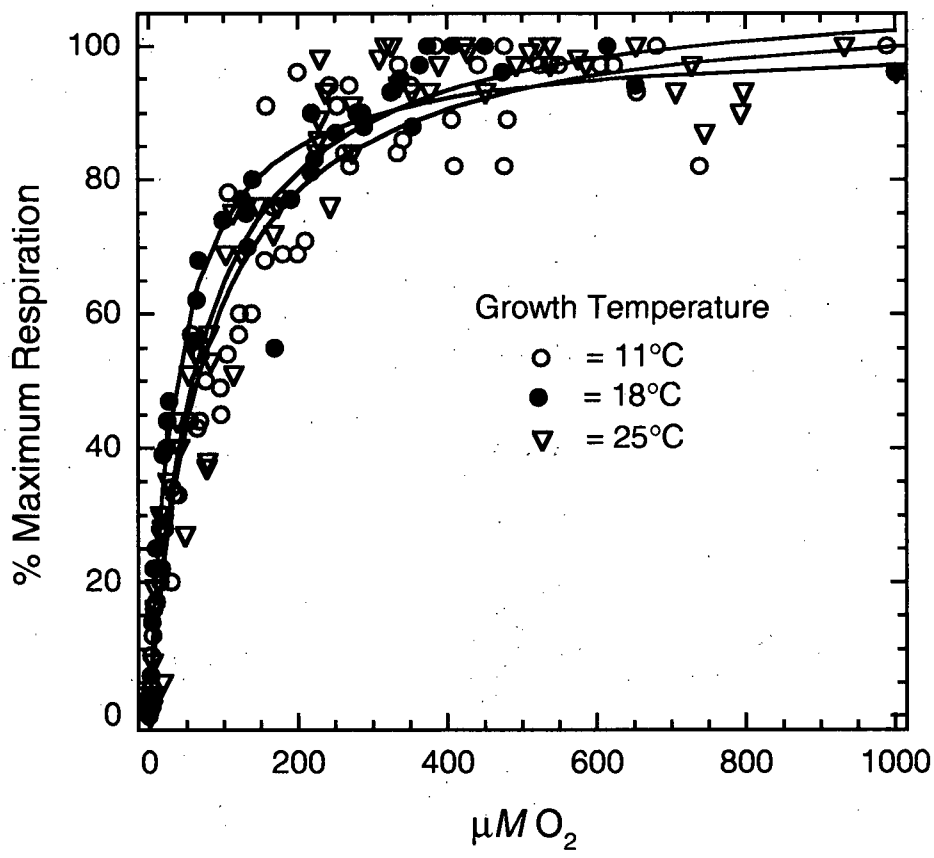

Figure 15: Oxygen response curves for root TDR in the coastal provenance of western red cedar, measured at 25°C. Mean Km values for 11, 18 and 25°C growth temperatures are 75.3, 37.6 and 71.1 $\mu M O_2$, respectively. Three seedlings were sampled from each growth temperature.

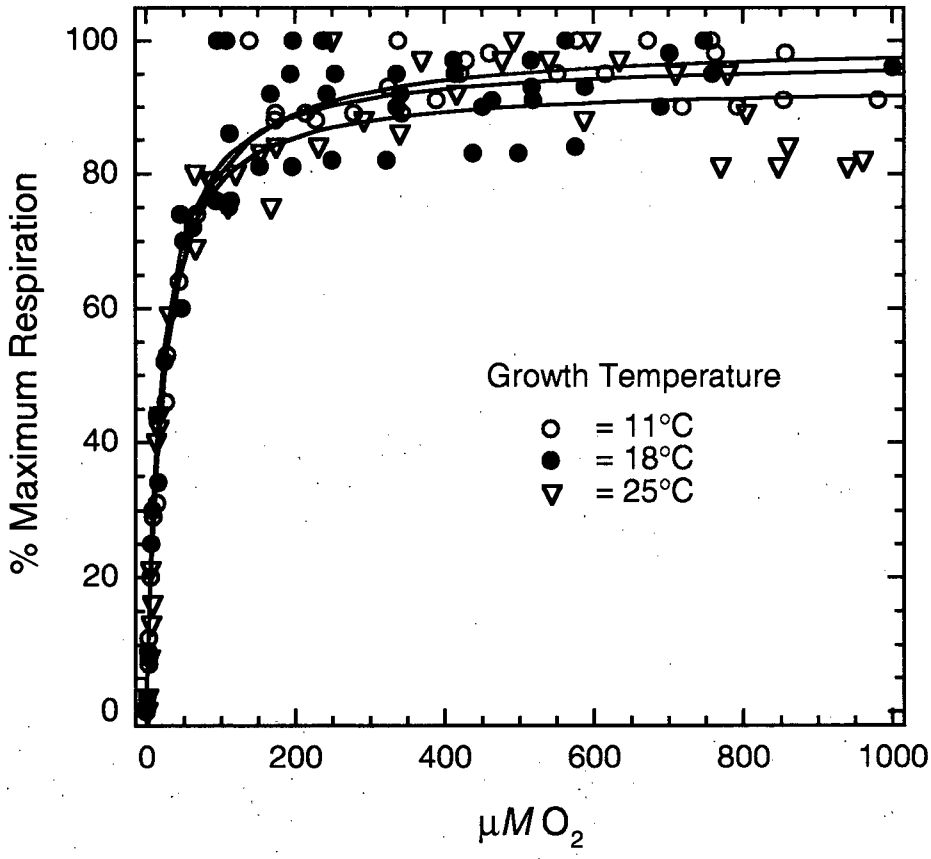

Figure 16: Oxygen response curves for root TDR in the interior provenance of western red cedar, measured at 11°C. Mean Km values for 11, 18 and 25°C growth temperatures are 24.4, 20.0 and 18.8 $\mu M O_2$, respectively. Three seedlings were sampled from each growth temperature.

