# Temperature Response and Acclimation of Coastal Douglas-fir Fine Root Respiration in the Laboratory and Field

by

Tyler Abbey

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## Abstract

The temperature response of Coastal Douglas-fir fine root respiration ($R_{FR}$) was investigated with a laboratory based experiment and during field studies. In the laboratory, a reciprocal transplant experiment was carried out to assess the temperature response of biomass-specific $R_{FR}$. Seedlings were moved from either 16 to 24°C or 24 to 16°C, to test for warm and cold acclimation, respectively. Following transfer, there was an elevation in $R_{FR}$ of all treatments. Neither this 'transfer effect', nor the subsequent results provided evidence of an acclimation response.

Over the 2004 and 2005 growing seasons, $R_{FR}$ was characterized at three stands that differ in age (55-y-old: Mature, 17-y-old: Young and 5-y-old: New). The results were interpreted for evidence of acclimation. Rates of $R_{FR}$ were low in spring 2004, however during that summer there was a significant and unexpected elevation in rate, at all sites. In 2005, rates began higher than the previous spring, indicating a possible 'carry-over effect' from 2004. The seasonal increase in $R_{FR}$ observed in 2004 was not repeated in 2005. The New stand was the only site to show a pattern consistent with acclimation, although these results may have arisen due to summer drought or a decline in substrate availability. Sensitivity (i.e. $Q_{10}$) of $R_{FR}$ appeared to change with overall capacity in 2004, increasing from ~1.3 to 2.4 at all sites. In 2005, $Q_{10}$ values changed little over the growing season; however, there were greater site differences in this year.

$R_{FR}$ in summer 2004 was much higher than most other published results for conifers. Given that there were no apparent methodological artifacts, it is suggested that because 2004 was such a hot and dry year, an endogenous stress reaction occurred in the trees and led to this result. With standing fine root biomass in May 2004 and a hypothetical mortality scenario, the measured rates of $R_{FR}$ were scaled to area to facilitate their comparison to ecosystem respiration (**ER**). The resulting model indicates that $R_{FR}$ may play an important role in maintaining levels of ER during drought years, when other less drought resistant components may have reduced activity.

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## List of Symbols and Acronyms

| | |
|---|---|
| AMC | aeroponic mist chamber |
| AOX | alternative oxidase |
| C | carbon |
| $CO_2$ | carbon dioxide |
| DW | dry weight, g |
| ER | ecosystem respiration, μmol $CO_2$ $m^{-2}$ $h^{-1}$, g C $m^{-2}$ $d^{-1}$ |
| FCRN | Fluxnet Canada Research Network |
| FW | fresh weight, g |
| GEP | gross ecosystem productivity, μmol $CO_2$ $m^{-2}$ $h^{-1}$, g C $m^{-2}$ $d^{-1}$ |
| IAV | inter-annual variability |
| MT | measurement temperature, °C |
| MES | 2-(N-morpholino) ethanesulfonic acid |
| N | nitrogen |
| NPP | net primary productivity |
| NEP | net ecosystem productivity |
| $O_2$ | oxygen |
| PPFD | photosynthetic photon flux density, μmol $m^{-2}$ $s^{-1}$ |
| $Q_{10}$ | simplifier describing the proportional change in respiration following a 10°C change in temperature |
| RQ | respiratory quotient |
| RGR | relative growth rate |
| $R$ | respiration |
| $R_{FR}$ | fine root respiration, μmol $O_2$ g $FW^{-1}$ $h^{-1}$, μmol $O_2$ g $DW^{-1}$ $h^{-1}$ |
| $R_H$ | heterotrophic respiration |
| $R_R$ | rhizosphere respiration |
| $R_S$ | soil respiration |
| SWC | soil water content (volumetric), % |
| TBCA | total belowground carbon allocation |
| 1WA | one week average of data for a variable |

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As with any big endeavor, it's taken a lot to get here, to the end of this project, this part of my life and I couldn't have done it alone. It has been a transformative and foundational time for me; there have been so many lessons about work, science and life interwoven throughout the process of completing this degree. Before the people I first have to thank the work for providing the hardest lessons and for forcing me to become a stronger person. I am grateful to plants first and foremost for what they do day after day and secondly for the challenge and fascination they offer as subjects of study. Over the course of this research I developed a greater appreciation for the scientific method because despite its arduous nature, it is a means through which to interpret the world in a manner which enlightens, and inspires. And as part of that method, I must also thank the instruments; for without them I'd have no data, less patience and I would not have gained such an appreciation for dealing with unplanned surprises.

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# 1. The dynamic response of plant respiration to changes in temperature environment

As sessile organisms, plants have evolved mechanisms that allow them to adjust as their surrounding environment changes. One of the most dynamic and ubiquitous variable that they routinely encounter is temperature and the responses of plant respiration (***R***) to a changing temperature environment are complex and variable by nature (Atkin and Tjoelker 2003). Depending on its habitat a particular species may have to accommodate either minimal to moderate daily and seasonal change (i.e. tropical/temperate), or significant shifts in the diurnal growth temperature regime, along with a reduced growing season (i.e. boreal/arctic). Interpreting the temperature response of terrestrial plant life is complicated by separation of the plant body; i.e. into shoots and roots which operate within different environments (i.e. aboveground vs. soil or substrate) and perform separate primary functions (i.e. photosynthesis and water/nutrient uptake, respectively) (Raven et al. 2005). Although *R* is a process shared by both, the differences between these organs complicate the assessment of either whole-plant or organ-specific temperature response (Dewar et al. 1999). Despite their distinctions shoots and roots are tightly coupled through complex allocation patterns (Pregitzer et al. 2000), which can favour investment in growth of either the above or belowground component as temperatures change (Atkin et al. 2006). Historically the belowground plant component has been investigated to a much lesser degree than that aboveground (Norby and Jackson 2000, Bhaskar 2003) and our understanding of the controls over allocation is still very incomplete (Widén and Majdi 2001). In the face of future changes in climate, these gaps in our understanding will need to be breached in order to accurately represent how the terrestrial biosphere is going to respond. Put another way, a comprehensive understanding of plant metabolism, and its response to temperature change will significantly improve our ability to project expected changes in the cycling of carbon (**C**) within ecosystems, and between the biosphere and atmosphere.

Assessments of yearly C exchange by terrestrial ecosystems vary, but it is believed that on a global basis there is currently a net sink of C into the biosphere (i.e. + net ecosystem productivity (**NEP**)) (Woodwell et al. 1998, Barford et al. 2001, Schimel et al. 2001). Recent estimations of the amount of C re-entering the atmosphere due to plant *R* fall around 60 GT C $yr^{-1}$ (Atkin and Tjoelker 2003, Gifford 2003, King et al. 2006). Given that this is nearly eight times the amount being released due to anthropogenic activities (i.e. fossil fuel burning, cement manufacturing and land use change; ~ 7.9 GT C $yr^{-1}$ in the 1990s – Schimel et al. 2001), it is vital that patterns of plant *R* and the factors controlling it be understood at global, regional and local spatial scales and over decadal, yearly, seasonal, and daily temporal scales (Atkin and Tjoelker 2003, Gaumont-Guay 2005, Trumbore 2006). However, a major hurdle in most ecosystem models has been that *R* is not as accurately represented as photosynthesis (Gifford 2003, Lambers et al. 2002), partly due to the fact that photosynthesis has been more thoroughly studied (Hopkins 1999), but also because the belowground component of ecosystem respiration (**ER**) presents greater challenges for investigation than the aboveground (Norby and Jackson 2000, Clark et al. 2001). Over the past three decades the functional placement of *R* in C budgets has grown due to increasing empirical evidence and the development of theoretical, functional (Ryan 1991) and process-based models using regressions and computer simulations (Allen et al. 2005). With the possibility that terrestrial ecosystems may be collectively becoming a global source of $CO_2$ (Woodwell et al. 1998, Schimel et al. 2001), and that positive feedbacks could lead to further $CO_2$ release (Houghton et al. 1998, Cox et al. 2000), accounting for the role of *R* in carbon-climate coupled future scenarios will continue to be an area of research requiring advancement.

One of the more recent developments aiding the formulation of future-directed models has been the deployment of eddy covariance towers across the landscape for continuous, multi-year monitoring of bio-meteorological fluxes. These towers were first applied in a Canadian research network during the BOREAS project in the mid-1990s, for studying C flux in boreal forest ecosystems. Since then these sites and others like them have provided a wealth of data to assist modelers in testing predictions of how complex ecosystem processes like *R* are controlled (Braswell et al. 2005). However

despite this innovation, *R* is still often only represented in models as a direct function of temperature (Curiel Yuste et al. 2003, Ryan and Law 2005) or even indirectly through its inclusion within growth parameters (Gifford 2003). When the temperature response of *R* is characterized, it is usually through the use of Arrhenius or van 't Hoff derived equations, which were originally designed to describe the exponential increase in metabolic rate that typically follows short-term temperature elevation (Arrhenius 1889, van 't Hoff 1898). These functions are often used under the assumption that temperature sensitivity is static, which if held to in a model can lead to inaccuracy (Curiel Yuste et al. 2003, Davidson et al. 2006). The application of these equations is usually referred to as a $Q_{10}$, which describes the proportional change in *R* for a 10°C change in temperature (Atkin et al. 2000, Tjoelker et al. 1999). This simplification has also been extended into what is called a seasonal or annual $Q_{10}$ which often masks the variability that is seen on shorter time scales but is a convenient means of comparing the sensitivity of *R* over different years and between sites and forest types (Curiel Yuste et al. 2004, Gaumont-Guay 2005). Unfortunately, the exclusive use of $Q_{10}$ algorithms does not account for the myriad of other factors that influence rates of *R* (Davidson et al. 2006), which can vary considerably across species and among plant organs (Atkin et al. 2005). $Q_{10}$ values have also been repeatedly shown to vary under different conditions, with the range of measurement temperatures (**MT**s) used and when *R* is evaluated with different methods (Atkin et al. 2000, 2005). Regardless of its drawbacks $Q_{10}$ can be an effective means of demonstrating the degree of respiratory change within a given temperature range. Given this significant attribute, this simplifier may continue to have worth in future modeling efforts as long as modelers recognize that there are other factors influencing *R* that need to be accounted for (Davidson et al. 1998, Davidson et al. 2006, Saiz et al. 2006) and that using long-term $Q_{10}$ relationships can lead to inaccuracies.

A further issue that must be recognized when interpreting the effect of temperature on *R* is the possibility that as changes in temperature become long-term, *R* may acclimate (Teskey and Will 1999, Tjoelker et al. 1999, Atkin et al. 2000, King et al. 2006). Typically this is understood as a compensatory adjustment in plants, whereby rates of *R* will either decline when plants grow under a prolonged warming or will increase

following exposure to lengthy periods of cold. It is now recognized that many species possess the capability to acclimate (see Ch2), however this phenomenon is not ubiquitous. Incorporating acclimation into C models is still rare (Ryan and Law 2005), which could result in misleading projections; for example due to overestimations of C loss if *R* is down-regulated during prolonged warming (King et al. 2006). Some recent, novel efforts have been made to develop acclimation algorithms that could account for this process (Wythers et al. 2005). These attempts may be premature however, due to insufficient empirical data to determine when or even whether acclimation is relevant enough in forests to necessitate its incorporation into models.

In considering the *R* budget for ecosystems there are two primary components: 1) the aboveground which accounts for autotrophic *R* from leaves, stems, boles and epiphytic plants and; 2) the belowground which represents the many contributors to C efflux from the soil. Of these two ecosystem components, the latter often accounts for more than two thirds of ER (Valentini et al. 2000, Janssens et al. 2001). The primary concern of my research has been with belowground processes and more specifically with the autotrophic component, although effort has been made to place the results within a whole-stand context. Given the requirement for knowledge concerning the functional role of root *R* in the C budget, and the uncertainty over whether acclimation processes should be accounted for in forests, the focus of this work had been to investigate the temperature response of fine root respiration ($R_{FR}$) and to interpret whether acclimation was a factor within that response. The project is part of the Fluxnet-Canada Research Network (**FCRN**) initiative, which was established in part to further understand the fluxes of carbon dioxide ($CO_2$) from terrestrial and peatland ecosystems, findings from which are being applied to the development of regional and landscape-scaled C models. This network is composed of various stations in a latitudinal transect across Canada, each station having a number of sites with eddy covariance towers for the continuous measurement of standardized variables. In the B.C. Fluxnet "station" there are now up to seven permanent, developing or temporary sites involved in the network. The three most established and long-running of these were used for field-based studies on the central-eastern coast of Vancouver Island, B.C. Coastal Douglas-fir (*Pseudotsuga menziesii*

Mirb. Franco) was the species under investigation; as it was the primary stand constituent at these sites. This commercially valuable, sub-climax species is naturally distributed from the central Californian coast to the central coast of B.C. It is a dominant presence in the Pacific Northwest region and as such has significant relevance for monitoring and modeling the C balance.

Three primary questions directed this research, the first two of which were pursued through a temperature controlled laboratory experiment. The results from that study are detailed in Chapter 2 of this thesis. In order to interpret the response of Coastal Douglas-fir $R_{FR}$ in the field, it was necessary to characterize that response in the laboratory. The purpose of the experiment was to isolate the response of $R_{FR}$ following abrupt changes in growth temperature. The nature of this response would help to answer the question of whether roots from this species would acclimate to the altered temperature conditions. If there was evidence of acclimation, a follow-up objective was to quantify the rate of respiratory adjustment. To make the results relevant for the field sites under study, seedlings from an eastern Vancouver Island provenance of Douglas-fir were used.

In Chapter 3 the results from field research at the aforementioned Fluxnet sites are described. The primary goal of this work was to determine how the seasonal temperature response of Douglas-fir fine roots compared for stands of differing maturity. If the observed patterns of $R_{FR}$ were found to relate to stand age, it was of interest if there was evidence of acclimation sharing similar relationships. The same measurement protocol used in the laboratory was adapted to characterize $R$ of fine, white roots from three stands that varied significantly in age but not in site characteristics. A preliminary field season in 2004 led to some interesting findings which were further pursued in 2005. The resulting phenology and inter-annual variability (**IAV**) of respiratory capacity and sensitivity (i.e. $Q_{10}$) are interpreted and effort is then made to scale these findings, to facilitate their comparison with ER data from these sites and years.

Chapter 4 summarizes the major findings from these two studies and concludes upon the role of Coastal Douglas-fir $R_{FR}$ as part of the C budget.

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# 2. Does Coastal Douglas-fir fine root respiration acclimate following an abrupt increase or decrease in growth temperature?

## 2.1 - Introduction

### 2.1.1 - Acclimation as specialized adaptation

All plant species are ultimately restricted in their ability to adapt to temperature change (Dahlhoff and Somero 1993). Latitudinal boundaries, growing season length, elevation gradients and the climatic zones within these demarcations are often used to characterize the range or habitat type that a species or population is capable of occupying (Aber and Melillo 1991). The intrinsic reason that specific groups of plants do well in certain regions is that their physiology is adapted to those environments. Adaptation involves the synchronization of many processes, all of which depend on the biochemical function of enzyme activity. Enzymes are well known to respond to changes in temperature such that there is an optimal zone for their activity. Usually this is somewhere between 20 and 30°C, whereas below 5°C and above 40°C activity is minimal (Raven et al. 2005). Although enzymes are certainly not the only determinant of respiratory activity (Atkin et al. 2000a), plants and plant respiration (***R***) are active within this 35°C range and it has been known for over 100 years that as temperature rises from 5 to 40°C, *R* generally increases exponentially (Arrhenius 1889). As discussed in Chapter 1, this exponential increase is usually represented in modeling ventures by a simplifier that describes the sensitivity of *R* to temperature change (i.e. $Q_{10}$). Despite this general pattern, changes in the rate of *R* are influenced by many factors and each plant species will have different ideal temperature conditions. When considering how respiratory activity relates to other physiological process such as photosynthesis, it is also important to recognize that each process will have its own optimum (Cunnigham and Read 2003) and therefore though ambient conditions may enhance *R*, it could be an extreme and even detrimental condition for other aspects of plant function.

Characterizing optimal temperature conditions for *R* is useful for describing relationships between growth rate and metabolism but, when temperature changes over longer periods, assessing the role and persistence of optima becomes more complicated (Pregitzer et al. 2000). Following patterns of long-term temperature change, *R* may acclimate (Atkin et al. 2000a, Pregitzer et al. 2000, Atkin and Tjoelker 2003), especially if the temperature condition has become sub-optimal or if a plant is developing in an extreme environment (Arnone and Körner 1997). An increasing number of studies have pursued the importance and ubiquity of acclimation responses to temperature change both to characterize its commonality (Zimmerman et al. 1989, Larigauderie and Körner 1995, Atkin et al. 2006a) and to determine the extent that it may influence carbon (**C**) modeling (Cox et al. 2000). Although some authors may argue that this term should only be applied to laboratory studies (Piersma and Drent 2003), in the interests of this work acclimation will be considered as a compensatory response by the respiratory system to temperature conditions, that either stress plants temporarily or are regularly extreme; for example in the arctic (Arnone and Körner 1997) or deserts (Palta and Nobel 1989). This leads us to a number of labels that can be used when describing the occurrence of this specialized process.

Piersma and Drent (2003) discuss the term *Phenotypic Flexibility* which refers to reversible adjustments of physiological machinery that occur when an organism faces significant environmental change. This form of phenotypic adjustment is partial and as such is similar to the designation of Type I acclimation by Atkin and Tjoelker (2003) and Atkin et al. (2005). This refers to a change in the capacity of plant *R* following long-term temperature change; however, only a segment of the temperature response curve compensates and therefore there is a concomitant alteration in the sensitivity (i.e. $Q_{10}$) of *R*. Type I acclimation occurs most often in older tissues that developed under a former temperature condition and were transferred to a new one (Atkin et al. 2005). Conversely full compensation (Type II acclimation - Atkin et al. 2005), involves a more integrated and complete adjustment, or homeostasis, which as a result leads to no change in the $Q_{10}$ of *R*. This form of compensation usually requires that new plant tissues are able to develop within the new temperature environment (Atkin et al. 2005). Homeostatic

acclimation can be likened to another concept presented by Piersma and Drent (2003), *Developmental Plasticity*. This describes the ability of different individuals within a species to grow under different temperature conditions but exhibit equal rates of $R$ when measured at growth temperature. Although this concept is discussed by Piersma and Drent (2003) in the context of a determined change by adapting sea urchin populations, it is essentially synonymous to Type II acclimation. Given that plants have the ability to produce new organs these two labels will be considered the same, with the recognition that a plant will always have limits in the ability to redefine its temperature phenotype. As an extension of these terms there are two basic acclimation processes that should be recognized: 1) *Cold acclimation* in which higher rates of $R$ are typically expressed in plants growing at lower temperature compared to warm grown counterparts; and 2) *Warm acclimation* which is characterized by lower $R$ in plants experiencing a higher growth temperature compared to $R$ of plants grown at moderate temperature. Simply put, these two categories of acclimation can be defined as an evolved physiological capacity for adjustment when the temperature environment has become too 'inhibitory' (i.e. *Cold acclimation*) or too 'costly' (*Warm acclimation*). This adjustment or acclimation of metabolism will of course have a mechanistic underpinning; however, a clear picture of how it occurs is still lacking. The following section presents an overview of the mechanistic control of $R$ and some examples of biochemical changes that have been shown to occur during acclimation responses.

### 2.1.2 - Mechanisms of acclimation

Crossing temperature thresholds naturally triggers significant variation in the biochemical make-up of metabolism, even when respiratory compensation is absent (Atkin et al. 2000a). When a plant with the capability to acclimate alters the machinery of $R$, it is more likely to be an extension to usual temperature response than new pathways of activity (Seppänena and Fagerstedt 2000, Sung et al. 1999). It is clear that the primary mechanisms involved in the regulation of $R$ depend on the temperature of exposure. At the temperature extremes for metabolism, ATP production is limited by enzymatic activity, whereas at moderate temperatures ratios of adenylates (i.e. ADP/ATP) and the

availability of substrate are the primary determinants of respiratory capacity (Atkin et al. 2000a, 2005, Atkin and Tjoelker 2003).

When considering how acclimation may alter *R*, the mechanisms involved could therefore be different in cold versus warm acclimation responses; however, there is still too little evidence on warm acclimation to draw such a conclusion. Mechanistic changes can occur at coarse levels of control (Farrar 1985) such as alterations in tissue structure/protein concentrations and at finer levels through the activity of particular proteins or the inhibition of reduction pathways at various points in the biochemical matrix. Following chilling, increases in mitochondrial density and ultrastructure complexity have been seen for leaves of *Arabidopsis* (Armstrong et al. 2006). In a separate study with this species, mRNA accumulation increased during cold treatment in 20% of 8000 genes that were analyzed (Provart et al. 2003). Another example of genetic change was observed by Santoiani et al. (1993), who found that the translation of sucrose metabolizing enzymes increased in the roots of wheat when grown at chilling temperature. Boosted capacity of the alternative oxidase pathway (**AOX**) was for a time considered as a standard mechanism behind the increases in *R* reported at cold temperature (McNulty et al. 1988, Purvis and Shewfelt 1993). However, more recent studies with improved methodology indicate that increases in AOX may be more functionally related to the reduction of reactive oxidants (Gonzàlez-Meler et al. 1999, Atkin et al. 2000a, Atkin et al. 2002). As a few other examples, increases in the cytochrome pathway have been seen after chilling (Kurimoto et al. 2004, Armstrong et al. 2006), while in cells of cold-tolerant varieties of winter wheat, it has been discovered that pre-chilling microtubules disassemble and are replaced by cold-stable microtubules (Abdrakhamanova et al. 2003). Overall the mechanistic understanding of *R* and acclimation responses to temperature is growing; however, it is still unclear whether the same biochemical changes associated with acclimation in one species, are relevant in others. Of course not all species will even possess the mechanisms and therefore the capability to acclimate when faced with challenging environmental conditions. In the following section some of the species that have been found capable of acclimation and others that appear to lack this type of response are reviewed.

### 2.1.3 - Species demonstrating capacity for acclimation

It is clear from the literature that temperature acclimation of *R* varies extensively among species and groups and even within species (Atkin et al. 2000a). Interpreting this data presents a number of challenges to researchers such as whether functional traits can be used for generalizing the acclimation response (Atkin et al. 2006b); for example whether its occurrence can be correlated with relative growth rate (**RGR**) (Covey-Crump et al. 2002, Loveys et al. 2002, 2003). Data from studies on acclimation can also vary depending on the methods that are used (Atkin et al. 2000a) and most studies differ in whether they looked at whole-plant, shoot, leaf or root *R* when coming to their conclusions. An example of the contradictions that can arise due to this latter problem has been shown in the case of sugar maple, whose leaves were found to show rapid acclimation following only a 4°C elevation in temperature (Gunderson et al. 2000), while in a separate study, *R* of roots demonstrated no evidence of acclimation to seasonal increases in soil temperature (Burton and Pregitzer 2003). Out of the studies that have investigated the role of acclimation, the majority focus on leaf *R*. There have been numerous findings to indicate that cold acclimation might be more common for plants growing in cold-typical environments such as the alpine or arctic (Arnone and Körner 1997, Xiong et al. 2000, Loveys et al. 2002, 2003); however, studies considering a greater number of species, have found little relationship between the presence of an acclimation response and plant origin (Larigauderie and Körner 1995, Collier 1996). In a synthesis study of leaf *R* at ambient temperature across 20 sites and 208 species of woody plants, Wright et al. (2006) indicated that *R* at warmer sites typically exceeded that of cooler sites; a finding which contradicts the idea that cold growing plants will demonstrate cold acclimation. Another interpretation of this result is that woody plants have less ability to cold-acclimate, although other studies oppose this (Bolstad et al. 2003, Tjoelker et al. 1999). Wright et al. (2006) pointed out that the majority of evidence for a link between cold acclimation and cold climates has come from studies on herbaceous plants (Zimmerman et al. 1989, Gunn and Farrar 1999, Loveys et al. 2002, 2003, Kurimoto et al. 2004), and in support of this, the few herbaceous species that were included in their survey demonstrated cold acclimation.

Numerous trees have often been found to acclimate following long-term changes in temperature: loblolly pine [whole-plant] (Teskey and Will 1999), five boreal species [roots, shoots and whole-plant] (Tjoelker et al. 1999), sugar maple [leaves] (Gunderson et al. 2000), and red and white oaks [leaves] (Bolstad et al. 2003). Conversely, other work has found no evidence for acclimation in white spruce (Weger and Guy 1991) or sugar maple roots (Burton and Pregitzer 2003). Obviously, the occurrence of acclimation is species specific and thus far the only consistent pattern is that the capability to acclimate may be linked to a strong genetic component (Dahlhoff and Somero 1993). This can be shown by studies on different populations of a species, which can acclimate but depending on their degree of spatial and temporal separation, may not do so equally. Pearcy (1977) demonstrated one such occurrence with a desert and temperate population of the evergreen shrub *Atriplex lentiformis*, the latter of which had diverged from the former. When grown at contrasting growth temperature both populations acclimated; however, the temperate plants had a markedly reduced capacity to acclimate compared to the desert variety. Given the genetic component, it is feasible that acclimation ability of unstudied species or populations may some day be characterized through the use of genetic markers (Provart et al. 2003).

Although the results are by no means clear cut, a great deal of research has already been done to build a better understand of acclimation responses and given concerns over how plants will react to the projected increases in global temperature (IPCC 2001), interest in this subject will likely grow. The majority of studies mentioned above did not involve the transfer of plants to different growth temperatures (Exceptions: Loveys et al. 2002, 2003, Covey-Crump et al. 2002, Bolstad et al. 2003). Instead species were grown at contrasting ambient temperature and rates of $R$ compared. Although useful for generating information as to which species demonstrate the capability for acclimation, only Type II acclimation is considered using these methods. The primary interest of this study was to determine whether acclimation was a relevant part of temperature response by Coastal Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) fine root $R$ ($\boldsymbol{R_{FR}}$), following abrupt changes in growth temperature. With nursery grown seedlings, a reciprocal transplant experiment was set-up primarily to test for Type I acclimation; however, over the course

of the growth period Type II acclimation would also have been possible. If acclimation did occur, a secondary goal was to determine the rate of respiratory adjustment. Both the potential for warm and cold acclimation were studied through transferring seedlings to a higher or lower growth temperature, respectively.

## 2.2 - Methods

### 2.2.1 - Experimental set-up and pre-transfer growth of seedlings

Preliminary preparations for the experiment began in February 2004. Half-way through March of that year, 300 Douglas-fir seedlings originating from wild-stand collected seed (seedlot #3071, 49'07 124'15, average elevation – 381 m) were received from a representative of Timberwest Forest Corp. The seedlings were grown in the fall of 2003 at the Mount Newton Seed Orchard in Saanichton, B.C. They were hot-lifted in early-January 2004 and had been in -4°C storage at Castle Coolers in Duncan, B.C. prior to their delivery to Vancouver, B.C. Upon their arrival they were thawed for 2 weeks in a 4°C cold room at the Forest Sciences Centre, UBC.

During this time four aeroponic mist chambers **(AMCs)** were placed into a walk-in growth chamber (Conviron CMP 3023, Controlled Environments Ltd., Winnipeg, MA, Canada) in the basement of the Forest Sciences Centre. The AMCs were originally constructed in the Botany shop (Faculty of Botany, UBC) with a design adapted from similar chambers used by Hubick et al. (1981). Set-up and use of the AMCs closely followed work done by Budge (1996). A diagram of one AMC is shown in Figure 2.1.

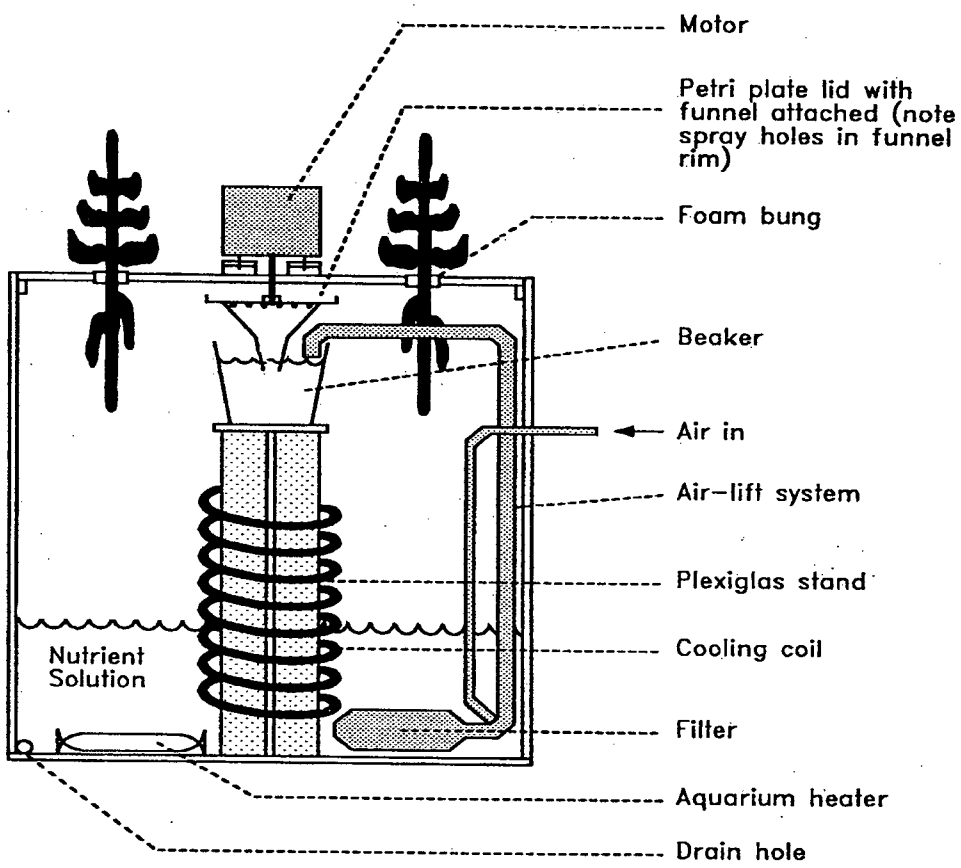


Figure 2.1. Diagram of an aeroponic mist chamber (AMC) (from Budge 1996).

Each AMC was built primarily out of Plexiglas and could house up to 48 seedlings in holes within the top panel. Individual plants were held in place by foam plugs as the root system was left hanging within the internal compartment. During operation, nutrient solution was slowly drawn through a nylon mesh surrounding rubber tubing on the bottom of the chamber, and then upward by pressure created using an Elite 802 115 AC air pump (Rolf C. Hagen, Inc.). Eventually it reached and filled a small plastic beaker that sat on the internal central column. From there the solution was drawn up into a cone fastened onto a plastic Petri dish via the centrifugal action of a rotating Magnetek 115 V / 60 Hz motor (Universal Electric™ Motor). The fluid was then propelled through spray holes cut into the cone, thereby creating a continuous mist inside the chamber. The solution was changed approximately every four days while there were seedlings in the

AMCs. Nutrient concentrations followed Budge (1996) [3.600 g 20N:8P:20K fertilizer, 2.287 g $MgSO_4.7H_2O$, 1.550 g $CaSO_4.H_2O$, 0.300 g $CaCO_3$, 0.180 g $FeSO_4$, and 0.068 g Stem Micronutrients per 18 liters of solution]. Macro and Micronutrients (Plant Products Inc.) were obtained from Westgrow Sales Inc. in Delta, BC.

Following thawing, seedlings were removed from the cold room and rinsed free of their soil. At this point 160 were selected for uniform size and placed in a water bath for 6 days in the laboratory to remove any remaining soil particles. The bath was nutrient free, at room temperature and under lights with a PPFD of ~130 μmol $m^{-2}$ $s^{-1}$, for a 16/8 h photoperiod. After soaking, the seedlings were randomly placed into one of four AMCs within the large growth chamber. Temperature within the AMCs was not controlled at first; therefore both roots and shoots experienced a 25°C day and 18°C night, as established by the growth chamber during a 16/8h photoperiod. PPFD within the chamber was ~300 μmol $m^{-2}$ $s^{-1}$.

From April 6$^{th}$ to June 2$^{nd}$ (~8 weeks) seedlings grew within the AMCs under the above conditions. On June 2$^{nd}$ both the roots and shoots were pruned because they had overgrown during delays in establishing temperature control within the chambers. A few days later temperature control commenced and was working to acceptable standards by June 14$^{th}$. The two temperature levels for the AMCs were chosen as 16 and 24°C in order to represent realistic levels of root growth temperature that this species/provenance might experience (based on soil temperature range and extremes at field sites on Vancouver Island, Ch-3) and to provide a sufficient shift in temperature for the transfer treatments. Each temperature was replicated with two AMCs. Type-T thermocouples (Omega Engineering, Inc.) were placed inside the root zone within the AMCs to monitor for deviations from the set temperature. Set values were maintained via on/off initiation of heating or cooling sources as controlled by DASYlab 8.0 monitoring software (Dasylab Software, National Instruments Inc.). During cooling the control software would send an analog output to an external relay box to activate an AC-2CP-MP centrifugal pump (March MFG Inc.). This pump would then circulate 6°C water from baths underneath the AMCs and through the metal cooling coils that surrounded the internal column (Fig-2.1).

Alternatively, during heating the software would initiate a similar signal to activate 50 W (in 16°C chambers) or 200 W (in 24°C chambers) Sera Precision aquarium heaters situated within the nutrient solution. Once temperature had been brought back across a programmed threshold, another signal was sent to turn off either device. Both the tubing and AMCs were insulated in order to minimize heat absorption from the growth chamber and oscillation during day-to-night, night-to-day temperature shifts.

### 2.2.2 - Initiation of experiment

After over two weeks of steady temperature control, pre-transfer measurements were carried out on June 29$^{th}$ and then again on July 1$^{st}$ and 2$^{nd}$ to characterize $R_{FR}$ at both growth temperatures. Following measurements on July 2$^{nd}$ the experiment was initiated with the transfer of every seedling from its original AMC into one of the other three. Depending on its former temperature and new temperature, individual seedlings became one of four treatments: Controls at 16°C (**C:16**), Controls at 24°C (**C:24**), Growth Temperature Increase from 16 to 24°C (**GTI:16-24**) or Growth Temperature Decrease from 24 to 16°C (**GTD:24-16**). All seedlings were moved in order to equate transfer stress (cf. Norris et al. 2001). When transferring was complete, each of the four AMCs had 20 control seedlings and 20 at a new growth temperature. Seedlings were colour-coded with labels by treatment (Fig-2.2).

Figure 2.2. Picture of the aeroponic mist chambers (AMCs) and seedlings following transfer. From left to right the chambers are at 16, 24, 16 and 24°C.

Post-transfer measurements began on the day after seedling movement and followed every 2-4 days for over two weeks, then every 5-7 days for another week and a half.

### 2.2.3 - Respiration measurements

$R_{FR}$ measurements were done in aqueous phase and represented the biomass-specific rate of oxygen (**$O_2$**) consumption as measured with a Clarke-type $O_2$ electrode (DW1 $O_2$ electrode unit, Hansatech Instruments Inc., Norfolk, England). During sampling a treatment was randomly chosen and actively growing white roots were obtained either by removing a seedling from the back of an AMC or through the door at the front. Roots of 1-2 mm in width were excised and placed into distilled water. These segments had their root caps removed with a razor blade and 4-5 mm long pieces were cut from the 0.1-10 mm region of root length until 15-25 mg of fresh weight (**FW**) was collected. Individual plants were sampled only once during the experiment. During measurement, roots were immersed in 2ml of 20 m*M* 2-(N-morpholino) ethanesulfonic acid (**MES**), pH 6.0,

0.5 m*M* $CaSO_4$ buffer within a 2.5 ml borosilicate reaction chamber, surrounded by a water jacket through which temperature-controlled water was pumped (Hansatech Instruments Inc.) (see App. A for picture). While in the chamber roots lay on a small screen under which a miniature stirring bar actively homogenized $O_2$ levels and disturbed the boundary layer. Prior to each new sample the electrode was calibrated to zero using sodium dithionite and at each measurement temperature (**MT**) an $O_2$ 'air-line' was established through saturation of the buffer with air pumped from a plastic syringe. Calibration curves were created using $O_2$ concentration values for air saturation at MT as calculated with an equation from DW1 $O_2$ electrode unit: Notes for Users (Hansatech Instruments Inc.). During $O_2$ consumption by roots, voltage output from the electrode to a CB1-D3 Electrode Control box (Hansatech Instruments Inc.) was recorded by an AD128 datalogger (Omega Engineering Inc.) for 15-20 minutes. Measurements were done at 10, 16 and 24°C for each sample taken pre-transfer and at 16 and 24°C for samples taken after transfer. As mentioned above, temperature control was established through pumping water around the reaction chamber. The control system included a LC-035 Liquid Cooler (TE technology Inc.) whose output was controlled using a TC-24-25 RS-232 Temperature Controller (TE technology Inc.) via programming with a laptop computer (see Fig-3.2, Ch-3). Water was pumped to the water jacket by a PQ-12-DC pump (Cole-Parmer Instrument co.). On each sampling day treatments were progressively sampled in a random order, until they were all replicated twice. Following measurement, roots were removed from the vessel, blotted dry, put into bags and cooled until they could be taken to the laboratory for determination of FW. $R_{FR}$ rates were calculated using these weights, the raw data from $O_2$ consumption and the calibration curves (example in App. B).

### 2.2.4 - Statistics and $Q_{10}$ calculations

Analyses were done using SAS 9.1.3 programming software (NC, USA). Normality was tested for and equal variance confirmed using the Barlett's procedure. 2-way ANOVAs (Time and Treatment) were carried out for all the post-transfer measurements and separately on data from days 10-38 (Fig-2.6). Calculation of $Q_{10}$ values followed Atkin et al. (2000a).

## 2.3 - Results

The first major finding from the experiment was an unexpected elevation of $R_{FR}$ rates 3-7 days following seedling transfer. This 'transfer effect' was apparent at both MTs and for all treatments. By day 10 rates leveled off and remained at this plateau for the rest of the measurement period (Fig-2.3).