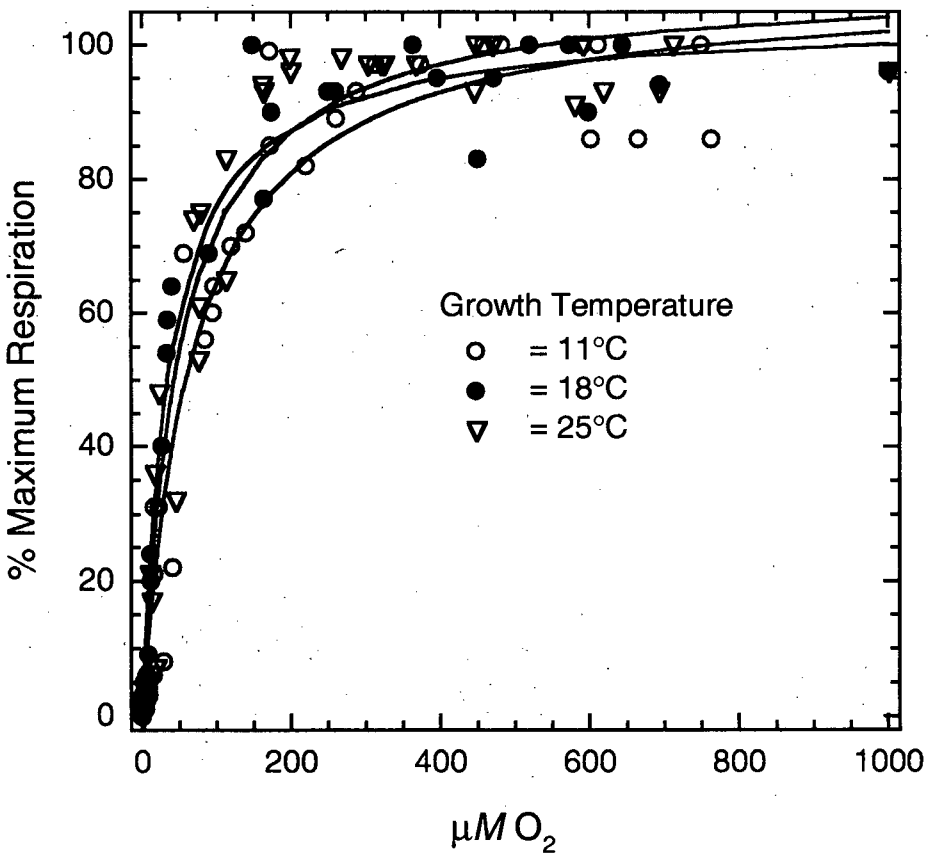

Figure 17: Oxygen response curves for root TDR in the interior provenance of western red cedar, measured at 25°C. Mean Km values for 11, 18 and 25°C growth temperatures are 68.8, 37.1 and 52.7 $\mu M\, O_2$, respectively. Three seedlings were sampled from each growth temperature.

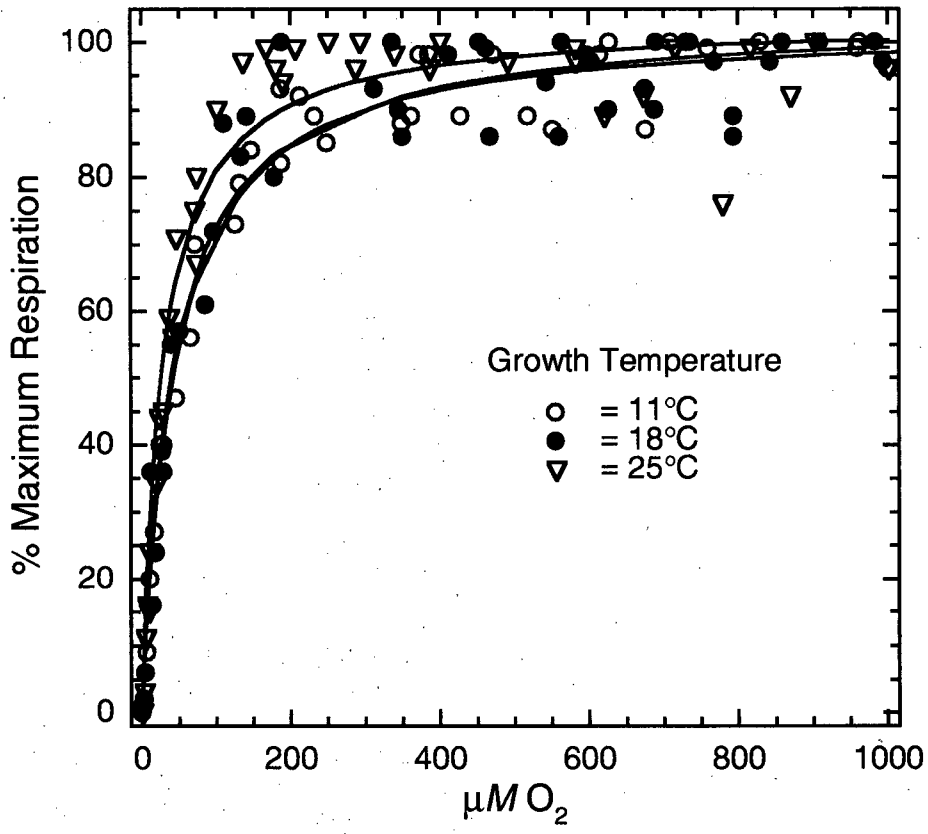

Figure 18: Oxygen response curves for TDR in the coastal Douglas-fir, measured at 11°C. Mean Km values for 11, 18 and 25°C growth temperatures are 45.4, 41.3 and 27.7 $\mu M O_2$, respectively. Three seedlings were sampled from each growth temperature.