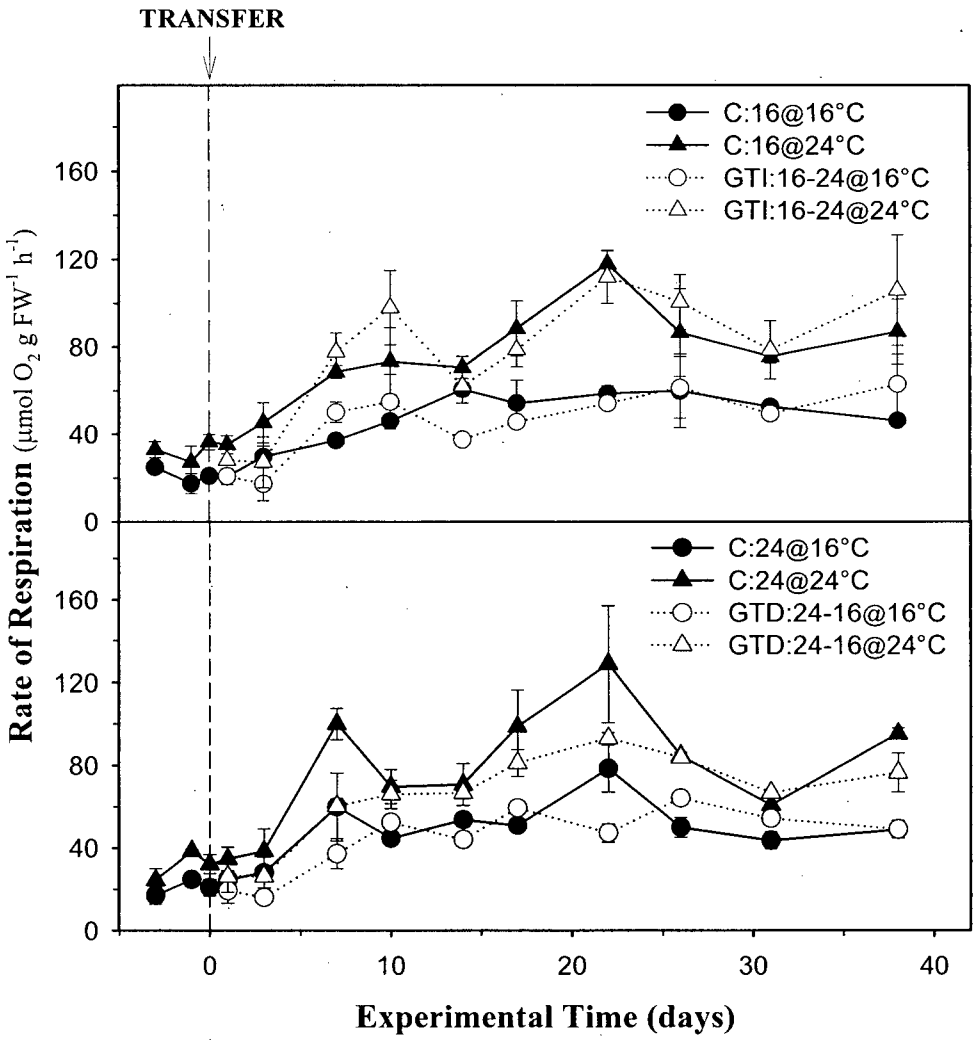


Figure 2.3. Rates of fine root respiration ($R_{FR}$) (n=2) both before and after transfer for C:16 / GTI:16-24 treatments (top panel) and C:24 / GTD:24-16 treatments (bottom). Solid lines show control treatments, dotted lines are temperature change treatments. Symbols designate measurement temperature (MT); 16°C (circles) and 24°C (triangles). Error bars represent standard error of mean.

The transfer effect resulted in 50-200% increases in $R_{FR}$ and these varied both with treatment and MT. Interestingly, the low temperature control treatment (C:16) experienced the smallest increase while its counterpart transfer treatment (GTI:16-24) demonstrated the greatest elevation in rate out of all the treatments and for both MTs (Fig-2.4).

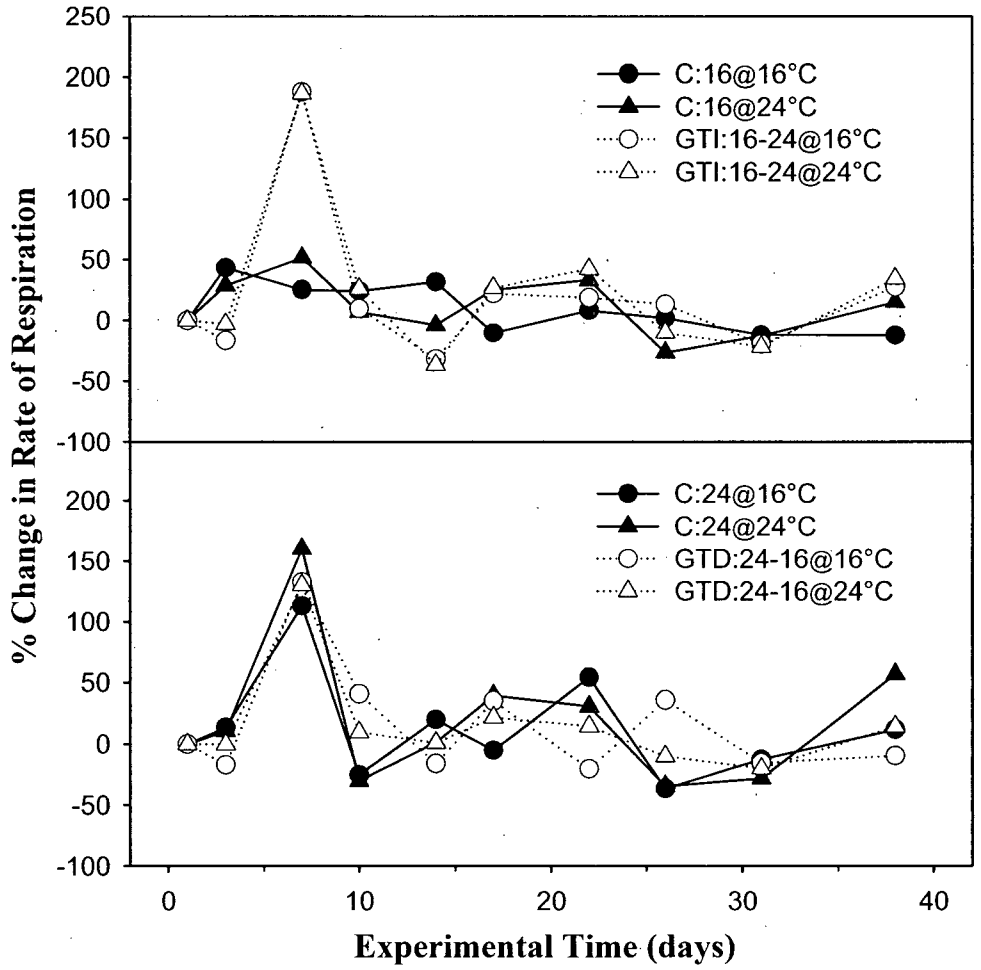


Figure 2.4. Percentage change in rates of fine root respiration ($R_{FR}$) between measurement days. % values represent the change in rate from the previous measurement day to the day where the value is shown. Solid lines show control treatments, dotted lines are temperature change treatments. Symbols designate measurement temperature (MT); 16°C (circles) and 24°C (triangles).

For the remaining post-transfer measurements, $R_{FR}$ oscillated without any systematic deviation from the level established by the transfer effect. The amplitude of this oscillation was between 20 and 50% from one measurement period to the next (Fig-2.4). Despite no apparent upward or downward trend for any of the treatments, a split-plot analysis was done by MT, because they clearly exhibited different rates ($p << .0001$, Fig-2.5), in order to test for differences over time and between treatments (see Table 2.1).

Table 2.1. Results from split-plot analysis on rates of fine root respiration ($R_{FR}$) (n=2) over the 1-38 day post-transfer measurement period. Mean square, F and p values for Treatment, Time and Interaction are shown, by measurement Temperature (MT).

| *MT (°C)* | *Effect* | *Mean Square* | *F Value* | *p* |
|---|---|---|---|---|
| **16** | Treatment (TR) | 57.90 | 2.43 | 0.2051 |
| | Time | 1426.65 | 14.34 | **< .0001** |
| | TR * Time | 135.14 | 1.36 | 0.1934 |
| **24** | Treatment (TR) | 754.01 | 5.80 | **0.0612** |
| | Time | 5052.19 | 20.36 | **< .0001** |
| | TR * Time | 185.58 | 0.75 | .7811 |

Given that this first analysis included all of the post-transfer measurements (with transfer effect) it was not surprising that time had a significant effect for both MTs. Through a Bonferroni's multiple comparison it was confirmed that the transfer effect had led to this result. The treatment effect was nearly significant for rates measured at 24°C; however, this was likely due to a more pronounced oscillation in rates at that temperature rather than an actual trend. Taken together, these results indicate that there was no form of respiratory compensation following seedling movement to the new growth temperatures. To investigate whether the transfer effect could have biased the analyses of post-transfer $R_{FR}$, rates from days 10-38 were pooled and treatments compared in Figure 2.5.

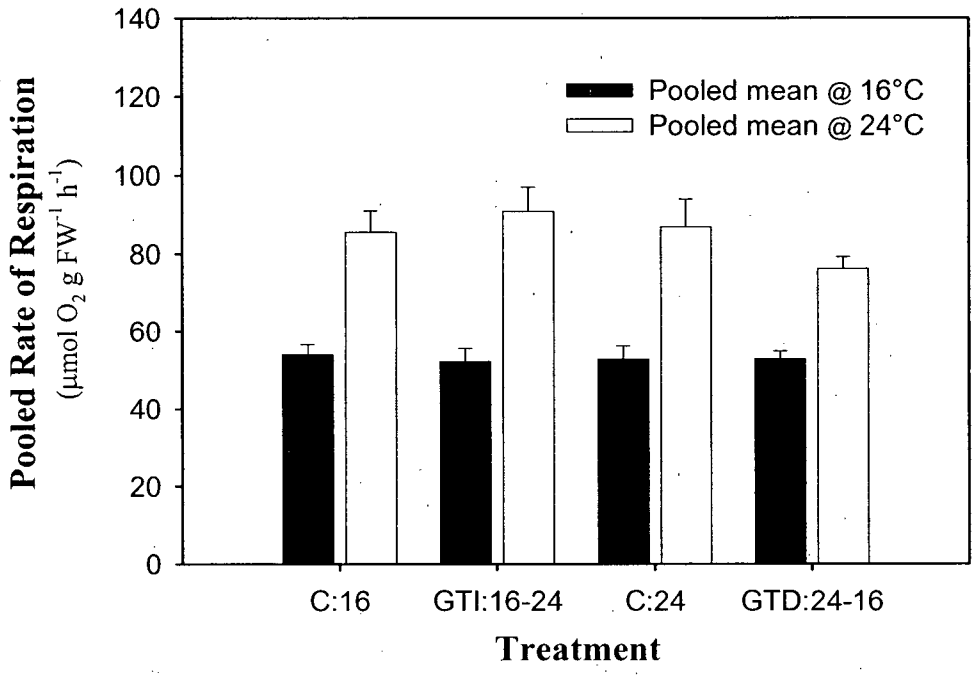


Figure 2.5. Pooled rates of fine root respiration ($R_{FR}$) from days 10-38 following transfer. All treatments are shown for both measurement temperatures (MTs) (16 and 24°C). Error bars represent standard error of mean.

Again we see that there were no apparent differences between treatments, even when the transfer effect and subsequent oscillation of $R_{FR}$ were removed by pooling. One of the most commonly used methods for assessing acclimation is to measure $R$ at a moderate MT for plants that have been grown at or shifted from different temperatures (Gunn and Farrar 1999, Teskey and Will 1999). In the presence of acclimation we would expect that warm-transferred seedlings would respire at a lower rate than cold-grown when measured at moderate temperature and visa versa for a cold-transfer. From Figure 2.5 it's clearly apparent that pooled rates measured at 16°C were not different and although there appears to have been greater variation at 24°C, the trend is not in line with an acclimation response (i.e. the GTD:24-16 treatment has a lower pooled rate instead of higher). An additional ANOVA on rates from days 10-38 (by MT, $p << .0001$) found that there was no significant difference between treatments (see Table 2.2). As in the case of the first

analysis, time had a significant effect for 24°C, likely due to greater oscillation at this temperature.

Table 2.2. Results from split-plot analysis on rates of fine root respiration ($R_{FR}$) (n=2) from days 10-38 of post-transfer measurements. Mean square, F and p values for Treatment, Time and Interaction are shown, by measurement Temperature (MT).

| *MT (°C)* | *Effect* | *Mean Square* | *F Value* | *p* |
|---|---|---|---|---|
| **16** | Treatment (TR) | 57.90 | 2.43 | 0.9631 |
| | Time | 1426.65 | 14.34 | 0.1461 |
| | TR * Time | 135.14 | 1.36 | 0.1069 |
| **24** | Treatment (TR) | 754.01 | 5.80 | 0.2064 |
| | Time | 5052.19 | 20.36 | **0.0005** |
| | TR * Time | 185.58 | 0.75 | 0.8663 |

This pooling exercise further indicates that these Douglas-fir seedlings were either incapable or did not require an acclimation response following the temperature adjustments experienced.

## 2.4 - Discussion

### 2.4.1 - Interpreting the transfer effect

There is no apparent reason for the jump in $R_{FR}$ rate following transfer; however, the most likely explanation is a general stress response to compensate for root damage from handling. Similar increases in basal $R$ have occurred in other growth chamber experiments involving seedling movement (Budge 1996). It is of interest that this increase was transient and that rates did not return to the pre-transfer level. A reduced effect of transfer on the C:16 treatment suggests that the metabolic state of these seedlings was more stable than the others. If we take temperature sensitivity as an indicator of metabolic state, the average $Q_{10}$ values over the experiment [C:16 – 1.81, C:24 – 1.79, C:16-24 – 1.89, C:24-16 – 1.64] do not indicate that seedlings from C:16 were any less responsive to temperature change. This comparison also shows that C:24-16 had the lowest overall sensitivity, a result that is contrary to the widely held belief that plants growing at low temperature usually have higher $Q_{10}$ values (Atkin et al. 2000b, Atkin and Tjoelker 2003).

### 2.4.2 - Absence of an acclimation response in Coastal Douglas-fir fine root respiration

If acclimation had occurred we would expect that rates of $R_{FR}$ for roots measured at a new growth temperature would approach rates for roots measured at the original temperature (i.e. $R_{FR}$ of GTI:16-24 @24°C would approach $R_{FR}$ of C:16 @16°C). It is clear however that besides a few random events, there was no indication of this pattern for either an increase or decrease in growth temperature (Fig-2.6).

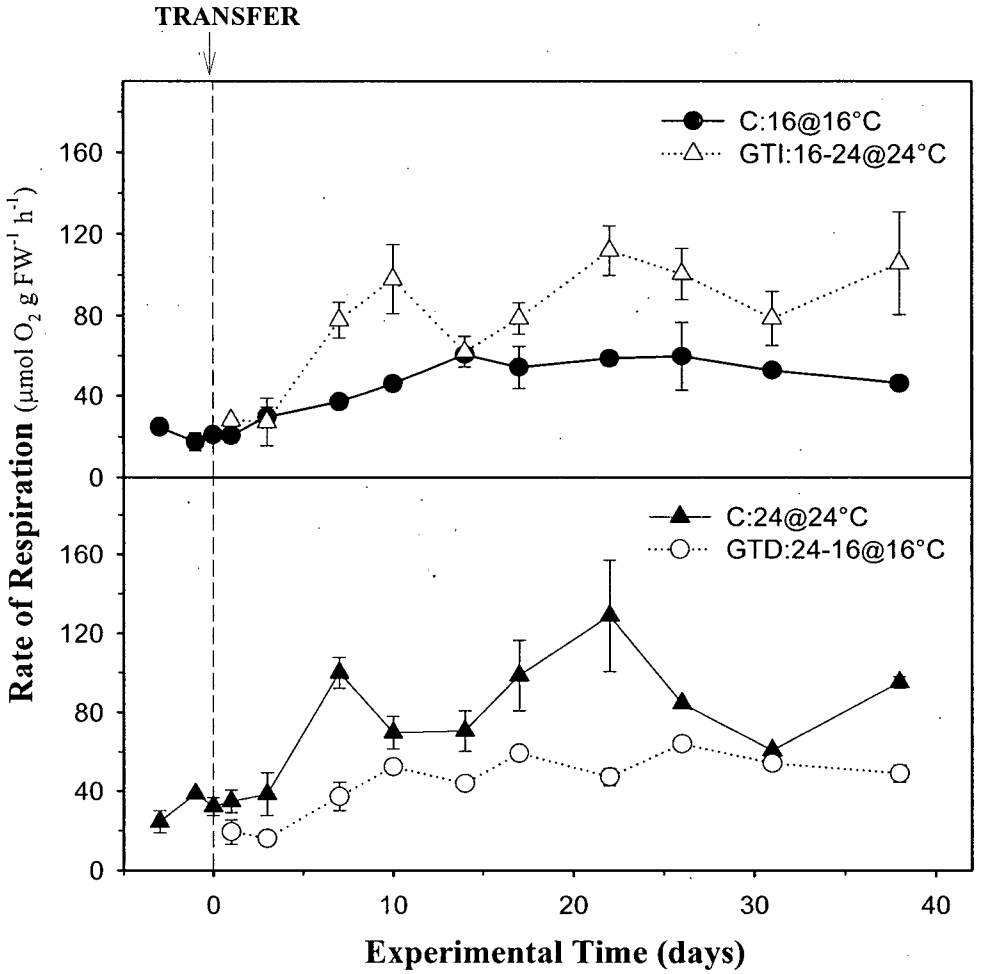


Figure 2.6. Rates of fine root respiration ($R_{FR}$) (n=2) both before and after transfer, for treatments measured at their respective growth temperatures. Both scenarios for potential warm acclimation (top panel) and cold acclimation (bottom) are presented. Solid lines show control treatments, dashed lines are temperature change treatments. Symbols designate measurement temperature (MT); 16°C (circles) and 24°C (triangles). Error bars represent standard error of mean.

Testing for an acclimation response can also be done in a more quantitative manner using ratios. Loveys et al. (2003) presented three methods to quantify temperature acclimation in plants that developed under one temperature and then shifted to another. The most simple of these is the *Homeostasis Method* in which a ratio is calculated between rates of $R_{FR}$ for cold-grown plants versus warm-grown plants measured at growth temperature (i.e. C:16 @16°C / GTI:16-24 @24°C and GTD:24-16 @16°C / C:24 @24°C). This particular method is only useful for demonstrating full acclimation and in a

case of homeostasis values would be equal to or greater than 1.0. Based on this criterion, Figure 2.7 demonstrates again that there was no consistent pattern towards acclimation following the transfer of seedlings to new growth temperatures. The ratios that are > 1 can again be attributed to the random effects of rate oscillation.

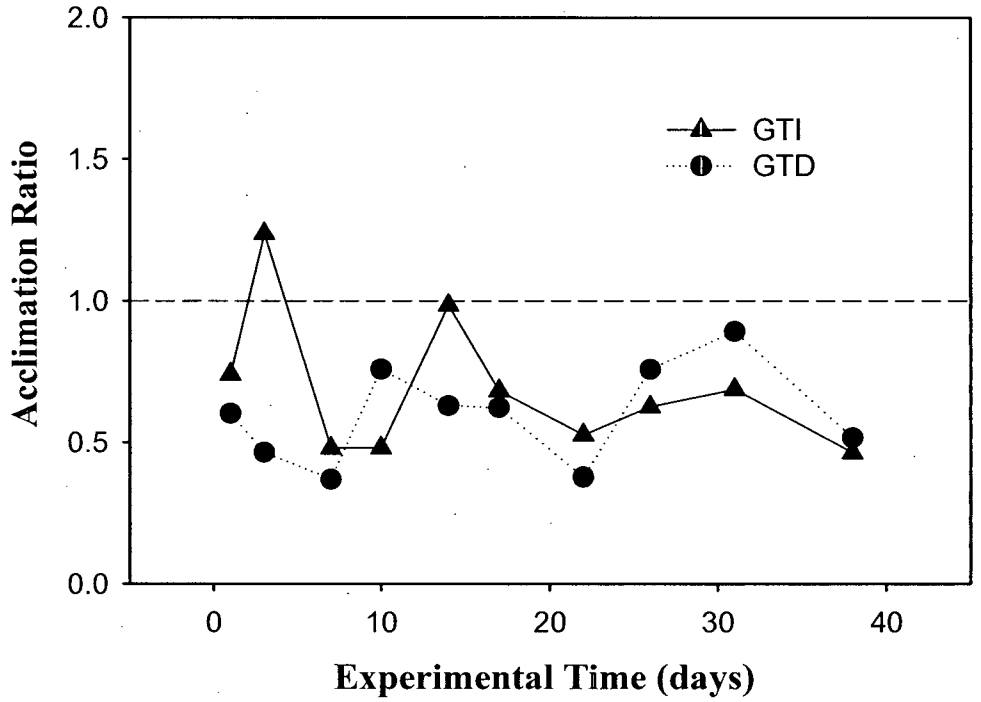


Figure 2.7. Acclimation ratios calculated from rates of fine root respiration ($R_{FR}$) for seedlings that experienced either a Growth Temperature Increase (GTI) or Decrease (GTD). The *Homeostasis Method* from Loveys et al. (2003) was used for calculation of the ratios. If greater than or equal to 1.0 then full acclimation has occurred.