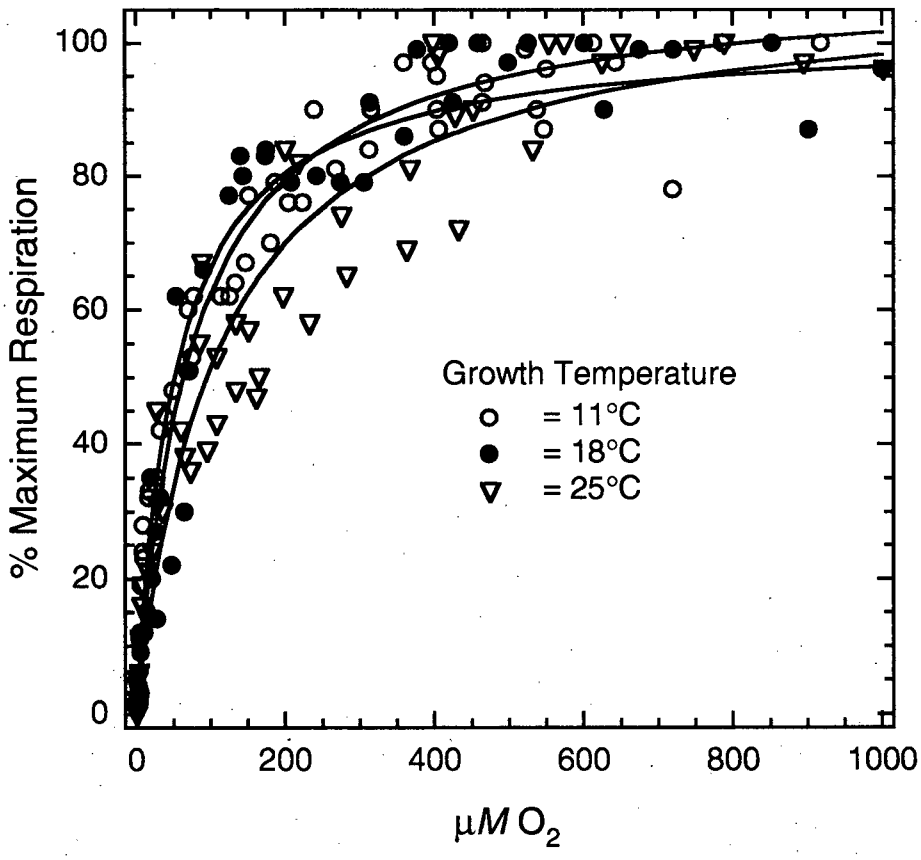

Figure 19: Oxygen response curves for root TDR in the coastal Douglas-fir, measured at 25°C. Mean Km values for 11, 18 and 25°C growth temperatures are 51.8, 84.9 and 83.1$\mu M\ O_2$, respectively. Three seedlings were sampled from each growth temperature.

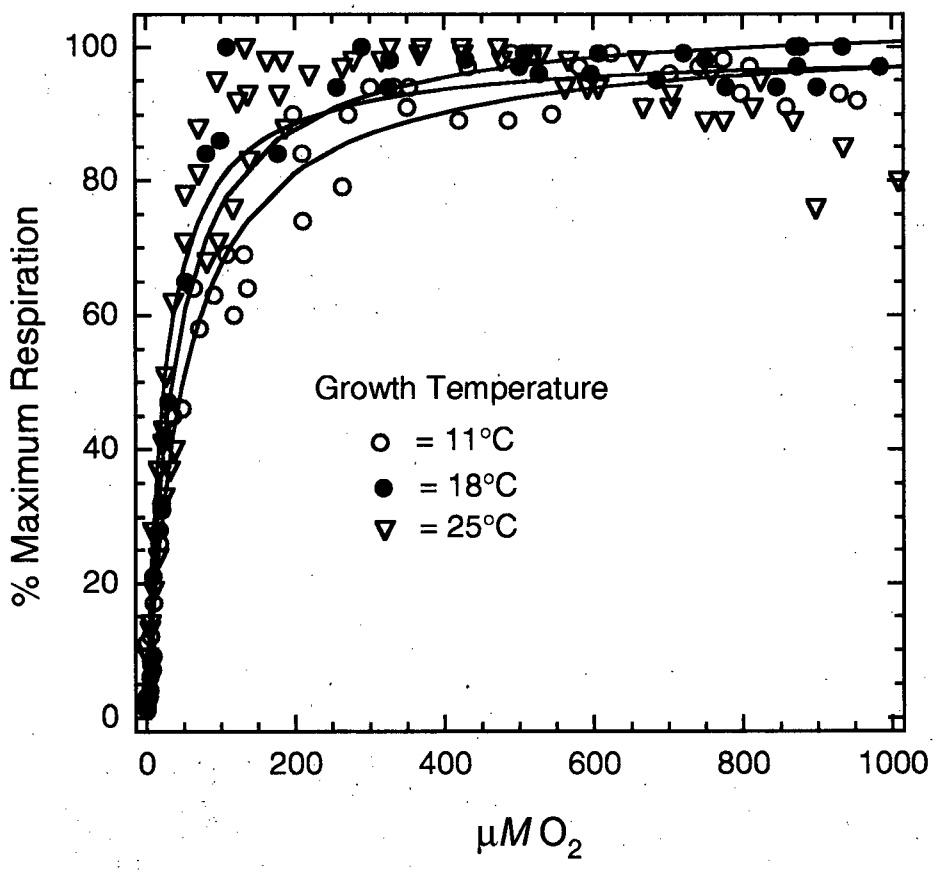

Figure 20: Oxygen response curves for TDR in the interior Douglas-fir, measured at11°C. Mean Km values for 11, 18 and 25°C growth temperatures are 51.6, 37.7 and 24.3 $\mu M\ O_2$, respectively. Three seedlings were sampled from each growth temperature.