Despite the lack of evidence for an acclimation response during this experiment, Coastal Douglas-fir $R_{FR}$ did acclimate in a study by Budge (1996) following at least four weeks of growth in seedlings at 11, 18 and 25°C. Budge also investigated Interior Douglas-fir which interestingly did not appear to acclimate. In that study, done under very similar conditions to the present work, rates of $R_{FR}$ at all MTs were clearly higher in cold-grown seedlings versus those grown at higher temperatures. Testing seedlings following a growth temperature shift was not part of that project; however, it did provide

evidence that some Coastal Douglas-fir provenances are capable of Type II acclimation. In Budge's study seed from near Squamish, B.C. (seedlot #1276) was used, which suggests that the climatic forces or growth season length in that region selected for *Developmental Plasticity* in roots. Shoot *R* of Coastal Douglas-fir (Sorensen and Ferrel 1973) has also been shown to acclimate to contrasting growth temperatures and root *R* of jack pine and black spruce have been reported to acclimate (Tjoelker et al. 1999). In contrast, no evidence for acclimation of root *R* was found for white spruce following four weeks of growth in water-baths at different temperatures (Weger and Guy 1991), or for roots of Englemann spruce and subalpine fir (Sowell and Spomer 1986).

The lack of acclimation in the current study could be due in part to the variable temperature history experienced by the seedlings. Over the course of the growth period they grew up to three times their original size (visual estimation) and over half of that time was spent developing under a temperature regime set by the growth chamber (i.e. roots and shoots were at 25/18°C day/night). Given this history, it's feasible that the seedlings had become adapted to the initial temperature regime to a degree that they became metabolically fixed, in particular because they had flushed and experienced the majority of their growth before temperature control. However, the roots were growing throughout the experiment, and therefore it is very likely that new tissue would have eventually become part of samples after transfer. In fact by the end of the experiment, the root tips used for measurement had likely all grown during the post-transfer period. It has been proposed that when plants are under a stressful or new temperature, that the development of new tissues is required for Type II (i.e. full homeostasis) acclimation to occur (Atkin et al. 2005). Even by the end of this experiment however, there was still no indication of an acclimation response or in this case *Developmental Plasticity,* even though new tissue had developed under a changed temperature.

Transferring the seedlings would have first provided the opportunity for Type I acclimation, given that the metabolism of pre-grown root tissue had the capability. In seedlings of radiata pine, Type I acclimation was found to occur for both photosynthesis and *R* within two days of their transfer to a new growth temperature (Rook 1969).

Transfer treatments in that study involved moving seedlings grown at 15/10°C to 33/28°C and visa versa. Given the 18°C shift in temperature that those trees acclimated to, it is possible that the temperature levels chosen for treatment in the current study were not sufficiently extreme to require an acclimation response by the seedlings. It has been proposed that for an acclimation response to occur, a temperature threshold must be crossed (Bryla et al. 2001). Considering this possibility, it may be appropriate to apply these results to established stands, as summer soil temperatures in closed canopy forest usually fall between 16 and 24°C; however, for seedling fine roots in a clearcut area, soils temperature extremes of over 30°C may be experienced in the summer (i.e. New stand, Ch-3, data not shown), a situation which the current work did not cover. Making assertions based on experiments with laboratory seedlings should also be done cautiously (Pregitzer et al. 2000), given that the physiology of young and mature trees growing in the field may operate differently. Due to this fact and the disparity that may have been introduced by the temperature range chosen for growth and transfer, it is difficult to conclude whether roots from this Coastal Douglas-fir population would be incapable of acclimating under extreme temperature conditions in the field. Nevertheless the experiment provided no evidence for either a Type I or II acclimation response under the imposed conditions.

## 2.5 - Conclusions

The reciprocal transplant experiment did not lead to indications of an acclimation response by Coastal Douglas-fir $R_{FR}$, to either an increase or decrease in root growth temperature. Interpreting seedling response was in part complicated by the presence of a transfer effect following seedling movement; however, the increase in rates that occurred was transient. Despite a lengthy period at growth chamber temperature, the seedlings did grow new fine roots under the different temperature conditions and therefore had the opportunity to exhibit a Type II acclimation response. No evidence of such a response was found, however, and no apparent Type I acclimation followed transfer. The shift in temperature presented a reasonable coverage of conditions that occur in the field; however, it is possible that a temperature threshold for acclimation was not crossed. Regardless, under the range tested, there were no signs of even partial acclimation and this is contrary to results from a study that used seedlings from a Squamish Douglas-fir provenance. This suggests that there may be variability among populations in the capability to acclimate to changing temperature conditions. Further study using multiple provenances from different climatic zones along the coast, would help to characterize the extent of this variability.

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# 3. The temperature response of Coastal Douglas-fir fine root respiration across a three-site chronosequence: evidence of inter-annual variability in phenology

## 3.1 - Introduction

### 3.1.1 - The carbon balance and soil respiration

Due to projected changes in global climate, there has been a steady increase in scientific interest and activity pertaining to the flows of carbon (**C**) within the biosphere. Eddy covariance flux towers and their associated research components have recently become a valuable means of support for the development of regional C budgets through continuous, multi-year measurement of micrometeorological and biological fluxes in terrestrial ecosystems. Interpretation of whether the flux area (footprint) under study acts as a net source or sink of carbon dioxide ($\mathbf{CO_2}$) to the atmosphere begins with the balance between gross ecosystem productivity (**GEP**) and ecosystem respiration (**ER**), which will lead to a net C sink (+ net ecosystem productivity (**NEP**)) if the former is greater, or a net C source (– NEP) if the latter is in excess. In forests, ER commonly has greater inter-annual variability (**IAV**) than GEP and as a result is usually the determinant of whether C is sequestered or lost (Valentini et al. 2000, Morgenstern et al. 2004). ER is the sum of total aboveground respiration (***R***) (including efflux from leaves, stems, boles and epiphytic plants) and soil respiration ($\boldsymbol{R_S}$), the latter of which can account for 60-80% ER (Davidson et al. 1998). Globally $CO_2$ efflux from soils corresponds to up to 80% of mean annual GEP (Janssens et al. 2001), a trend which is indicative of the inseparable link between above and belowground processes.

The advent of continuous data sets for $R_S$ through the use of automated chambers has led to an improved ability to correlate short-term variations in $R_S$ with litter moisture, soil and air temperature and patterns of plant phenology (Trumbore 2006). Despite this range of potential influences, $R_S$ is often still represented in models with static Arrhenius $Q_{10}$

functions utilizing temperature as the only predictive variable (Körner 1995, Davidson et al. 2006a). A $Q_{10}$ value describes the sensitivity of $R$ to temperature change and can be used to predict the proportional change in $R$ for a 10°C change in temperature. Of the $Q_{10}$ expressions that have been proposed (reviewed in Fang and Moncrieff (2001)), the Arrhenius derived exponential functions (Arrhenius 1889) appear to best-fit empirical data (although $R$ may be underestimated at lower temperatures). Short-term $Q_{10}$ values typically fall within the range of 1.1-2.9 (Lambers et al. 2002), but are usually assumed to be near 2.0 (Atkin et al. 2000). They are also known to depend on respiratory source, over time, and the range of measurement temperatures (**MT**s) used for developing them (Atkin et al. 2000, Curiel Yuste et al. 2003). Given the complexity of the soil community and the processes that interlink them, simplifiers like the $Q_{10}$ concept are attractive from a modeling standpoint (Davidson et al. 1998). It is now common to see this descriptor applied over longer time scales (i.e. seasonally to yearly), under the assumption that the phenology of temperature response and the resulting cumulative flux can be accurately generalized (Curiel Yuste et al. 2004, Davidson et al. 2006a). When modeling $R_S$ over time however, projections are usually more accurate if parameters fluctuate as opposed to being static (Tjoelker et al. 2001, Curiel Yuste et al. 2003, Wythers et al. 2005), a fact confirmed by Lloyd and Taylor (1994) and Fang and Moncrieff (2001) through testing the predictive ability of different $Q_{10}$ functions. If $Q_{10}$ relationships are built only on temperature, they omit the possibility that other variables such as soil water content (**SWC**) play a role in determining rates. One of the most commonly cited drawbacks of $Q_{10}$ is that during times of drought or flooding (Davidson et al. 1998), correlation of $R$ to temperature is significantly reduced. Given these uncertainties a cautionary approach should be taken if generalizing the response of $R$ to temperature change through the use of fixed and/or long-term $Q_{10}$ relationships.

### 3.1.2 - Components of soil respiration

Sources of soil $CO_2$ efflux are commonly lumped into two categories: 1) Rhizosphere respiration ($\boldsymbol{R_R}$), which includes efflux from live plant roots in the soil matrix and their associated microbial and mycorrhizal communities; and 2) Heterotrophic

respiration ($R_H$), which is the sum of metabolic $CO_2$ primarily from decomposer organisms (+ parasites and fauna) involved in the breakdown of litter, dead roots and other more recalcitrant forms of C. Various methods have been devised to partition these components, in order to understand their relative contributions to $R_S$ over time and under different conditions (Hanson et al. 2000). These can be grouped based on the degree of disruption to the soil system (with potentially associated artifacts) into component integration and trenching (Ewel et al. 1987, Boone et al. 1998, Gaumont-Guay 2005), which are considered to be more intrusive than girdling (Högberg et al. 2001, Scott-Denton et al. 2006) and isotopic methods (Rochette et al. 1999, Cisneros-Dozal et al. 2006). Despite this disparity in disturbance to soil physical properties, all of the above methods have their own sources of error contribution and financial or practical drawbacks. From the plethora of partitioning studies that have been done, the relative contribution of $R_R$ to $R_S$ has been found to vary considerably, as is summarized in various reviews citing ranges from 33-60% of total $CO_2$ efflux (Atkin et al. 2000), to 30-90% (Widén and Majdi 2001) and 10-90% (Hanson et al. 2000). This wide variability in the reported contribution of $R_R$ can be largely explained by vegetation type (Raich and Tufekcioglu 2000), but may result in part from methodological differences (Hanson et al. 2000). The relative contribution of $R_R$ has also been found to vary on yearly, seasonal (Trumbore 2006), and diurnal time scales (Lipp and Anderson 2003, Misson et al. 2006), in a manner that is likely site-specific (Trumbore 2006).

It has been suggested that $R_R$ and $R_H$ may also be differentially controlled by abiotic and biotic factors (Lee et al. 2003, Scott-Denton et al. 2006). $Q_{10}$ values have been found to differ in some studies with the rhizosphere contribution having a greater sensitivity to temperature (Kirschbaum 1995, Boone et al. 1998); findings that may be explained by increases in root exudation at higher temperatures (Boone et al. 1998). However, higher sensitivity of $R_R$ may simply be an artifact of assuming a seasonal $Q_{10}$. Bhupinderal-Singh et al. (2003) suggested that it is actually the high seasonality of $R_R$ and its link with the pattern of photosynthesis that caused greater $Q_{10}$ values in the above studies, as opposed to any real difference in sensitivity to temperature. This assertion was supported by a relatively insensitive change in efflux from $R_R$, following a 6°C reduction in soil

temperature during the mid-summer peak in $R_S$ (Bhupinderal-Singh et al. 2003). Results from Boone et al. (1998) and others (Epron et al. 2001, Lavigne et al. 2003) do however indicate that models may need to incorporate controls other than temperature, and that these determining factors are not necessarily consistent over space and time.

### 3.1.3 - Environmental and substrate controls over soil respiration

Temperature and moisture are commonly purported to be the primary environmental controls over $R_S$. It has often been shown that $R_S$ decreases as a result of drought (Davidson et al. 1998, Borken et al. 2002, 2006, Saiz et al. 2006), primarily through reduced activity of the heterotrophic component. $R_R$ does not appear to have the same response during drought and is often found to have no respiratory change, even in severely dry soils (Scott-Denton et al. 2006), although some studies have shown otherwise (Gansert 1994, Bryla et al. 1997, 2001). The development of empirical models including both temperature and moisture functions has been shown to explain 90% (Trumbore 2006) and up to 98% (Curiel Yuste et al. 2003) of variation in $R_S$. Efforts to explain the variability of $R_S$ over larger spatial scales using temperature and moisture have not met with as much success. In a comparison of $R_S$ across 31 Ameriflux and CarboEurope sites representing various deciduous, evergreen and mixed sites, Hibbard et al. (2005) found that empirical relationships between $R_S$, temperature and moisture trends were only robust at individual sites, while across sites these relationships broke down. There is a growing pool of evidence that when modeling $R_S$ over larger spatial scales and between forest types other factors such as productivity (Janssens et al. 2001, Davidson et al. 2002, Campbell et al. 2004) or litterfall (Raich and Nadelhoffer 1989, Raich and Tufekcioglu 2000, Davidson et al. 2002) may become more important. This supports the idea that photosynthetic substrate is a limiting factor of $R$ in soils (Bond-Lamberty et al. 2004a). In roots, high $Q_{10}$ values may result from a combination of the direct effects of temperature on metabolism and the indirect influence of the seasonality displayed by photosynthesis and belowground allocation (Davidson et al. 1998). Following the flow of C through the rhizospheric and inevitably heterotrophic pathways is still difficult, however, as estimates of fine root biomass are sparse (Norby and Jackson 2000,

Hendricks et al. 2006) and the partitioning of C allocation to $R_R$ versus structural root growth remains poorly understood (Trumbore 2006).

### 3.1.4 - Seasonal root dynamics and stand age

Despite the imbalance of information on belowground versus aboveground plant production, the last decade yielded methodological and theoretical advances that are clarifying the role of belowground C movement. Of the methods used to estimate the production and turnover of fine root biomass, the recent increase in the employment of minirhizotrons has helped to improve the accuracy of fine root estimations (Hendrick and Pregitzer 1996, Johnson et al. 2001, Hendricks et al. 2006). A recent comparison of all current methods by Hendricks et al. (2006), found that assessing belowground production with minirhizotrons was far more accurate than either the sequential or in-growth coring methods or through estimation with site nitrogen (**N**) budgets. It has long been recognized that these latter methods have high sampling error and often underestimate production (Kurz and Kimmins 1987, Publicover and Vogt 1993). Future studies may require the application of a number of the above techniques to cross-check findings and increase the accuracy of root biomass estimations (Clark et al. 2001).

Improved assessment of root growth dynamics is crucial for understanding their influence on C cycling, given that as much as 33% of annual global net primary productivity (**NPP**) goes into the production of ephemeral fine roots (Jackson et al. 1997); while they typically represent less than 2% of total ecosystem biomass (Vogt et al. 1996). Approximately 1/3 of belowground allocation is believed to go towards structural root growth (Nadelhoffer and Raich 1989, 1992, Högberg et al. 2002), while the remainder is released directly through root *R* (Högberg et al. 2002) and passed into the rhizosphere as exudates (Neumann and Römheld 2001). Patterns of root growth and seasonal root *R* appear to be strongly linked with photosynthesis (Pregitzer 2003, Saiz et al. 2006). This is demonstrated in the spring growth pattern of boreal and temperate forests through an increase in root *R* with a coinciding flush of fine root biomass. Following this initial rise there is usually a rapid or gradual peak in activity sometime

during the summer (Epron et al. 2001, Widén and Majdi 2001, Misson et al. 2006). Reduction in $R_H$ due to summer drought can coincide with this peak in root activity resulting in $R_R$ becoming the predominant source for $CO_2$ flux from soils (Thierron and Laudelout 1996, Lee et al. 2003). Root growth and respiratory phenology will of course depend on the biome, climate and stand under study. Different forests invariably have dissimilar soil temperature and moisture thresholds which are considered to influence the timing of root growth with other phenological processes such as the expansion of spring foliage and the rise of photosynthesis (Misson et al. 2006). $R_S$ has been shown to vary with the age and structure of stands due to influences of tree age on fine root production and the quality of substrates being added to soils (Saiz et al. 2006). It is generally accepted that following clearcut harvesting and planting, an establishing stand will go through a period of rapid root growth, which eventually peaks and falls to a steady state during maturity (Claus and George 2005). Both an elevated rate of root production and the higher overall specific $R$ rates of younger plants (Lambers et al. 2002) may help explain why new stands initially act as a net source of $CO_2$ (Humphreys et al. 2005). New stands have also been found to exhibit higher $R_S$ (Law et al. 1999, Saiz et al. 2006), which typically decreases with stand age and stabilizes in mature forests.

Clearly projections of ecosystem response to future scenarios of climate change will need to incorporate the role of stand age (Irvine and Law 2002, Claus and George 2005) and associated fine root dynamics. Not only have patterns of $R_S$, nutrient cycling and C flux been found to change with stand age, but the physiological activity of plants changes throughout maturation. It has been suggested that acclimation of root $R$ or the other contributors to $R_S$ could complicate predictions of C flux (Atkin et al. 2000, 2005, Atkin and Tjoelker 2003). Some forest tree species have been found to acclimate (Tjoelker et al. 1999a), while others have not (e.g. Burton and Pregitzer 2003, reviewed in Ch-2). The central goal of the present study was to investigate the seasonal pattern of biomass-specific fine root $R$ ($\boldsymbol{R_{FR}}$) in Coastal Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco), across a three-site chronosequence. These sites are part of the B.C.-Fluxnet station, which is one of seven, established in a latitudinal transect across Canada by the Fluxnet-Canada Research Network (**FCRN**) (www.fluxnet-canada.com). Along with interpreting the

environmental and biological controls over the variability of $R_{FR}$ between sites and over time, a secondary objective was to look for evidence of acclimation response.

### 3.2 - Methods

The FCRN sites used for this work are all located on the central-eastern coast of Vancouver Island, B.C., within the driest subzone of the Coastal Western-Hemlock zone, under the Biogeoclimatic Classification System of British Columbia. Mean annual temperature and precipitation for the area is 9.5°C and 1500 mm. During the spring of 2003 four 8m by 8m measurement plots were established at each of the three sites. Shortly thereafter, type-T thermocouples (Omega Scientific Inc.) were installed to a depth of 5 cm in all plot corners, to obtain average soil temperatures. These values were continuously recorded with CR-10X dataloggers (Campbell Scientific Inc.). Plots were established within the tower footprint, in areas having a least five trees.

#### 3.2.1 - Site descriptions

The study areas are on private forest property (Timberwest Forest Corp.) between Nanaimo and Campbell River, B.C. For the purpose of this paper sites will be considered as a **Mature** stand (55-y-old), **Young** stand (17-y-old) and a **New** stand (5-y-old). The FCRN designations are IDF, DF88 and HDF00 (I = Intermediate, H = Harvested, DF = Douglas-fir), respectively. The Mature (49°52.137'N, 125°20.120'W) and New stands (49°52.330'N, 125°17.537'W) are found within 5 km of each other, ~15 km south of Campbell River, B.C. The Young stand (49°31.180'N, 124°54.105' W) is located ~50 km south of the others (Fig-3.1). Slight differences in elevation exist between the three sites; Mature-300 m, Young-200 m and New-180 m.