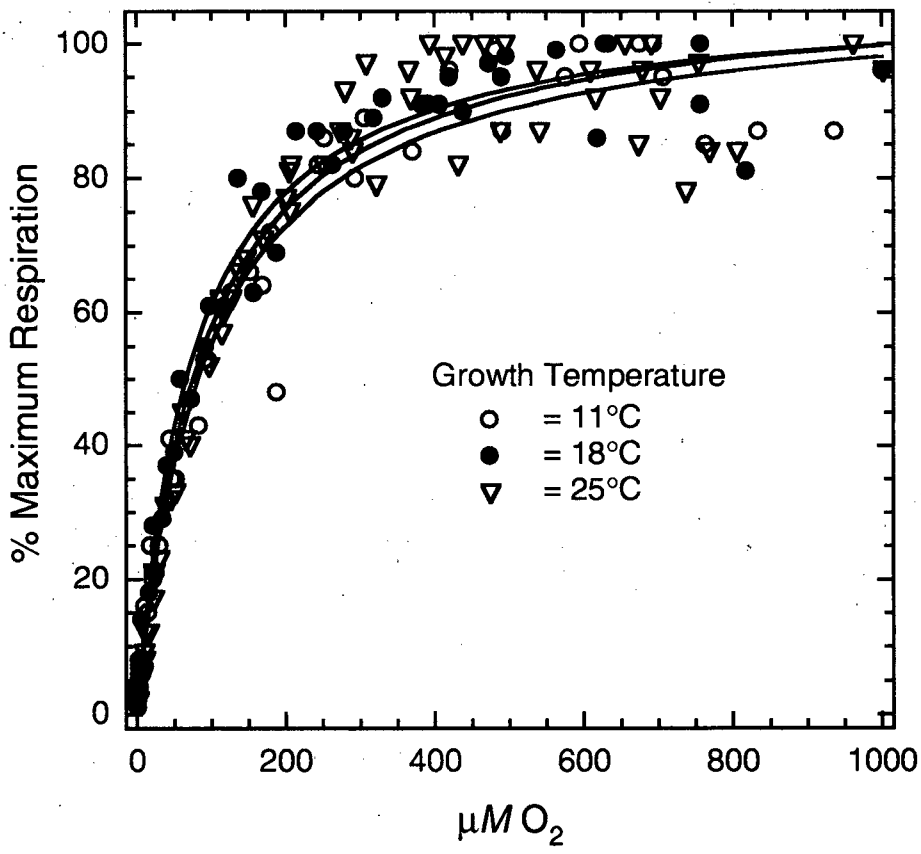

Figure 21: Oxygen response curves for root TDR in the interior Douglas-fir, measured at 25°C. Mean Km values for 11, 18 and 25°C growth temperatures are 94.26, 77.03 and 88.15 $\mu M\, O_2$, respectively. Three seedlings were sampled from each growth temperature.

measured at 11°C (P=0.032). Oxygen concentration at air saturation is 347 μ*M* at 11°C and 258 μ*M* at 25°C. It is clear from the figures that respiration at 25°C became O_2-limited at or just below air saturation level, regardless of growth temperature. Curves were similar when measured at 11°C, but shifted to the left such that respiration was saturated at somewhat lower O_2 concentrations.

4.2 Effect of Root Temperature on Water Relations

4.2 i. Effect of Root Temperature on Shoot Xylem Water Potential (Ψ_x)

Mean Ψ_x values for western red cedar and Douglas-fir seedlings grown at different root temperatures are presented in Table 3. ANOVAs run on each provenance determined that Ψ_x was not significantly affected by temperature ($0.056 \leq P \leq 0.170$), except in coastal Douglas-fir (P=0.003). A Tukey multiple comparisons test performed on the means found that Ψ_x was more negative at lower growth temperatures in every case for this provenance.

4.2 ii. Effect of Root Temperature on Shoot Stable Isotopic Composition

Table 4 contains mean $\delta^{13}C$ values for shoots of each provenance grown at different root temperatures. Since the 25°C-grown seedlings were sampled one month after the others, only the 11 and 18°C growth temperatures could be compared. T-tests were used to determine that shoot $\delta^{13}C$ was less negative at a root growth temperature of 11°C than 18°C for all provenances ($P \leq 0.002$).

4.3 Effect of Root Temperature on Seedling Physiology

Figure 22 depicts total plant dry weights for each provenance grown at the different root temperatures. Root growth temperature significantly affected total plant dry weight for every provenance ($P \leq 0.001$). The use of Tukey multiple

Table 3: Shoot xylem water potential (Ψ_x) of seedlings grown at different root temperatures (n = 5)

Provenance	Growth Temp.	Mean Ψ_x (MPa)	SEM
Western red cedar (Coastal)	11°C	-11.8	0.715
	18°C	-9.7	0.603
	25°C	-10.1	0.469
Western red cedar (Interior)	11°C	-11.4	0.737
	18°C	-9.3	0.515
	25°C	-9.8	0.395
Coastal Douglas-fir	11°C	-14.5	0.583
	18°C	-13.4	0.594
	25°C	-11.1	0.470
Interior Douglas-fir	11°C	-11.4	0.649
	18°C	-12.5	0.617
	25°C	-13.1	0.433

Table 4: Shoot $\delta^{13}C$ values of seedlings grown at different root temperatures. Shoots at 25°C were harvested one month later than those at 11 and 18°C. (n = 5)

Provenance	Growth Temp.	Mean $\delta^{13}C$ (‰)	SEM
Western red cedar (Coastal)	11°C	-27.09	0.4257
	18°C	-29.58	0.3278
	25°C	-28.96	0.2809
Western red cedar (Interior)	11°C	-27.10	0.2809
	18°C	-29.40	0.3457
	25°C	-28.97	0.2476
Coastal Douglas-fir	11°C	-28.46	0.1011
	18°C	-29.83	0.2902
	25°C	-29.52	0.3072
Interior Douglas-fir	11°C	-28.08	0.2048
	18°C	-30.76	0.2822
	25°C	-28.97	0.5572

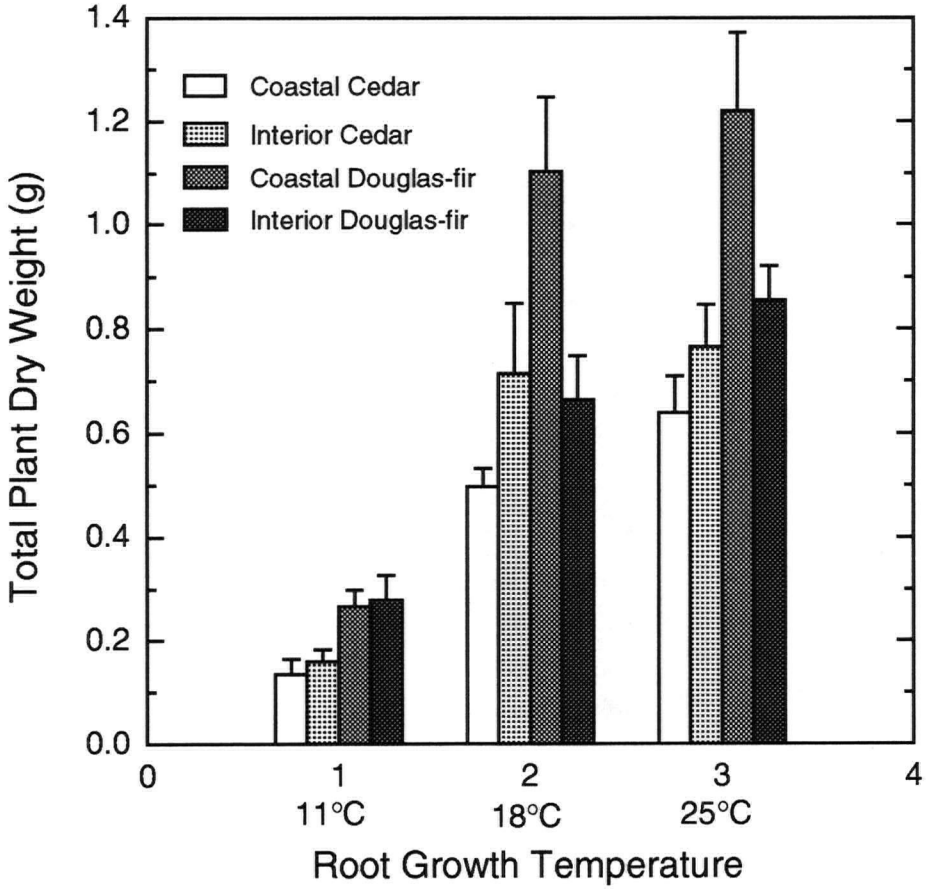

Figure 22: Bar graph depicting total plant dry weight at the three root growth temperatures for all four provenances. (n = 5; error bars = SEM)