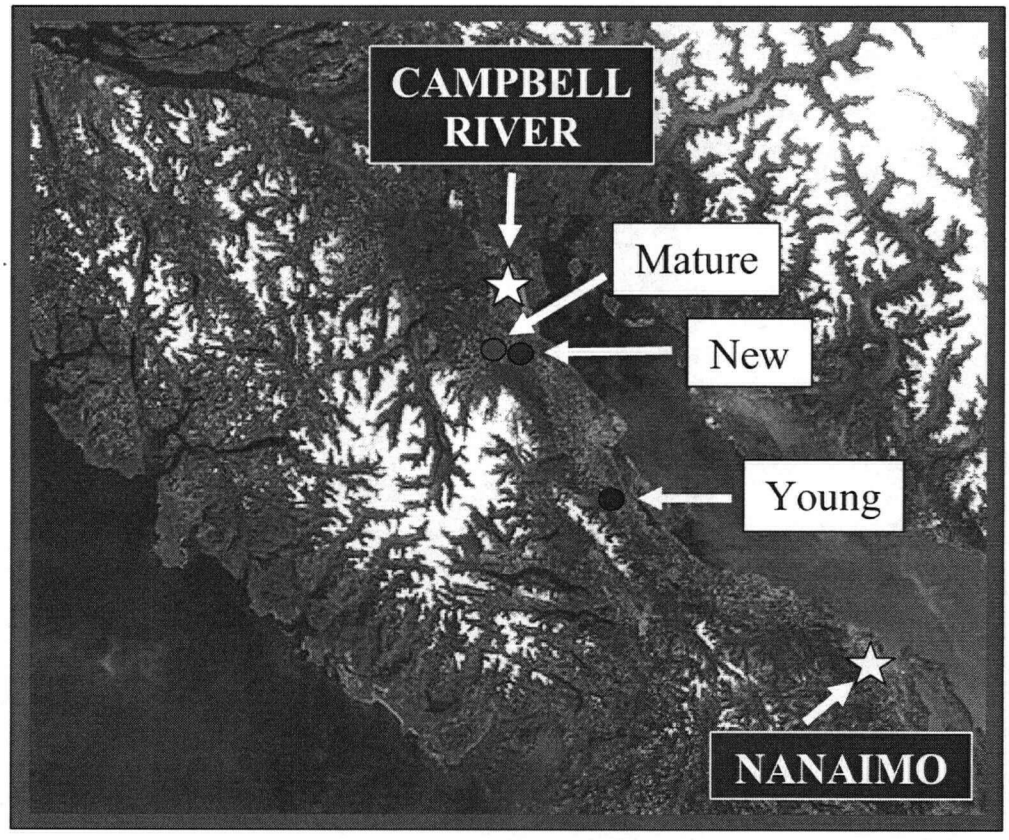


Figure 3.1. Location of study sites on Vancouver Island, B.C., Canada.

All of these stands are dominated by Douglas-fir, which at the time of measurement represented > 80% of trees at the Mature stand and > 97% of Young and New stands. Coastal western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western red cedar (*Thuja plicata* Donn ex D. Don) make up the majority of other trees present. All stands were initiated by planting, following clearcut harvesting and broadcast or pile burning. Soils are humo-ferric podzols with a gravelly loam texture at the Mature and New stands and loam at the Young stand. The Mature stand has well developed horizons, while soil at the Young stand is partially developed and moderately compacted. At the New stand the soil is still in the early stages of developmental and the forest floor is littered with significant slash. For further site information the reader is referred to Humphreys (2004).

$R_{FR}$ measurements were undertaken during seven excursions to Vancouver Island from May to August 2004 and on three follow-up trips during the 2005 growing season

(Table 3.1). On all trips each site was visited for one day, beginning with the Young stand and followed by the Mature and then New stands.

Table 3.1. Measurement periods in 2004 and 2005. Excursions were three days in length, one day per site. The Young stand was always visited first followed by the Mature and New stands.

| Year | Spring | Spring | Summer | Summer | Summer | Summer | Summer |
|---|---|---|---|---|---|---|---|
| **2004** | May 25-27 | June 08-10 | June 21-23 | July 06-08 | July 20-22 | August 03-05 | August 17-19 |
| **2005** | --- | June 07-09 | --- | --- | July 19-21 | --- | Aug 23-25 |

### 3.2.2 - Root sampling and measurement

Over the course of a measurement day the four plots were sampled for fine, white roots only (coarse, mycorrhizal and suberized roots were avoided), from between the 5 and 15 cm soil depths. At the Mature and Young stands, sampling involved randomly turning over a ~400 $cm^2$ block of soil with a shovel. Fine roots were then excised from seminal roots when emerging from the same primary root. If the first random sampling area did not yield enough tissue, a new area and primary root was sampled. At the New stand, individual trees were sampled by uncovering primary roots emerging from the root ball. Roots of Douglas-fir were distinguished by a striated periderm that peeled easily, pink to red phloem and fine roots that were typically > 1 mm in width and radiated from small bundles. Clean white to partially suberized roots were excised above the region of interest, put into a beaker with distilled water and immediately taken to a vehicle housing instruments. Prior to measurement, roots were cut with a razor blade to isolate the white to partially suberized regions which were accepted when 2-4 mm long and 0.5-2.5 mm thick. After final preparation ~8-12 mg of fresh weight (**FW**) root material was used.

*R* measurements were done in aqueous phase and represented the biomass-specific rate of oxygen ($\mathbf{O_2}$) consumption, as measured by a Clarke-Type Hansatech $O_2$ electrode

(DW1 $O_2$ electrode unit, Hansatech Instruments Inc., Norfolk, England). Each root sample was used for a maximum of four measurements at different temperatures. Measurements involved sealing the roots within a 2.5 ml borosilicate reaction chamber that was surrounded by a water jacket, through which temperature-controlled water was pumped (see App. A for picture). Roots respired while fully saturated in 2 ml of 20 m*M* 2-(N-morpholino) ethanesulfonic acid (**MES**), pH 6.0, 0.5 m*M* $CaSO_4$ buffer, as they lay on a small screen under which a miniature stirring bar actively homogenized $O_2$ levels and disturbed the boundary layer. $O_2$ consumption by roots reduced the voltage output from the electrode to a CB1-D3 Electrode Control box (Hansatech Instruments Inc.) which was recorded for ~15-20 minutes by an AD128 datalogger (Omega Engineering Sci.). Prior to measurement of new samples, the electrode was calibrated to zero using sodium dithionite, and at each MT an $O_2$ 'air-line' was established through saturation of the buffer with air pumped from a plastic syringe. The zero and airline values were used to calibrate voltage output by the electrode to $O_2$ concentration values which were calculated with an equation from DW1 $O_2$ electrode unit: Notes for Users (Hansatech Instruments Inc.), for air saturation at the different MTs.

The temperatures used for measurement were 10, 16, 22 and 28°C during most trips; except for in the first two measurement periods during 2004 when 8, 15, 22 and 28°C were used. The original temperature scheme was changed due to instrument limitations when ambient temperature was high. The temperature control system was housed in a portable box and was made up of a LC-035 Liquid Cooler (TE Technology Inc.) whose output was controlled using a TC-24-25 S232 Temperature Controller (TE Technology Inc.) via programming with a laptop computer. Water was pumped outside of the box to the water jacket by a PQ-12-DC pump (Cole-Parmer Instrument co.) (Fig-3.2).

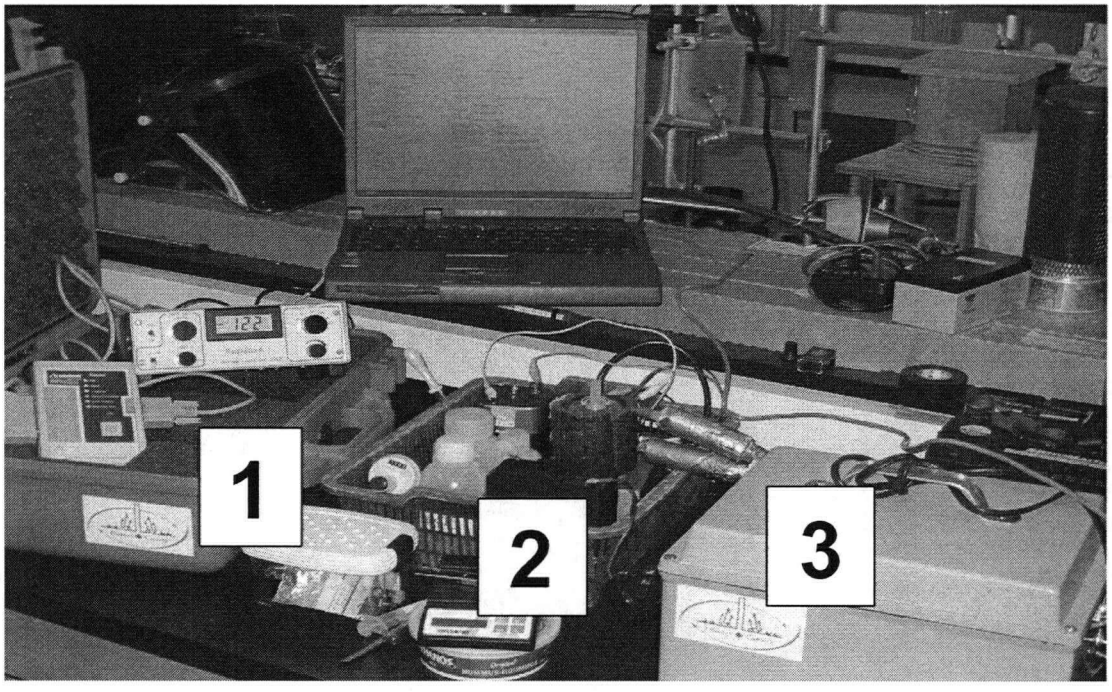


Figure 3.2. Portable respiration ($R$) apparatus. Numbers represent: 1) Datalogger for voltage data; 2) Water-jacketed root cuvette with electrode; 3) Temperature control box with computer-operated Peltier thermoelectric module and circulating pump.

Total time from sampling to the end of the last measurement amounted to a maximum of three hours. Tests in the laboratory using seedlings confirmed that $R_{FR}$ in Douglas-fir remains consistently active for this period following excision. Following the final measurement, roots were blotted dry, put into tinfoil and onto dry ice before they could be freeze-dried and weighed at UBC. These dry weights (**DW**s) were used in conjunction with the calibration curves and raw $O_2$ consumption data for calculating biomass-specific rates of $O_2$ consumption (example in App. B). Mean rates (typically n=3-4, a few n=1-2) are presented for each MT, for all measurement days and sites.

### 3.2.3 - Follow-up research

To test for artifacts in the $O_2$-based $R$ method, a laboratory test and drought experiment were performed in the summer and fall of 2005, respectively. In July, 100 hot-lifted seedlings (seedlot #7401) were acquired from the Sylvan Vale nursery in Black Creek, B.C. They were washed free of their soil and placed in a water bath (room

temperature, under PPFD ~130 μmol $m^{-2}$ $s^{-1}$), in a laboratory at UBC. The nutrient mixture in the bath [3.600g 20 N: 8 P: 20 K fertilizer, 2.287g $MgSO_4.7H_2O$, 1.550g $CaSO_4.H_2O$, 0.300g $CaCO_3$, 0.180g $FeSO_4$, and 0.068g Stem Micronutrients per 18 litres of solution] was changed weekly. Seedlings were left to adjust to the bath for ~two weeks prior to beginning a test on how *R* varies over root length. Samples were taken from five regions along the length of white, to partially suberized growing roots. Measurements of *R* were done at 22°C for up to five samples per region, from different seedlings.

It was hypothesized that the measured rates of $R_{FR}$ in the field may have been high when trees were under drought conditions, due to an interaction with the aqueous nature of the $O_2$ method. This possibility was pursued through a drought experiment using the remaining seedlings from the test above. Seedlings were transferred from hydroponics into cones with a greenhouse soil mixture. They were left to adjust for two weeks under the same light regime as above and with regular watering. Following this period, half of the seedlings were subjected to a drought lasting four weeks. At the end of weeks 1, 2 and 4, seedlings were sampled and $R_{FR}$ measured at 22°C. Five samples were taken from both controls and seedlings under drought. After a seedling was sampled, shoot water potential was measured using a pressure chamber (Hoskin Scientific Ltd.).

### 3.2.4 - $CO_2$ method for testing the respiratory quotient

A $CO_2$-based method for measuring $R_{FR}$ was developed in the fall of 2005 for testing the respiratory quotient (**RQ**). Before and after running an $O_2$-based measurement, a 0.5 ml sample of buffer (start volume 2.5 ml) was removed and injected into a 12 ml vial containing 0.3 ml 16N $H_2SO_4$, which had been previously filled with $N_2$ gas and sealed with a septum. This method was adapted from Miller et al. (1984) who followed a similar procedure but within a syringe. Heat generated by the buffer addition was dissipated by placing the vial in water at the 22°C MT. When pressure in the vial had returned to normal, it was shaken vigorously to release $CO_2$ into the $N_2$ atmosphere. A 0.5 ml gas sample was then removed and injected into a LICOR-6251 IRGA (Omega Engineering Sci.) using a $N_2$ carrier gas for determination of the $CO_2$ concentration. Rates of $CO_2$

production represented the change in buffer $CO_2$ concentration, per unit biomass, over the 0.25 h period from start to end of an $O_2$-based measurement.

### 3.2.5 - Other data sources and $Q_{10}$ calculations

Data support was provided by the main BC-Fluxnet group (PI – Andy Black). This included volumetric SWC data measured as site average using CS616 water reflectometers (Campbell Scientific Inc.), as well as daily GEP and ER exchange, both of which were calculated from flux tower measurements. Root C and N data were obtained from mass elemental analysis at the Department of Earth and Ocean Science (UBC), for three samples, from the six trips that coincided over the two years (i.e. early-June, late-July, late-August).

$Q_{10}$ values were calculated for each site and day following Atkin et al. (2000). The method involved plotting $\log_{10} R_{FR}$ against MT and using the linear slope (b) in the following equation:

$$Q_{10} = 10^{10\,b}$$

### 3.2.6 - Statistics

All analyses were done using SAS 9.1.3 programming software (NC, USA). For seasonal $R_{FR}$ data, stepwise multiple linear regressions were run by year and MT using a range of independent variables. For 2004, the inverse transformation for all variables was included in the regression as $R_{FR}$ had exponential relationships with most variables in that year. Regressions were run by site and also across sites, using site as an indicator (dummy) variable. ANOVA was employed for analysis of the $Q_{10}$ data to test for differences between sites and over time again as separate analyses for the 2004 and 2005 seasons. To account for the lack of replication of $Q_{10}$ values, the analysis included a 'single degree of freedom for nonadditivity' test to ensure that the interaction between site and time was not significant.

## 3.3 - Results

### 3.3.1 - Phenology and inter-annual variability of Douglas-fir fine root respiration

The seasonal pattern of $R_{FR}$ followed a very different trend over the two growing seasons. This is demonstrated in Figures 3.3a & b for rates measured at 22°C.

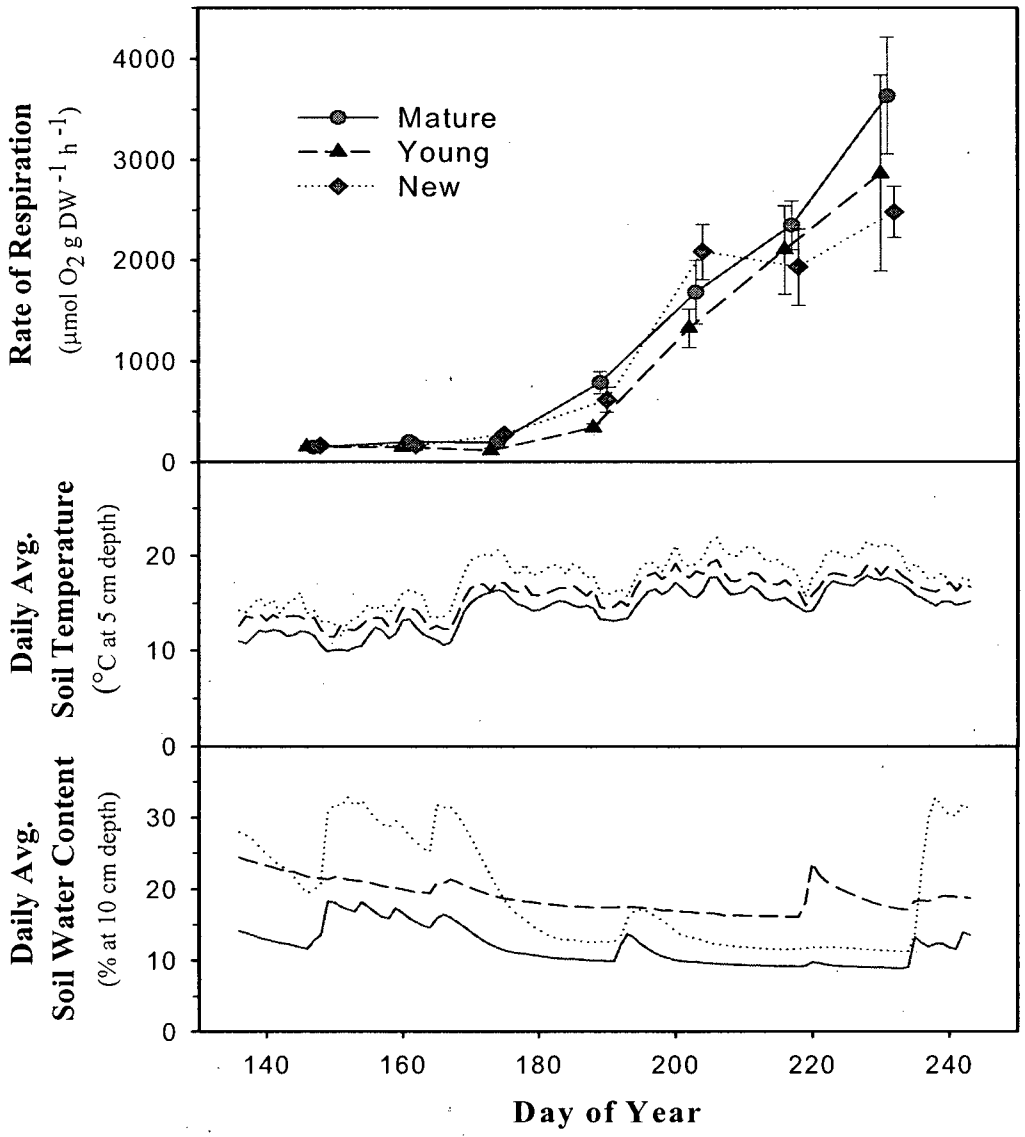


Figure 3.3a. Cross-site comparison of fine root respiration ($R_{FR}$) rates measured at 22°C (top panel), daily (24-h) average soil temperature (middle) and daily (24-h) average soil water content (SWC) (lower), over the 2004 growing season. Error bars represent standard error of mean.