comparison tests determined that dry weight was greater at root growth temperatures of 18 and 25°C than at 11°C in every case, while no differences existed between the 18 and 25°C treatments.

Root to shoot dry weight ratios of all provenances from different root growth temperatures are presented in Table 5. ANOVAs revealed that temperature did not significantly affect biomass allocation between roots and shoots ($0.078 \leq P \leq 0.888$), except in the interior provenance of western red cedar ($P=0.027$). A Tukey multiple comparisons test was performed on the means, determining that the 11°C treatment resulted in a smaller root/shoot ratio than the 18°C treatment for this provenance.

Table 6 presents the density of roots grown at different temperatures for the four provenances. There was no significant temperature effect on root density ($0.096 \leq P \leq 0.771$).

For all four provenances, there were few visible differences between seedlings grown at root temperatures of 18 and 25°C. However, seedlings grown at these temperatures were larger, greener and appeared healthier than those grown at 11°C. The shoots of both provenances of 11°C-grown cedar seedlings were reddish brown at the branch tips (Figure 23). When seedling roots were dried for many hours and then rehydrated (the result of an equipment failure), cedar shoots from the 18 and 25°C treatments turned a similar colour. The roots of 18°C- and 25°C-grown seedlings were thinner and longer than, and were not as white as, those grown at the 11°C root temperature. Roots grown at 25°C appeared to be slightly longer, thinner and dingier in colour than 18°C-grown roots. Roots grown at 11°C seemed to be more turgid and brittle than those from the other temperatures, particularly for cedar (i.e., they would snap instead of fold when bent). Cold-grown seedlings often had fewer lateral roots and these had a

Table 5: Dry root/shoot biomass ratios of seedlings grown at different root temperatures (n = 5)

Provenance	Growth Temp.	Root/Shoot	SEM
Western red cedar (Coastal)	11°C	0.2103	0.0340
	18°C	0.2260	0.0215
	25°C	0.2250	0.0174
Western red cedar (Interior)	11°C	0.1615	0.0183
	18°C	0.2354	0.0197
	25°C	0.2118	0.0116
Coastal Douglas-fir	11°C	0.4501	0.0624
	18°C	0.3479	0.0259
	25°C	0.4489	0.0170
Interior Douglas-fir	11°C	0.3280	0.0259
	18°C	0.4389	0.0322
	25°C	0.4823	0.0671

Table 6: Root density of seedlings grown at different root temperatures (n = 4)

Provenance	Growth Temp.	Density (g/cm^3)	SEM
Western red cedar (Coastal)	11°C	1.042	0.0235
	18°C	0.994	0.0030
	25°C	1.031	0.0185
Western red cedar (Interior)	11°C	0.996	0.0135
	18°C	0.994	0.0375
	25°C	1.017	0.0140
Coastal Douglas-fir	11°C	1.065	0.0085
	18°C	1.046	0.0035
	25°C	1.003	0.0355
Interior Douglas-fir	11°C	0.986	0.0180
	18°C	1.038	0.0355
	25°C	1.088	0.0345

Figure 23: Photograph of seedlings from the interior provenance of western red cedar which are representative of the 11°C (left) and 25°C (right) treatments.

smaller inclination from the horizontal than in the 18 or 25°C treatments. A photo of a warm- and cold-grown Douglas-fir seedling is presented in Figure 24.

4.4 Species and Provenance Comparisons

Total dark respiration increased with measurement temperature in a similar manner between and within species (Figures 6-9); Q_{10}s were the same in all four provenances (Table 2). Seedlings of both of the western red cedar provenances and coastal Douglas-fir were able to acclimate TDR rates to root growth temperature, while interior Douglas-fir seedlings were not. Generally, TDR was greater in Douglas-fir than in cedar. Douglas-fir tended to respire relatively more through the AP than cedar (13 vs. 8% max.) while cedar had a greater APC (40 vs. 29% max.). There were no clear intraspecific differences in TDR, APA or APC. Total dark respiration in all provenances showed similar O_2 responses except for the coastal provenance of western red cedar measured at 11°C, wherein root growth temperature appeared to influence O_2 response ($P=0.032$).

Stem xylem water potential tended to be more negative in Douglas-fir than cedar (Table 3). Only in coastal Douglas-fir did growth temperature significantly influence Ψ_x. Values of $\delta^{13}C$ were slightly more negative in Douglas-fir than in cedar (Table 4). Douglas-fir seedlings were generally larger than cedar (Figure 22) and had relatively more roots (Table 5). Growth temperature appeared only to affect biomass allocation in the interior provenance of western red cedar. Coastal Douglas-fir seedlings were slightly larger, on average, than interior plants. Root density was similar in all provenances (Figure 6). Western red cedar roots tended to be thicker, whiter and had a smaller inclination from the horizontal than Douglas-fir roots.

Figure 24: Photograph of interior Douglas-fir seedlings representative of the 11°C and 25°C treatments.

5.0 Discussion

5.1 Effect of Root Temperature on Respiration

5.1 i. Effect of Root Temperature on TDR

The observed increase in TDR with measurement temperature (Figures 6-9) was expected and concurs with other studies (Smakman & Hofstra, 1982; Weger & Guy, 1991). Root respiration rates were higher in Douglas-fir than in western red cedar. This was also easily predicted, given that they appeared to be growing faster. All provenances except interior Douglas-fir showed compensatory acclimation of TDR to growth temperature. It is interesting that coastal Douglas-fir was able to acclimate while the interior provenance was not. In a way, it is not surprising that they behaved differently since they are genetically distinct enough to be classified as separate varieties (Little, 1953). However, within these varieties, genetic variation is great (Haley, 1982). As reviewed in section 1.0, there are reasons to expect that plasticity should increase as heterozygosity decreases. Thus, it was hypothesized that the cedar provenances, with limited heterozygosity (Minore, 1990), would show the best acclimation, and that Douglas-fir would show little or no acclimation. Perhaps there is something about a coastal existence that results in a tendency towards plasticity. In any case, as others have observed (section 1.0), plasticity and heterozygosity are not always mutually exclusive.

The significant interaction effects between growth and measurement temperature in the interior provenance of cedar and in coastal Douglas-fir do not seem to tell us much. From Figure 7, it appears that TDR in 18°C-grown interior cedar roots did not increase as much as in the other treatments when temperature was raised from 25 to 32°C. Similarly, TDR in 11°C-grown coastal

Douglas-fir was relatively higher at 25°C than at the other measurement temperatures (Figure 8). These effects may be partly due to random error along with error in normalizing TDR to a percentage of control where control was one of two different values. Some points relied more on one control than the other.

Mean Q_{10}s for root TDR between 11 and 32°C were between 1.6 and 1.8 for every provenance (Table 2), well within the expected range and similar to those reported for respiration in other species (Smakman & Hofstra, 1982; Lambers, 1985). Although some have found that respiratory Q_{10}s are higher at low temperatures (Lambers, 1985), values here were similar for 11-25°C and 18-32°C. Breeze and Elston (1978) observed that the Q_{10} of respiration for field bean (*Vicia faba* L.) leaves was 1.7 when carbohydrate content was high and 2.1 when the level of carbohydrate was low. They suggested that this may have been due to incomplete hexose respiration, which occurs at high substrate concentrations, being less temperature-sensitive than complete hexose respiration, or respiration of a substrate which is not soluble carbohydrate. Since carbohydrate levels tend to be higher in cold-grown shoots and roots (Farrar, 1988), one might expect respiration of the 11°C-treated roots to be more temperature-sensitive. However, there was no difference in Q_{10}s between growth temperatures. It may be that carbohydrate content was high even in the warm-grown roots, given the conditions of long days and plentiful nutrients. The partitioning of electrons through the CP and AP is perhaps the biggest influence on respiratory Q_{10}s (McNulty & Cummins, 1987; Collier & Cummins, 1990), since the AP tends to be less sensitive to cold temperatures.

The only other studies done on temperature acclimation of respiration in conifers were performed on *Picea glauca* (Weger & Guy, 1991) and *Pinus radiata* (Rook, 1969). There was no evidence of acclimation in white spruce whereas Rook found complete thermal acclimation in radiata pine. Recent studies have

shown that photoperiod can influence photosynthetic acclimation to low temperature in some conifer species. Short days were necessary for acclimation in *Pinus contorta* and greatly enhanced it in *Picea glauca* (Silim et al., 1996). There was no shifting of the temperature optimum of photosynthesis upon lowering the temperature in *Thuja plicata*, even with a shortened photoperiod (Weger et al., 1993). *Picea glauca* and *Pinus contorta* are both species which require short days for the induction of dormancy and frost hardiness. Shoot growth in *Thuja plicata*, which lacks buds and therefore, by definition lacks bud dormancy, is more under direct temperature control. It makes sense, then, that photosynthetic acclimation to temperature be controlled in a similar manner since it can be seen as a facet of cold hardiness. Perhaps photoperiod also plays a role in the temperature acclimation of respiration in some conifers. Although Weger and Guy (1991) found no acclimation of respiration to temperature, a long-day photoperiod was used in their experiment. Douglas-fir also requires short days for dormancy induction and the development of cold-hardiness. It is feasible that root respiration in the interior Douglas-fir used in this study would have acclimated under a short day treatment. Coastal Douglas-fir may behave more like cedar by relying less on day length.

5.1 ii. Effect of Root Temperature on APA and APC

Although compensatory temperature acclimation of respiration to growth temperature was observed in three of the four provenances, it was apparently not due to increased electron partitioning through the AP. Weger and Guy (1991) found no acclimation of root respiration to growth temperature in *Picea glauca*. Neither the activity or capacity of the AP was affected by changes in measurement temperature (Figures 10-13). This is interesting considering that in most other species, APA tends to be less temperature-sensitive at cold and

moderate measurement temperatures than CP activity (CPA) (Cook & Cammack, 1985; Collier & Cummins, 1990). Weger and Guy (1991), however, found that APA and APC increased at higher temperatures in *Picea glauca*.

If the energy overflow hypothesis is correct, then it seems that the CP is able to accomodate electron flow just as well at cold growth or measurement temperatures as it is at warm ones. The AP was apparently rarely engaged at full capacity, particularly in the western red cedar. Although the cedar provenances had higher APCs than the Douglas-fir, they showed less activity. Interestingly, others have also found no correlation between APA and APC in several species (Hemrika-Wagner et al., 1983). The reason for this is unclear. Perhaps the AP has a higher capacity than is necessary for everyday stresses, but is there to protect the plant against rare and extreme stresses, temperature or otherwise. Activities of the AP as percentages of TDR for the four provenances (8-40%) were comparable to other crop and forest species.

From the evidence that has been mounting in the past few years, it seems that the role of the AP may be more significant and complicated than once believed. This is not to say that the overflow hypothesis is incorrect, for there is still every indication that the AP usually becomes engaged when the CP is saturated with substrate, as often occurs with low temperatures (Furuhashi et al., 1989). The AP is likely engaged to prevent damage of the more cold-sensitive CP. Superoxide and H_2O_2 are produced when the CP is impaired due to a high energy charge or stress-induced physical changes in the membrane (Purvis & Shewfelt, 1993). Thus, rerouting electrons through the AP would reduce the production of active oxygen species so that the scavengers are better able to minimize membrane damage. Perhaps it is sometimes necessary to redirect electrons before actual saturation of the CP in an effort to further reduce degradation, or even in anticipation of it. This would account for those instances

where the AP has been found to operate when the CP is not saturated. This is likely accomplished through the manipulation of organic acids, particularly pyruvate (see section 2.4 iv), which has been clearly shown to alter the ubiquinone reduction level at which the AP becomes engaged (Wagner & Wagner, 1995; Millar et al., 1996). Salicylic acid may play a role in AP engagement (Kapulnik et al., 1992).