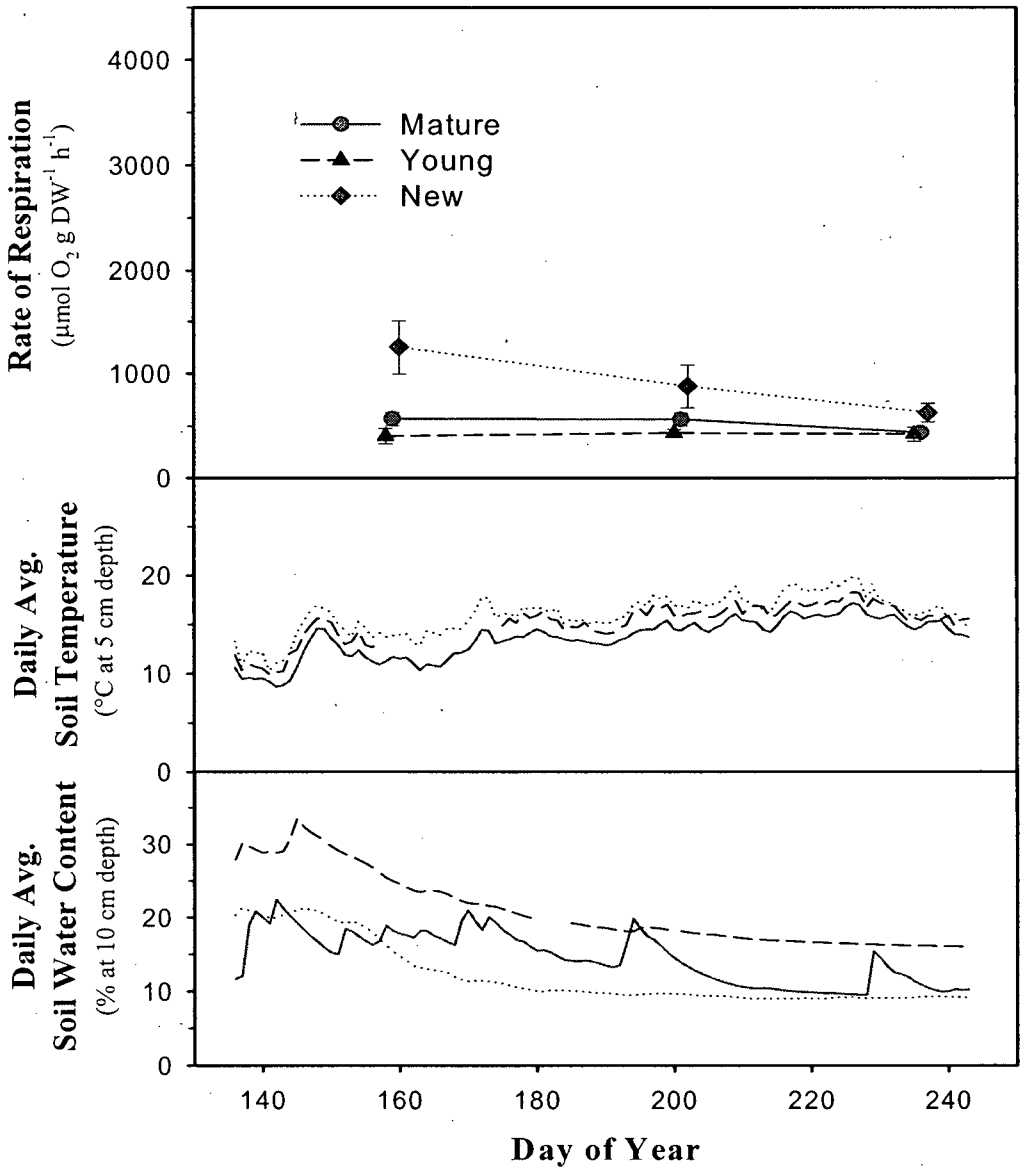


Figure 3.3b. Cross-site comparison of fine root respiration ($R_{FR}$) rates measured at 22°C (top panel), daily (24-h) average soil temperature (middle) and daily (24-h) average soil water content (SWC) (lower), over the 2005 growing season. Error bars represent standard error of mean.

In 2004, rates of $R_{FR}$ were relatively low in the spring but increased dramatically in the latter part of the season at all sites. This cross-site elevation of $R_{FR}$ started just after soil temperatures rose rapidly and as SWC was falling (Fig-3.3a). These three seemingly

coordinated events, took place between days 165-175 or from June 13-23. Interestingly, $R_{FR}$ began to climb first at the New stand, as it also experienced a greater shift in soil temperature and moisture relative to the other sites. The New stand demonstrated a more chaotic change in $R_{FR}$, while rates at the Mature and Young stands paralleled each other throughout the season. By the final measurement period in mid-August, $R_{FR}$ at the Mature and Young stands still appeared to be increasing while at the New stand it leveled off. During the 2005 season, rates of $R_{FR}$ began higher than the previous spring, possibly due to a 'carry-over effect' from the year before (Fig-3.3b). The significant increase in $R_{FR}$ seen in summer 2004 was not repeated in 2005. In contrast, rates remained relatively stable except at the New stand, where they decreased from a higher level to one more similar to the other sites. The leveling of rates at the New stand in 2004 and this decline in 2005, are patterns that could indicate a down-regulating acclimation of $R$ as soils warmed (Fig-3.3b).

The dissimilar phenology of $R_{FR}$ over the two seasons and the independent activity exhibited by the New stand might be explained by weather differences between years. 2004 was characterized by warm spring soil temperatures, which rapidly jumped to even warmer summer temperatures. As a result, all sites appeared to have reached minimum SWC by early-July. In contrast, soils in the spring of 2005 were cool in early May, followed by a rise in temperature similar to that in 2004, but occurring a month earlier. The overall change in temperature was less in 2005 however because following spring warming there was a more gradual elevation in temperature compared to 2004. As a result, the daily (24-h) average soil temperature maximum was reached later, in mid-August at 17.2, 18.3 and 19.8°C for the Mature, Young and New stands respectively. In 2004, the seasonal maximums occurred in late-July at 17.7, 19.5 and 22.0 °C. Changes in SWC in 2005 were also different, as water loss occurred more gradually and only the New stand appeared to reach a minimum. Stepwise multiple regressions by site and for all sites were performed on rates of $R_{FR}$ measured at 16 and 22°C. A variety of environmental and stand level variables were included for potential incorporation into the models. For analyses on 2004 data, the inverse of each variable was also tested (Table 3.2).

Table 3.2. Results from modeling 2004 rates of fine root respiration ($R_{FR}$) using multiple regression. Model results by site and for all sites (with site as an indicator variable) are shown for two different measurement temperatures (MT) (16 and 22°C). Partial $R^2$ values for variables having a significant contribution to the models ($\alpha$=0.05) are indicated.

| Variables | MATURE | | YOUNG | | NEW | | ALL SITES | |
|---|---|---|---|---|---|---|---|---|
| | 16°C | 22°C | 16°C | 22°C | 16°C | 22°C | 16°C | 22°C |
| **1WA* Max. Temp.** | 0.6481 | 0.6517 | --- | --- | --- | --- | --- | --- |
| **1/1WA Max. Temp.** | 0.1410 | 0.1322 | --- | --- | --- | --- | --- | --- |
| **1WA Min. Temp.** | --- | --- | --- | --- | 0.1568 | 0.1383 | 0.5350 | 0.5299 |
| **1/1WA Min. Temp.** | --- | --- | --- | --- | 0.0407 | --- | 0.0752 | 0.0758 |
| **1WA Avg. Temp.** | --- | --- | --- | 0.4280 | --- | --- | --- | --- |
| **% SWC†** | --- | --- | --- | --- | --- | --- | --- | 0.0169 |
| **1/1WA % SWC** | --- | --- | --- | --- | --- | --- | --- | 0.0384 |
| **GEP ‡** | --- | --- | --- | --- | --- | --- | --- | 0.0176 |
| **1/GEP** | --- | --- | --- | --- | 0.6548 | 0.7233 | --- | --- |
| **1/1WA GEP** | --- | --- | 0.5344 | --- | --- | --- | 0.0282 | 0.0299 |
| **1WA ER ‡** | --- | --- | 0.1809 | --- | --- | --- | --- | --- |
| **1/ER** | --- | --- | --- | --- | --- | --- | 0.0237 | 0.0157 |
| **New x Min. Temp.** | --- | --- | --- | --- | --- | --- | 0.0426 | --- |
| **New x 1/1WA % SWC** | --- | --- | --- | --- | --- | --- | --- | 0.0311 |
| **MODEL $R^2$** | 0.7890 | 0.7839 | 0.7153 | 0.4280 | 0.8524 | 0.8616 | 0.7047 | 0.7385 |

* One week average of data for a variable (**1WA**)
† Soil water content (**SWC**)
‡ Gross primary production (**GEP**) and ecosystem respiration (**ER**) in g C $m^{-2}$ $day^{-1}$
--- Signifies lack of input in model

As is apparent in Table 3.2, a significant amount of the variation in seasonal $R_{FR}$ was explained by the model variables (i.e. all models but one had $R^2$ values > 0.70), although those that were included changed from site to site. Usually at least one temperature variable was significant (exception – Young stand @16°C) and 1/ GEP was important, particularly in the two younger stands. The all-sites model predicted a strong relationship between $R_{FR}$ and one week average (**1WA**) Min. temperature, regardless of stand age. Overall, the results support the presence of an exponential phenology in this year, for both the trend of $R_{FR}$ and for the variables that became part of the models. This autocorrelation complicates the interpretation of these analyses and indicates that phenology may override the importance of variable correlation to $R_{FR}$. Regression models for prediction of $R_{FR}$ were usually similar between the two MTs. The best models for each site are shown below:

Mature (@ 16°C): $R_{FR} = -37072 + 1373.95\ 1WA\ MaxTemp + 250630\ 1/1WA\ MaxTemp$

Young (@ 16°C): $R_{FR} = -5172.86 + 13486\ 1/1WA\ GEP + 574.43\ 1WA\ ER$

New (@ 22°C): $R_{FR} = -3591.78 + 6486.56\ 1/GEP + 231.67\ 1WA\ MinTemp$

The respiratory trend in 2005 was far more static than the previous year and as a result fewer variables had a model contribution (Table 3.3). In this case the site models were not as strong ($R^2 < 0.42$), and for two of the sites @16°C (Mature and Young), there was no model following analysis. ER was the dominant predictive variable for the Mature and New stands, while in the Young stand the 1WA of daily temperature amplitude explained the most variation. The analysis across sites yielded a stronger model ($R^2 > 0.6$), although the only noteworthy result was a significant interaction between the New stand and 1WA of % SWC; indicating that at this stand $R_{FR}$ was influenced by soil moisture, whereas at the others it was not.

Table 3.3. Results from modeling 2005 rates of fine root respiration ($R_{FR}$) using multiple regressions. Model results by site and for all sites (with site as an indicator variable) are shown for two different measurement temperatures (MT) (16 and 22°C). Partial $R^2$ values for variables having a significant contribution to the models ($\alpha$=0.05) are indicated.

| Variables | MATURE | | YOUNG | | NEW | | ALL SITES | |
|---|---|---|---|---|---|---|---|---|
| | 16°C | 22°C | 16°C | 22°C | 16°C | 22°C | 16°C | 22°C |
| **1WA* Daily Temp. Amplitude** | --- | --- | --- | 0.4203 | --- | --- | --- | 0.0708 |
| **ER ‡** | --- | 0.3657 | --- | --- | 0.3719 | 0.3584 | --- | --- |
| **New x 1WA % SWC †** | --- | --- | --- | --- | --- | --- | 0.6017 | 0.5383 |
| **MODEL $R^2$** | No Model | 0.3657 | No Model | 0.4203 | 0.3719 | 0.3584 | 0.6017 | 0.6092 |

* One week average of data for a variable **(1WA)**
† Soil water content **(SWC)**
‡ Ecosystem respiration **(ER)** in g C $m^{-2}$ $day^{-1}$
--- Signifies lack of input in model

The best models for each site are shown below:

Mature: $R_{FR} = 782.08 - 29.41\ ER$ (@ 22°C)

Young: $R_{FR} = 594.96 - 56.83\ ER$ (@ 22°C)

New: $R_{FR} = 2167.44 - 259.59\ ER$ (@ 16°C)

In terms of explaining the differences between the two years, the analyses confirmed that soil temperature and 1/GEP were important factors in 2004 whereas in 2005, $R_{FR}$ followed ER and was influenced by soil moisture at the New stand but not at the others. This lack of continuity for the controlling factors over $R_{FR}$ across the two seasons, suggests that endogenous and phenological factors may be just as important as other controls over $R_{FR}$.

### 3.3.2 - Sensitivity of fine root respiration over two seasons

Temperature response curves for all measurements periods, sites and both years are presented in Figure 3.4. The curves shown are the individual $Q_{10}$ equations plotted as Antilog ($Log_{10} R_{FR} = b_o + b_1 x$).

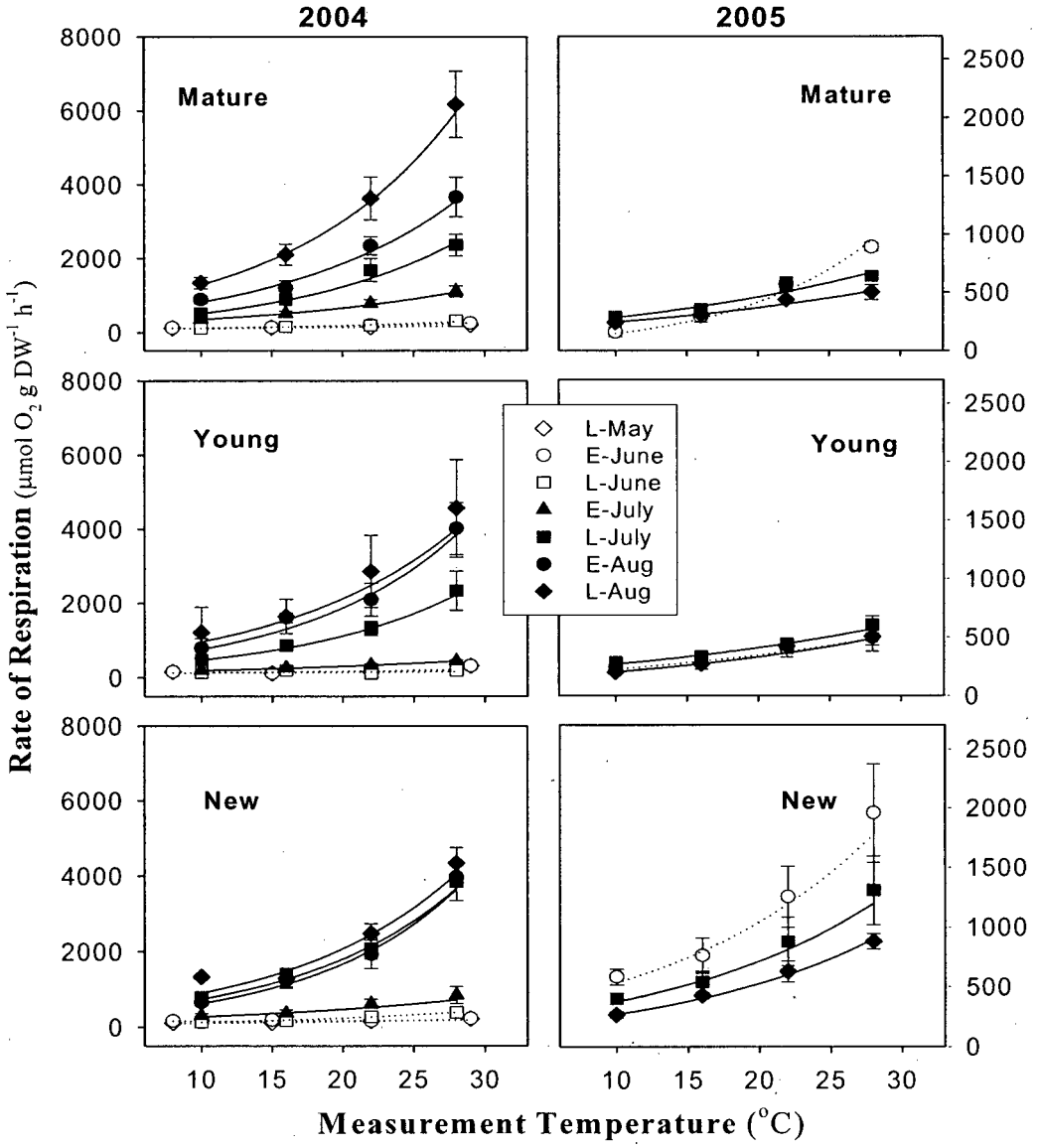


Figure 3.4. Rates of fine root respiration ($R_{FR}$) at different measurement temperatures (MTs). Presented by measurement period for all sites and both seasons. Light symbols and dotted lines represent measurements in May and June, while dark symbols and full lines are for measurement periods in July and August. Error bars represent standard error of mean. *Note scale differences between years*.

It is clear from Figure 3.4 that temperature response followed a different pattern in the two years. In 2004, changes in temperature response over the course of the season were obvious but among sites there was little difference. 2005 exhibited less variation over the season with the exception of rates at the Mature stand, which showed greater sensitivity in the spring compared to summer. As was observed for respiratory capacity in 2004, there was an increase in $Q_{10}$ at all sites over the growing season, indicating a greater sensitivity of $R_{FR}$ to short-term temperature change (Fig-3.5).

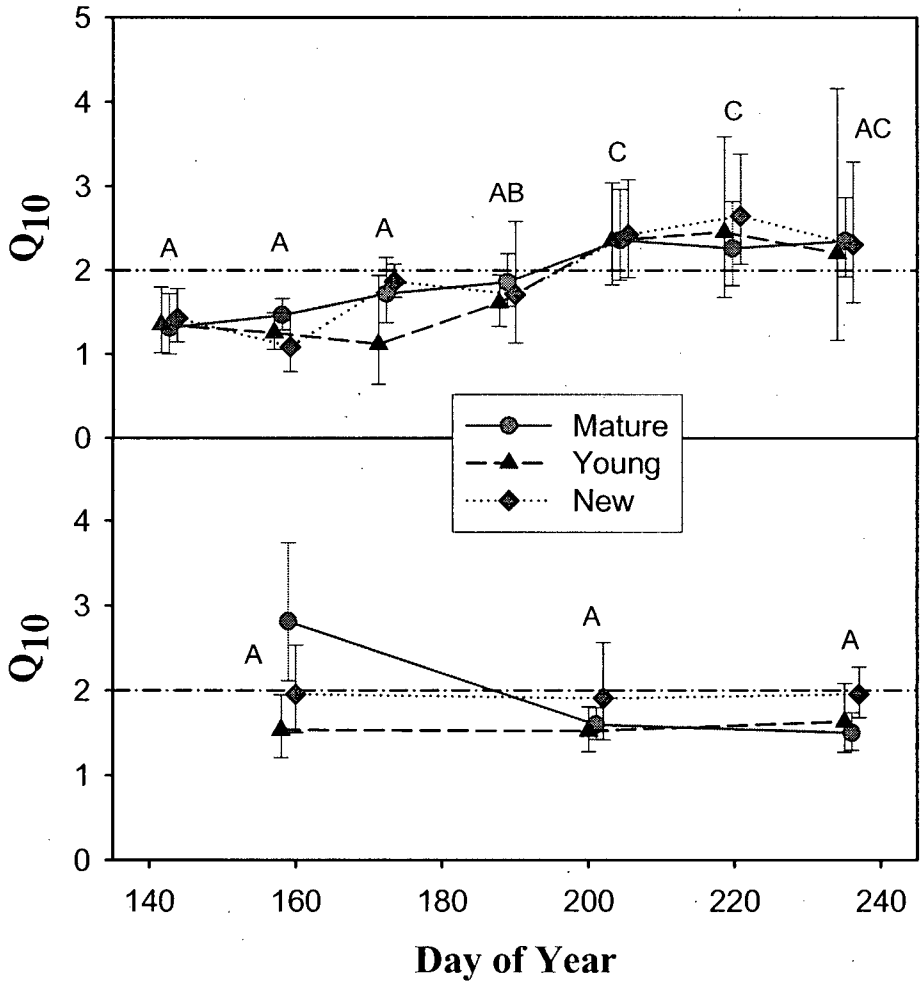


Figure 3.5. Cross-site comparison of $Q_{10}$ values calculated by measurement day. 2004 (top panel), 2005 (bottom). Letters indicate similarity or differences of $Q_{10}$ across sites, during the different measurement periods. Points are individual $Q_{10}$ values, error bars represent 95% confidence interval of value.