It is curious that the AP was not preferentially engaged at low growth temperatures, especially considering that TDR was influenced by growth temperature in three of the four provenances. Most of the acclimation studies that found that the AP increased at cold growth and measurement temperatures were performed on crop species. It is possible that the 11°C growth temperature was not cold enough to elicit a response in the AP in these conifer species, which may, in general, be more tolerant of low rooting zone temperatures. A colder growth temperature was initially selected but root growth was found to be severely inhibited below 11°C. It seems likely that the often-observed increase in APA and/or APC at cold temperatures is independent of compensatory respiration and may occur coincidentally with it simply to reduce the production of active oxygen species, which can be a problem at those temperatures. This view is supported by studies which have found that other factors inducing active oxygen production in plants, such as light (Atkin et al., 1993), drought (Weger & Dasgupta, 1993), disease (Molinari, 1991) and seed germination (McCaig & Hill, 1977), also seem to involve the AP.

Another possible explanation for the lack of preferential engagement of the AP at low temperatures in these conifers (or its apparent presence in other species) is to blame the technique. The methods used here and in all similar studies to estimate APA and APC are based on Bahr and Bonner's model of electron partitioning. As discussed, this model is extremely simplified and does

not account for the influence of hormones, SA, or organic acids on the critical reduction point of ubiquinone. Van den Bergen et al. (1994) have demonstrated that the Bahr and Bonner technique sometimes leads to a considerable underestimation of APA. Although it may be uncertain whether APA was accurately assessed, at least it is clear that it could not have exceeded APC. Measurements of APC were likely to have been accurate since there was no evidence of SHAM-stimulated O_2 uptake.

5.1 iii. Effect of Root Temperature on O_2 Response

Respiration began to become O_2-limited at or just below air saturation (258 μM O_2) for roots measured at 25°C, regardless of growth temperature (Figures 15, 17, 19 & 21). At 11°C the trend was similar (Figures 14, 16, 18 & 20) except that respiration became limited below 200 μM O_2 (air saturation of water is 347 μM O_2 at 11°C). It is not surprising that roots measured at 25°C became O_2-limited more quickly than those at 11°C, since they were respiring up to three times faster. At a given diffusional resistance, the higher the rate of TDR, the greater the difference in O_2 concentration between the external solution and the sites of oxygen uptake. Alternatively, since the CP has a higher affinity for oxygen than the AP (Purvis, 1988; Lambers & Smakman, 1978), it might be that the AP made up more of the TDR at 25°C than at 11°C. However, the data do not support this. Furthermore, the partitioning measurements were done at nonlimiting O_2 concentrations. Weger and Guy (1991) found that O_2 uptake in *Picea glauca* roots was saturated at the same O_2 concentration at 18°C and at 4°C, at a value just below air saturation for 18°C. Their study and this one indicate that plants seem to have a fixed oxygen response, so that they tend to become O_2-limited at or near air saturation. Note, however, that root respiration

of the marine macrophyte *Zostera marina* was severely limited at O_2 concentrations in equilibrium with air (Zimmerman et al., 1989).

Since TDR was found to be greater at lower root temperatures in three of the four provenances, one would expect that respiration of the cold-grown roots of these provenances would become O_2-limited at higher O_2 concentrations. Also, the cold-grown roots were accustomed to higher amounts of O_2 since O_2 solubility is higher at low temperatures. However, no differences in O_2 affinity were detected for TDR between growth temperatures, except for between 11 and 25°C in the coastal provenance of western red cedar, measured at 11°C. Given that the probability of type one error was 5% (α=0.05) and the F-value was barely significant (P=0.032), it is likely that no real difference exists.

It seems that the cold-grown roots may have found a way to increase the rate of delivery of oxygen to the mitochondria. This might have been accomplished by increasing the surface area to volume ratio (see section 5.3), creating more/larger intercellular airspaces and thus reducing root density (see section 5.3), or otherwise increasing root permeability to O_2 (e.g. across membranes, etc.). Another possibility is that the roots were able to adjust to the O_2 concentration of the buffer so quickly (by unknown means) as to make it appear that no differences existed between growth temperatures. No evidence for such a mechanism exists in normal plant roots, but dynamic control of diffusional resistance has been shown to exist in legume nodules (Layzell & Hunt, 1990). It would be difficult to prove that a similar phenomenon occurs in ordinary roots.

5.2 Effect of Root Temperature on Water Relations

Mean Ψ_x values were not significantly affected by temperature, except in coastal Douglas-fir (Table 3). At first glance, this might suggest that temperature

did not influence plant water relations in the other three provenances. However, shoot $\delta^{13}C$ was found to be less negative at the 11°C than at the 18°C root growth temperature for every provenance, indicating a higher WUE at the colder temperature. These data support the theory that plants can act by adjusting stomatal conductance to prevent or lessen a decrease in water potential, instead of just responding to it (Mansfield & Atkinson, 1990; Liang et al., 1996). The Douglas-fir seedlings seemed to be slightly more water stressed than the cedar, as indicated by lower Ψ_x values. However, the more negative $\delta^{13}C$ values in Douglas-fir shoots indicate that they had a lower WUE than cedar.

It is not possible to compare the $\delta^{13}C$ data from October (11 and 18°C) with that from November (25°C) of 1995 since the relative amount of ^{13}C in the atmosphere can vary significantly from month to month. This is linked to changes in CO_2 concentration, which, in turn, depend largely upon global net photosynthesis (Emanuel et al., 1994).

5.3 Effect of Root Temperature on Seedling Physiology

Total plant dry weight was greater at 18 and 25°C than at 11°C for every provenance (Figure 22). This was expected since both root and shoot growth tend to increase with root temperature (Bowen, 1991). The fact that no increase in biomass was observed between 18 and 25°C indicates that the optimal root growth temperatures for these species lie within this range. This seems to be a reasonable conclusion in that it is indicative of the environmental ranges of these trees. Lyr and Garbe (1995) found that maximum growth rates occurred at a root temperature of 15°C in *Pinus sylvestris*.

Douglas-fir seedlings were generally larger than western red cedar. This was expected since cedar is known to be a slow growing species (Curran & Dunsworth, 1987). The fact that seedlings of coastal Douglas-fir were slightly

larger than those from the interior was also not surprising, given that the interior variety tends to be smaller and slower growing (Hermann & Lavender, 1990).