In the spring of 2004, $Q_{10}$ values at all sites were significantly less than 2.0 and by summer had increased to a level nearly greater than 2.0, although this was not significant (i.e. confidence intervals do not and do cross the reference line, respectively, Fig-3.5). A 2-way ANOVA found summer $Q_{10}$ values to be different than those in spring to early summer. The same analysis did not show any difference in $Q_{10}$ between sites, at any point during the season. Sensitivity of $R_{FR}$ was more linear in 2005 and, in that year, $Q_{10}$ values were significantly different from 2.0 at the Mature and Young stands but not at the New stand. A 2-way ANOVA for 2005 found no significant difference between sites or over time.

### 3.3.3 - Results from laboratory tests and quotient work

To support the $R_{FR}$ field results, laboratory studies were carried out to: 1) investigate the variability of $R_{FR}$ over root length; 2) test for an interaction between the aqueous nature of the $O_2$ method and $R_{FR}$ of roots that have been under drought; and 3) compare $R_{FR}$ measured with an aqueous $CO_2$ method to rates measured by the $O_2$ method (i.e. RQ). In the summer of 2005, white primary roots from Douglas-fir seedlings growing in hydroponic solution, were sampled at different regions along their length for measurement. These results are considered applicable to 'fine roots' due to other tests (data not shown), that indicated no difference between $R$ of small white laterals (typically considered the fine portion) and the white primary. Mean rates of $R$ at these different root zones (0-45 mm) are shown in Figure 3.6.

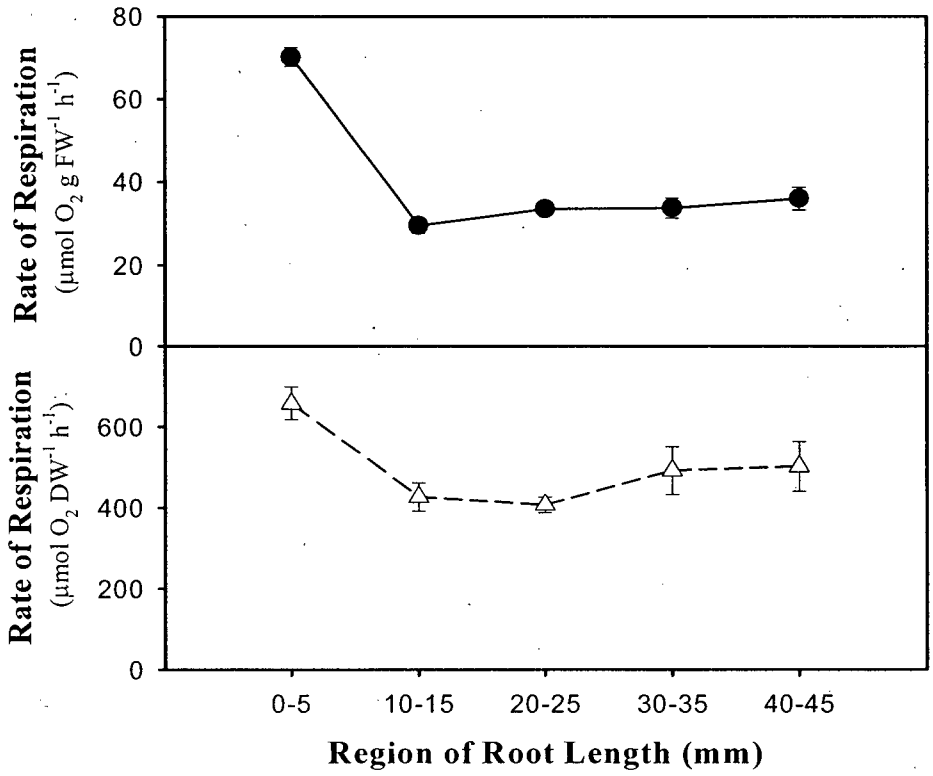


Figure 3.6. Rates of fine root respiration ($R_{FR}$) (n=5) at 22°C, for different root zones moving back from the tip to the 45 mm region of primary Douglas-fir roots. Rates were calculated based on root fresh weight (FW) (top panel) and dry weight (FW) (bottom). Error bars represent standard error of mean.

On a DW basis, $R$ at the tip region (0-5 mm) was 30% higher than average $R$ for the more distal root regions. The greater difference between tip and distal FW rates in Figure 3.6 can be explained by higher water content of the tip region. Following this test a drought experiment was undertaken on the same seedlings (after their transplantation to soil cones), to assess whether $R_{FR}$ of roots experiencing drought would be higher than control roots; due to a greater relative change in water content during measurement. As is shown in Figure 3.7, $R_{FR}$ was the same for roots after four weeks of drought as for control, indicating that rapid re-hydration did not influence their respiratory rate.

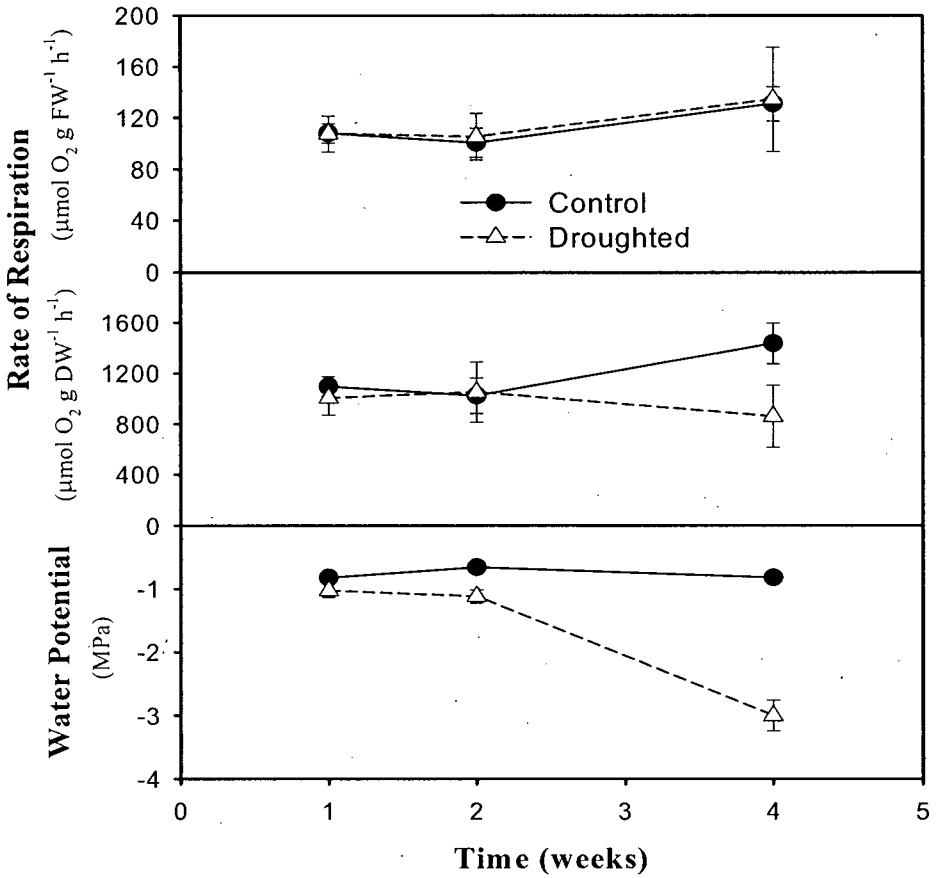


Figure 3.7. Rates of fine root respiration ($R_{FR}$) (n=5) at 22°C, for control seedlings and those under drought. Rates were calculated based on root fresh weight (FW) (top panel) and dry weight (DW) (middle). Shoot water potential is also shown for both treatments (bottom). Error bars represent standard error of mean.

To test the reliability of the $O_2$-based method, an aqueous $CO_2$ method was developed for calculation of RQ (i.e. $CO_2$ efflux / $O_2$ consumption). When RQ is near 1.0, it is assumed that predominantly carbohydrates are being metabolized, while RQ < 1.0 indicates more highly reduced substrates (i.e. proteins/lipids) are being respired, and RQ > 1.0 would suggest the use of more oxidized substrates (i.e. organic acids) (Hopkins 1999). Using the same seedlings from the previous tests, $CO_2$-based measurements were replicated in tandem with the aqueous $O_2$ method at 22°C and RQ calculated. Results from this work indicated an average RQ of 0.8 for fine, white roots, as the $CO_2$ method consistently demonstrated less efflux compared to $O_2$ consumption.

## 3.4 - Discussion

### 3.4.1 - Interpreting the high rates of fine root respiration

The magnitude of $R_{FR}$, particular in the summer of 2004, must be addressed prior to moving into a discussion of seasonal phenology and IAV. The change in $R_{FR}$ over that season was unexpected as rates from late-June onward were much higher than most previously reported values. Whether these rates are realistic is in question and the following discussion will attempt to cover all areas that may have led to error. To begin with, an evaluation of other reported rates of $R_{FR}$ is imperative but before doing this, it is important to clarify the units that will be used for comparison. Many studies present $R_{FR}$ in terms of $CO_2$ efflux as opposed to $O_2$ consumption (Kelting et al. 1998, Vose and Ryan 2002, Burton and Pregitzer 2003). For the time being a RQ of 1.0 will be used for conversion and a subsequent discussion will interpret how RQ < 1 may have contributed to the high rates. Typically $R_{FR}$ is expressed as nmol $CO_2$ production g $DW^{-1}$ $s^{-1}$, whereas my data are presented as consumption of $O_2$ in units of μmol $O_2$ g $DW^{-1}$ $h^{-1}$. The following rates from the literature were converted to these units for ease of comparison and if rates were on a FW basis, a 10-fold correction factor was applied (based on laboratory results, Figs-3.6 & 3.7).

Numerous laboratory-based studies (Ch-2, Qi et al. 1994, Budge 1996) have demonstrated rates of $R_{FR}$ for Coastal Douglas-fir ranging from 150-600 μmol $O_2$ g $DW^{-1}$ $h^{-1}$ when measured at moderate temperature (e.g. 16-18°C). These rates are already high in comparison to other conifers such as white spruce: ~40 (Weger and Guy 1991), tamarack larch: ~80 (Tjoelker et al. 1999a) and ponderosa pine: ~85 (Lipp and Anderson 2003), which indicates that Coastal Douglas-fir has a high metabolic rate compared to these other species. This tree is found primarily in the northwestern temperate rainforest and therefore is adapted to a longer growing season and higher winter temperature than other intra-continental or boreal species. Relative growth rate (**RGR**) has been shown to correlate with biomass-specific $R$ rate (Poorter et al. 1991, Loveys et al. 2002) and deciduous trees and herbaceous species typically exhibit higher RGRs and rates of $R$ than

conifers (Tjoelker et al. 1999a, Loveys et al. 2003, Covey-Crump et al. 2002). This is clear in comparison of the above rates to the roots of white oak: ~165 (Comas and Eissenstat 2004) and numerous grasses: ~150 (Loveys et al. 2002). Rates of *R* over 1000 μmol $O_2$ g $DW^{-1}$ $h^{-1}$ have been demonstrated in thermogenic tissues (Beevers 1961). Watling et al. (2006) recently reported rates of up to 3600 μmol $O_2$ g $DW^{-1}$ $h^{-1}$ during thermogenesis in sacred lotus flower receptacles. In the current study, $R_{FR}$ reached 6000 μmol $O_2$ g $DW^{-1}$ $h^{-1}$ when measured at 28°C in late summer 2004. Projected to soil temperature at sampling time (Fig-3.8), the highest rates were closer to 2500 μmol $O_2$ g $DW^{-1}$ $h^{-1}$. Even though these values were measured using the same apparatus and electrode and during the same time period as Douglas-fir roots in the laboratory (Fig-2.3, Ch-2), they were five times higher. Given this discrepancy, the $O_2$ method was thoroughly scrutinized for artifacts that could have caused larger rates in the field.

Use of the Clarke-type $O_2$ electrode dates back to the mid-1960s and has been the primary means of measuring *R* in many publications. These electrodes are typically used for laboratory studies and therefore adapting the method for field-based study could have influenced performance. There was no indication however that hot summer temperatures, humidity, or transport could have caused artifacts isolated to summer 2004, or that the electrode was operating more 'realistically' during other time periods. With no indication of instrumentation problems, artificial rate elevations may have arisen from some other aspect of the methodology. It's important to acknowledge that rates depend on the roots chosen for measurement. Smaller diameter roots (0.5-1 mm) can have 1.6-3.4 times higher rates than larger root classes (Cox 1975, Pregitzer et al. 1998, Pregitzer 2002) and different regions along the root have been shown to exhibit dissimilar rates of *R* (Machlis 1944, Pregitzer et al. 1997, Burton et al. 2002). Root tips in particular have been indicated as being 'respiratory hotspots' (Pregitzer 2003, Atkin et al. 2005), with higher rates often occurring along the elongation zone and especially in root hairs (Bhaskar 2003). Results presented from a laboratory test on hydroponic Douglas-fir seedlings, demonstrated that the tip region of white primary roots exhibited *R* rates that were 30% higher than the more distal zones. This suggests that samples with a higher proportion of tips would exhibit a greater relative rate of $R_{FR}$. This indicates the need to

acknowledge heterogeneity of metabolism in the root system, something that has been highlighted recently as a challenge to modeling belowground *R* (Pregitzer 2002, Schurr et al. 2006).

Whether the high rates were due to an artifact of the aqueous method was also investigated in the laboratory with a drought experiment (Fig-3.7). It was hypothesized that roots under drought conditions may respond to rapid re-hydration in the buffer by increasing *R* but this possibility was rejected given that roots under significant drought (< –3.0 MPa), did not have higher rates than control. Increases in $R_{FR}$ have been reported following excision but are usually attributed to wound *R*, which is transitory (Rakonczay et al. 1997). Conversely it has been observed that $R_{FR}$ may decline shortly after excision (Saglio and Pradet 1980, Brouquisse et al. 1991, Rakonczay et al. 1997), which is considered to result from substrate starvation. Excision unavoidably cuts off substrate supply and as a result may influence the RQ. The development of a $CO_2$-based method allowed for determining the RQ of excised Douglas-fir roots in the laboratory. This work led to an average RQ of 0.8, which suggests that a more reduced pool of substrate, such as proteins and/or lipids may have been used by Douglas-fir roots (Hopkins 1999). This value is comparable to other studies on tree roots [RQ=0.8 Burton et al. 1996, RQ=0.84 Lipp and Anderson 2003] and may not necessarily have resulted from excision, because near identical RQ values < 1 have been shown for excised and attached roots of ponderosa pine (Lipp and Anderson 2003). In the current study, converting the $O_2$ rates to $CO_2$ only partially reduces their magnitude. RQ was not measured during the field trials but if it had varied from 0.60-0.85, converting a rate of 2000 μmol $O_2$ g $DW^{-1}$ $h^{-1}$ (mid-summer 2004) to $CO_2$ would have yielded rates of 1200-1700 μmol $CO_2$ g $DW^{-1}$ $h^{-1}$.

In addition to the role of RQ in gas exchange, the concentration of $O_2$ and $CO_2$ has been shown to influence $R_{FR}$ (Beevers 1961, Qi et al. 1994, Widén and Majdi 2001), although an effect of the environmental $CO_2$ level on *R* is much more contested. The effect of $O_2$ depletion on *R* has been known since studies first began and it is clear that under high water content $CO_2$ efflux from soils is greatly reduced (Davidson et al. 1998, Borken et al. 2006). Over the last few decades the potential for inhibition of *R* by high

$CO_2$ concentration in soils has been in question; however, the general consensus is that it does not occur. Qi et al. (1994) presented evidence that $R_{FR}$ of Douglas-fir was uncoupled when measurements were taken at lower $CO_2$ concentration than in the soil. Other researchers, however, have also shown no inhibitory effect of high $CO_2$ concentration on the $R$ of citrus roots (Bouma et al. 1997), of roots in nine northern tree species (Burton and Pregitzer 2002), or leaves of numerous tree species (Davey et al. 2004). These authors point to a leakage artifact as the cause of previous inhibition results.

The role of RQ < 1 during measurement appears to be the only indication that observed rates may have been higher than *in situ* $R_{FR}$. Beyond this there were no obvious artifacts that could have led to an artificial increase in $R_{FR}$, therefore it is reasonable to assume that the observed patterns of $R_{FR}$ are accurate. Given the apparent lack of methodological error, the environmental and functional forces behind this surprising result will now be considered. The incorporation of dynamic fine root phenology within the IAV of climate and ecosystem properties is highlighted throughout.

### 3.4.2 - Seasonal and inter-annual variability of fine root respiration

Rates of $R_{FR}$ were projected to soil temperature at time of sampling through the use of the calculated $Q_{10}$ values from Figure 3.5. These estimations of *in situ* $R_{FR}$ are plotted by measurement day in Figure 3.8.

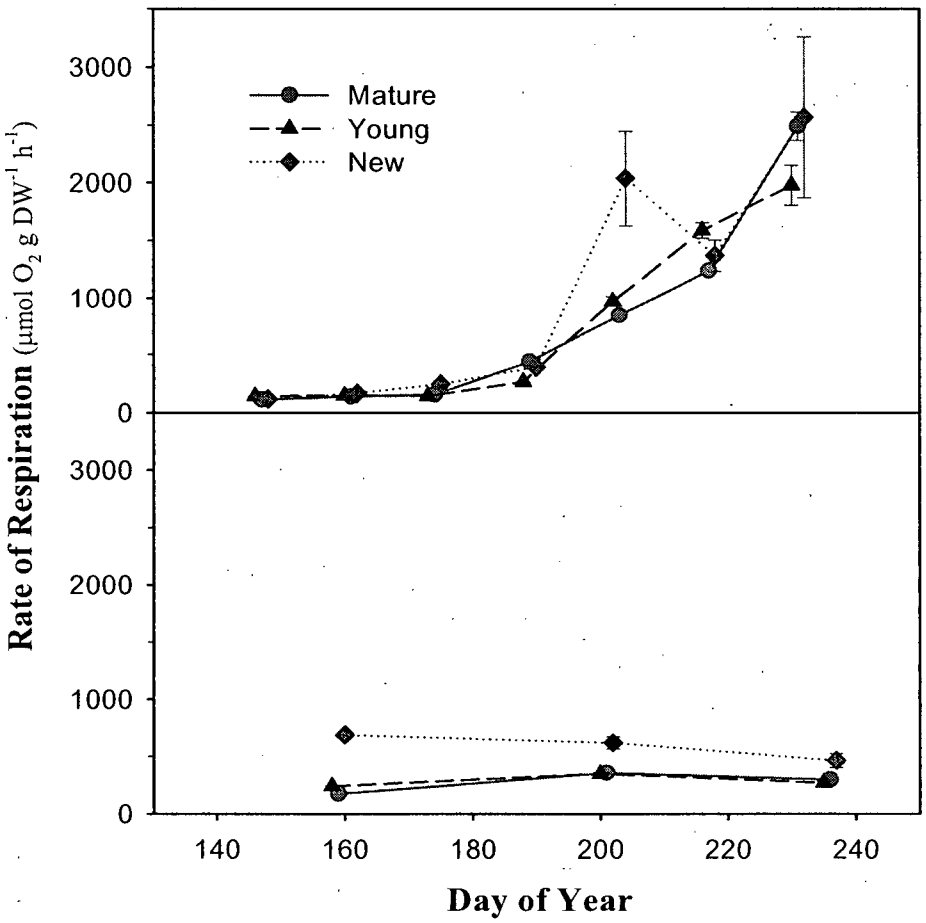


Figure 3.8. Cross-site comparison of fine root respiration ($R_{FR}$) rates, projected to soil temperature at time of sampling through the use of $Q_{10}$ relationships. Mean $R_{FR}$ is shown by measurement day for the 2004 (top panel) and 2005 (bottom) growing seasons. Error bars represent standard error of mean.