Biomass allocation between roots and shoots was not affected by root temperature, except in the interior provenance of western red cedar, where the root to shoot ratio was greater at 18°C than at 11°C (Table 5). There may be a tendency for young seedlings to favour shoot growth over root growth at cold root temperatures (Abbas Al-Ani & Hay, 1983; Bowen, 1991). However, taken together with the idea that plants from cold/temperate environments may allocate more resources to the roots in such a situation (Lyr & Garbe, 1995), these two factors may have served to cancel one another out. The root to shoot ratio in nine-month-old chaparral shrub *Ceanothus greggii* (Trel.) Jeps. increased with root temperature (Larigauderie et al., 1991) while it decreased in year-old *Pinus sylvestris* seedlings (Lyr & Garbe, 1995). In this study, Douglas-fir tended to have relatively more roots than western red cedar. In mature trees, Douglas-fir usually has a deeper rooting system than cedar (Minore, 1979). However, mature Douglas-fir and western red cedar trees of similar size growing on analogous soils have roots that penetrate to approximately the same depths and extend over similar areas (Minore, 1990).

An increase in root density with temperature would have explained the similarity in O_2 response curves between growth temperatures, given that a greater airspace in cold roots would have facilitated a higher rate of O_2 diffusion into the roots at a similar external concentration. This appears not to have been the case, however, since root density was found not to be affected by growth temperature (Table 6). The displacement method used for determining root density was rather crude and I am not completely convinced as to the accuracy of these results. A pycnometer might provide more accurate results.

A greater surface area to volume ratio would also promote O_2 diffusion by providing a relatively larger O_2-absorbing surface. However, roots grown at 11°C tended to be larger in diameter (and thus had less relative surface area) than those grown at 18 or 25°C (Figure 24). The fact that the cold-grown roots were less dingy and discoloured than the warm-grown roots may mean that the root segments from 18 and 25°C were somewhat suberized, which might also impair O_2 diffusion. The discolouration may not have been indicative of suberization, however, since root suberization actually tends to occur further from the apex at optimal temperatures (Nambiar et al., 1979; Bowen, 1991; Kaspar & Bland, 1992). Others have also found that roots grown at cold temperatures are whiter than those grown at warm temperatures (Kaspar & Bland, 1992).

Shoots of warm-grown roots appeared greener and healthier than those from the 11°C treatment, presumably because the optimal root temperatures lie between 18 and 25°C for these species. The increase in root thickness at low root temperatures has been observed in many other species, such as *Pinus radiata* (Nambiar et al., 1979). The inclination of lateral roots from the horizontal was greater at optimal root temperatures in both species, as it is in *Glycine max* (Kaspar et al., 1981).

Others have noted that western red cedar leaves often turn red-brown when grown at low temperatures (Weger et al., 1993), with drought (Phil Burton, pers. comm.), or with over-storey removal (Deb DeLong, pers. comm.). A reddening of cedar foliage was also observed in this study when roots were cooled, and upon accidental drought stress due to equipment failures. Weger et al. (1993) demonstrated that the colour change was due to the synthesis and accumulation of the carotenoid rhodoxanthin. They suggested that it might serve to protect photosynthetic capacity at low temperatures by decreasing the amount of light reaching the photosynthetic apparatus. The effects of air temperature,

drought and light may act directly upon the shoot to induce rhodoxanthin accumulation. However, since air temperature was held constant and Ψ_x was not affected by root temperature, it appears that root signalling could also be involved.

6.0 Summary and Conclusions

Although many researchers have studied photosynthetic acclimation to temperature, considerably fewer have looked at acclimation of respiration, especially in conifers. This is curious considering the potential benefits of such experiments in the face of global climate change. The main objective of this study was to determine whether Douglas-fir and western red cedar seedlings could exhibit compensatory acclimation of root respiration to growth temperature. It was hypothesized that western red cedar might be better able to acclimate, given that it has less genetic heterozygosity, which may be linked to greater plasticity. The results showed that both the coastal and interior provenances of western red cedar and the coastal provenance of Douglas-fir were able to acclimate, while interior Douglas-fir was not. Acclimation did not appear to involve an increase in electron partitioning through the AP. Considering these results and other recent studies, it was suggested that the increase in APR that is often observed at low temperatures is independent of compensatory respiration, but may sometimes occur coincidentally with it to reduce the damage caused by active oxygen species, which are likely to be a problem at those temperatures.

Interestingly, increased respiration rates at cold growth temperatures did not increase the O_2 concentration at which respiration became limited. This suggests that the cold-grown roots may have had a lower resistance to O_2 diffusion. This was not accomplished by increasing the root surface area to volume ratio or, apparently, by reducing root density. Although Ψ_x measurements showed that cold-grown plants were not water stressed, $\delta^{13}C$ analysis revealed that they had a higher WUE than warm-grown seedlings. These data support the theory that plants can adjust stomatal conductance to

prevent or limit a drop in Ψ_x , before stress develops. The optimal root growth temperature was between 18 and 25°C for both species.

It is important that more forest species and provenances be tested for temperature acclimation potential. According to Larigauderie and Körner (1995), acclimation of dark respiration to temperature is a missing link in efforts to predict the effects of global warming on plant communities. In future studies of conifers, the manipulation of photoperiod along with growth temperature may prove to be informative. The long-term effect of root-zone temperature on photosynthesis also needs further exploration. This is particularly so for coastal evergreen species that may accomplish much of their net carbon fixation outside the normal growing season, when soil temperatures are low. Since there is evidence that the use of inhibitors to estimate APA and APC may give misleading results, further studies of this sort should employ alternative methods of assessment, such as oxygen isotope analysis.

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Appendix A: Example ANOVA table for root temperature effects

Source	df	SS	MS	Comp. Var.	F-Test	P-Value
Meas. Temp. (M)	3	_	_	$12\sigma^2_M + 4\sigma^2_{MxG} + \sigma^2_E$	MS_M/MS_E	_
Grow. Temp. (G)	2	_	_	$16\sigma^2_G + 4\sigma^2_{MxG} + \sigma^2_E$	MS_G/MS_E	_
M x G	6	_	_	$4\sigma^2_{MxG} + \sigma^2_E$	MS_{MxG}/MS_E	_
Error (E)	36	_	_	σ^2_E		
Total	47					