Variability in climate and ecosystem function is an unavoidable challenge to understanding C flow. Over the year, plant phenology interacts with climatic variables to produce observed trends in GEP and ER, the primary indicators of ecosystem function. These fluxes are a cumulative representation of above and belowground processes, which directly regulate C turnover and transfer in an interdependent fashion. For each of the Douglas-fir stands under study, there was marked IAV in the seasonal pattern of $R_{FR}$ (Fig-3.8), as well the indication of a carry-over effect linking the two years. Seasonal patterns of $R_{FR}$ and the variables that control these patterns have been shown to depend

on vegetation cover. In a spruce/pine boreal forest, Widén and Majdi (2001) found a doubling of $R_{FR}$ from May to July which declined thereafter. Studies in coniferous and deciduous forests have indicated peaks in $R_S$ that occur late in the growing season for the former, versus in spring for the latter (Lavigne et al. 2003). Vose and Ryan (2002) found that in a pine forest $R_{FR}$ was low during the spring and summer but increased significantly in early autumn, presumably due to a resumption of growth. As in other studies (Gansert 1994, Epron et al. 1999, 2001, Burton and Pregitzer 2003), there was a link between $R_{FR}$ of Douglas-fir roots and temperature, especially in 2004. However, concluding that temperature is always a primary control would be erroneous in this case because in 2005 temperature had much less influence over $R_{FR}$. To generalize the two seasons, 2004 was hot and dry while in 2005 there was moderate temperature increase and moisture loss. It has been suggested that roots are more temperature sensitive than other components of $R_S$ (Boone et al. 1998), which may explain why the extreme conditions in 2004 led to a stronger correlation with temperature. Warmer sites have been found to display higher root contributions to $R_S$ (Lavigne et al. 2003); however it should be acknowledged that the effect of temperature covaries with other variables (i.e. SWC and GEP) throughout the season (Bääth and Wallander 2003), thereby making it difficult to identify the most influential variable. Short-term temperature response in 2004 was coupled to changes in respiratory capacity as $Q_{10}$ increased with climbing temperatures. In contrast, numerous authors (Kirshbaum 1995, Rayment and Jarvis 2000, Luo et al. 2001) have observed that $Q_{10}$ typically decreases with SWC loss and temperature increases in the summer. This suggests that the $Q_{10}$ relationships in the current study may be describing an endogenous pattern that controlled the temperature sensitivity of metabolism; which would not be surprising given that $Q_{10}$ is actually less of a pure temperature parameter and more of an "integration of several confounding ecosystem processes" (Janssens and Pilegaard 2003). To assess seasonal sensitivity, $Q_{10}$ values were calculated using the same $Q_{10}$ equation for short-term values and with the rates projected to soil temperature (Fig-3.8). These are presented in Table 3.4 below.

Table 3.4. Seasonal $Q_{10}$ values for all sites during 2004 and 2005.

| | Mature | Young | New |
|---|---|---|---|
| **2004** | 28.1 | 15.9 | 6.0 |
| **2005** | 5.89 | 1.69 | 1.21 |

Clearly, seasonal $Q_{10}$ values were high for 2004, particularly at the Mature stand. This resulted from a lower relative change in temperature at this site compared to other stands. Very high values of $Q_{10}$ (up to 23) have been reported by Janssens and Pilegaard (2003) for $R_S$ in a beech forest but the values in that study were calculated on a weekly time step and during the winter. Despite this complication, the above values indicate the importance of considering site specific temperature regime when generalizing sensitivity, as the site with the lowest seasonal change in soil temperature (the Mature stand), had greater apparent sensitivity, despite exhibiting similar absolute rates of $R_{FR}$ to the other sites. Obviously these long-term $Q_{10}$ values are less informative than the seasonal progression of short-term $Q_{10}$ shown in Figure 3.5 and may actually be misleading if one wanted to compare sites (e.g. in 2004 the short-term values show little difference between the sites, while the long-term values above are very different). The recent use of 'moving-window' approaches to describe the masked variability within long-term $Q_{10}$ values is a development that will help to limit this kind of problem (Barr et al. 2004). Given the importance of properly predicting respiratory temperature response and the dynamic nature of that response, a blended approach such as this one will likely become more prevalent in future modeling efforts. This will allow modelers to continue to benefit from the simplicity of long-term $Q_{10}$ values, while still accommodating for the short-term fluctuations within them.

In 2004, the capacity and sensitivity of $R_{FR}$ was highest when there was maximum soil temperature and minimum moisture, a result that is opposite to the findings of Saiz et al. (2006) in Sitka spruce stands. These authors found that drought stress during high

temperature reduced the autotrophic contribution to $R_S$. Although drought did not appear to cause any significant depression in either year of the current study, Lu (1994) found that Douglas-fir $R_{FR}$ did decline with drought. Obviously there is a threshold where moisture loss is significant enough to slow activity of $R_{FR}$ (Bryla et al. 1997), thereby limiting its contribution to $R_S$. This point may have been reached at the New stand during both years, as $R_{FR}$ leveled in late summer 2004, and gradually fell throughout 2005. Acclimation of roots to warming soils could also be an explanation. It has been suggested that younger plants may have a greater capability to acclimate (Atkin et al. 2005) and the pattern observed in this year is consistent with that expected from down-regulation of the respiratory system (warm acclimation, Ch-2). As yet another possibility, roots at the New stand may have been substrate starved in the summer of both years, due to a reduction in GEP which was more significant in 2004 (Figure 3.9). This explanation is supported by the inverse relationship between $R_{FR}$ and GEP in that year (Table 3.2).

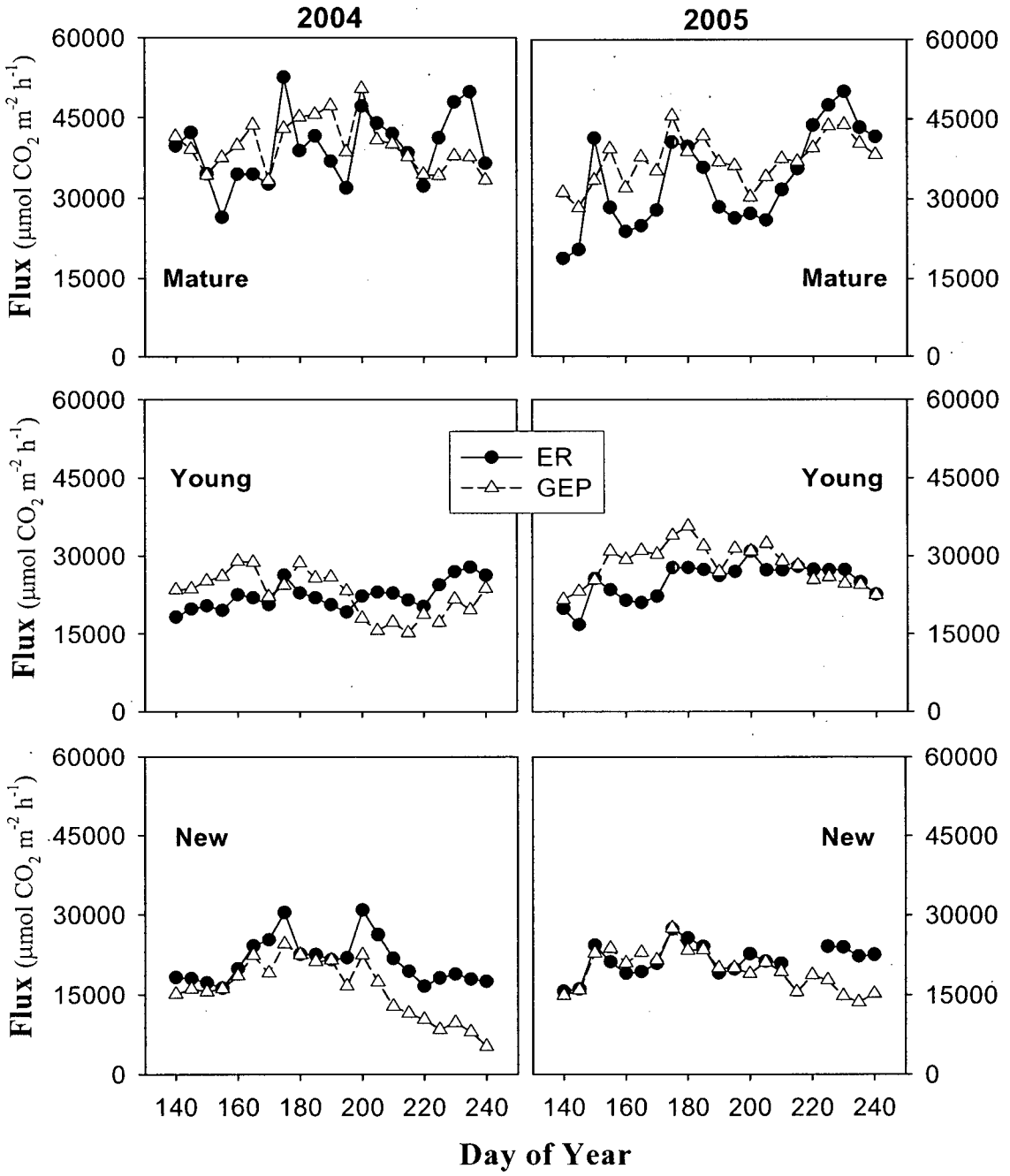


Figure 3.9. Seasonal pattern of ecosystem respiration (ER) and gross ecosystem productivity (GEP) for all sites and both years. Data points represent an averaged daily flux for the previous 5 days, converted to an hourly rate.

All sites were net sources of $CO_2$ by the end of summer 2004; however in 2005, only the New stand was consistently a source by late summer. The dissimilarities exhibited by

the New stand are likely a reflection of the more extreme environmental conditions at this site and its patterns of GEP, C allocation and root production compared to the other two, more established stands. Higher autotrophic contribution has been shown to occur in younger stands (Bond-Lamberty et al. 2004b) and $R_{FR}$ often decreases with stand age (Ohashi et al. 2000, Saiz et al. 2006). Greater levels of fine root production are also recognized in younger stands (Zerva et al. 2005, Saiz et al. 2006); which is likely a function of more allocation belowground (Smith and Resh 1999, Davidson et al. 2002). Another factor that will lead to greater variability of $R_{FR}$ with stand age, are maturity related differences in seasonal respiratory costs from fine root growth, maintenance and ion uptake (Lambers et al. 1983, Amthor 1984, 2000).

The differences in weather between 2004 and 2005 probably led to distinct patterns of nutrient mineralization, ion uptake, exudation and mycorrhizal colonization. Without direct evidence, I can only speculate on how the 2004 drought affected heterotrophic activity but as previously discussed a reduction in $R_H$ would likely have occurred. A combination of $R_H$ decline and increased $R_{FR}$ during drought would help to explain patterns of hysteresis that have been reported to occur for seasonal $R_S$ (Drewitt et al. 2002, Gaumont-Guay 2005). Drought induced changes in heterotrophic activity would also influence nutrient dynamics, potentially leading to a decline in nutrient availability (Norby and Jackson 2000). Perplexingly, such an occurrence would offset potential increases in nutrient uptake capacity at higher summer soil temperatures (Bassirirad 2000). Results on the N or C content of fine roots (see App. C) indicate a seasonal increase in root N content for the Mature and Young stands in both years, but a reduction at the New stand. N content of roots has been shown to explain a high proportion of the variability in $R_{FR}$ (Burton et al. 1996, Pregitzer et al. 1998, Tjoelker et al. 1999b, Burton et al. 2002), however, as in the current study, others have found little correlation between N content and $R_{FR}$ (Widén and Majdi 2001, Vose and Ryan 2002). Despite not being proportionate to the rate increase in summer 2004, the elevation in root N content by season's end suggests that there was either no lack of nutrients, or that the root system adjusted to reduced mineralization. Uptake capacity could have been enhanced during drought by increased total belowground carbon allocation (**TBCA**). As the second largest

flux after GEP (Davidson et al. 2002), TBCA has been shown to account for up to 75% of annual NEP (Nadelhoffer and Raich 1992) and only 1/4-1/3 of this amount is commonly used for structural growth (Davidson et al. 2002, Hendricks et al. 2006); thereby leaving the rest for *R* and exudation.

Under the conditions in 2004, the role of elevated $R_{FR}$ may have been to create a substrate sink to the roots for: 1) continued growth of white tips; 2) for mucilaginous protection of roots from water loss (Bhaskar 2003); 3) attracting mycorrhizal colonization; or 4) to fuel ion uptake and maintenance processes. All of these scenarios could have been part of a general stress reaction which led to the observed change in $R_{FR}$. In a C balance assessment of an Interior Douglas-fir forest, Mcdowell et al. (2001) assumed that 20% of TBCA went to mycorrhizae and other exudates (based on estimates from other authors); a proportion that may have been even higher in the current study. If such an increase in TBCA partitioning to the root zone had occurred, it would have lead to significant changes in rhizosphere dynamics (Neumann and Römheld 2001). Changes to the rhizosphere and the proportion of belowground C allocation, may have caused the apparent carry-over effect on $R_{FR}$. Through comparing the two seasons it seems clear that there was a sustained influence of 2004 on the metabolic activity of fine, white roots throughout 2005. Without the same high soil temperature and low SWC stress, $R_{FR}$ may have simply been maintained at a level pre-set by the previous conditions. Given the significant environmental differences between these seasons, we would also expect the phenology of TBCA, mycorrhizal colonization, and C exudation to have varied. The differences in TBCA that likely occurred and the presence and lack of an apparent stress response in 2004 and 2005, respectively, would also help to explain the significant decline of fine white root tips that was observed in 2004 compared to 2005.

Unfortunately the only biomass data currently available for these sites is for standing fine roots in May 2004. Data from minirhizotrons installed at all sites are being processed to provide a quantitative assessment of white root decline over the two seasons (T. Trofymow-personal communication). For the purposes of this chapter, the trend in fine white roots will be considered proportional to the degree of climatic stress between

the two years: i.e. in 2004 there was a rapid and significant decline in the presence of fine, white roots during a hot and dry season, while in 2005 the decline was much more moderate, as was the weather. Assuming this pattern, the remainder of this paper will consider how fluctuations in fine root biomass may alter the activity of $R_{FR}$, as well as its relative contribution to ER over the season.

### 3.4.3 - Fine root dynamics and the variability of soil respiration

It has been clearly shown that $R_S$ is influenced by the level of fine root biomass (Xu and Qi 2001, Butnor et al. 2003, Saiz et al. 2006) and that the high spatial variability often exhibited by $R_S$ relates to the proximity of measurement to vegetation (Law et al. 2001). Linear relationships between $R_S$ and fine root biomass (Pregitzer et al. 2000) and belowground production (Campbell et al. 2004) have been demonstrated. In coniferous forests, biomass of fine roots varies from 100-1260 g $m^{-2}$, with a mean standing biomass of 500 g $m^{-2}$ (Fogel 1983). Among other factors, these estimates depend on species, age, season, nutrient status of soils (Butnor et al. 2003) and allocation patterns. $R_{FR}$ is often estimated in models as a function of temperature and biomass (Chapin III and Ruess 2001); however, root biomass and moisture have distinct seasonal patterns which can covary with temperature (Janssens and Pilegaard 2003). This complication played out in the current study, as the observed decline of white roots in 2004 was proportional to moisture, whereas temperature was inversely related. Increased temperature has been implicated in root mortality (Eissenstat and Yanai 2002); however, dieback can also increase significantly following moisture loss (Deans 1979, Marshall 1986) or due to higher $R_{FR}$ rate (Norby and Jackson 2000); both of which are indirect effects of elevated temperature. Endogenous changes in C allocation belowground can also reduce fine root numbers (Butnor et al. 2003). In summary, it appears that both environmental and endogenous factors can combine to cause rapid root mortality (Stevens et al. 2002), which may lead to significant losses. As a global average, 56% of forest fine roots turnover within a year (Gill and Jackson 2000); however, under extreme conditions such as those displayed in 2004, dieback can no doubt be higher.

As previously mentioned, the only biomass data available for these stands is for standing fine roots in May 2004, which was obtained through soil core analysis (Trofymow 2004, in revision). Despite this deficit, these spring fine root levels can be used to project seasonal biomass decline during that summer. At the Mature stand, a live fine root density of 175 g $m^{-2}$ was measured vs. 100 g $m^{-2}$ at the Young stand, and only 25 g $m^{-2}$ for the New stand. These values are low in comparison to other biomass assessments on Douglas-fir (Santantonio and Hermann 1985, Vogt 1991, Mcdowell et al. 2001), possibly due to underestimation by the coring method or because these stands are highly productive (the site index of all stands is near 30 m) and therefore may require fewer fine roots. The ability to find fine, white roots during the summer contrasted greatly between the 2004 and 2005 seasons, with sampling taking an average of 1 hour longer to obtain 10 mg samples in summer 2004. As a rough visual estimation, ~90% of fine white root length was lost to mortality or colonization in the summer of 2004 vs. ~50% in 2005. It is proposed that the rapid and significant decline in biomass during summer 2004 is directly related to the observed change in $R_{FR}$. To develop a further understanding of how this could influence the C budget, the influence of these changes on the seasonal contribution of $R_{FR}$ to ER will now be investigated.

### 3.4.4 - A model for scaling fine root respiration

To represent the role of fine root dynamics in seasonal ER, a model was developed to scale $R_{FR}$ throughout the 2004 growing season. Given that comprehensive information on root biomass is lacking, this model is intended to be heuristic rather than an accurate explanation of what occurred. Biomass-specific $R_{FR}$ has been converted to an area based ($m^{-2}$) estimation of $CO_2$ exchange, using standing biomass in May 2004 and a hypothetical mortality scenario. A total of 92% mortality was imposed beginning in late-June, assuming that there was no production in the summer. As mentioned above, this degree of mortality is based largely on visual estimation at the sites but in support of this, significant seasonal fine root decline has been reported previously for Douglas-fir stands on the eastern coast of Vancouver Island (Kurz 1987). In that study Kurz (1987) reported 22-98% fine root mortality from May to August, for five mature stands differing

primarily in productivity. Listed below are the other assumptions and data corrections that have been incorporated into this scaling exercise:

1) The observed rates of $R_{FR}$ are accurate for the fine, white root regions measured.

2) A 0.8 RQ correction was applied when converting $O_2$ consumption rates to $CO_2$ production, to facilitate their comparison to ER (Burton et al. 1996).

3) Based on the laboratory test presented in Figure 3.6, a correction for higher $R$ of white tips was used during rate conversion. In spring 2004, the proportion of tips / distal root biomass was assumed at 50 / 50%. During the modeled mortality period, a ratio of 20 / 80% and then 10 / 90% was used under the rationale that as the summer progressed, the remaining biomass was composed of fewer root tips. For each individual date the respective proportions were used to calculate a corrected rate, assuming that the tip proportion respired at the rates from Figure 3.8 and that the distal proportion was active at the projected rates reduced by 30% (Fig-3.6).

The scaled and corrected seasonal rates are compared to average hourly ER for all sites in Figure 3.10.

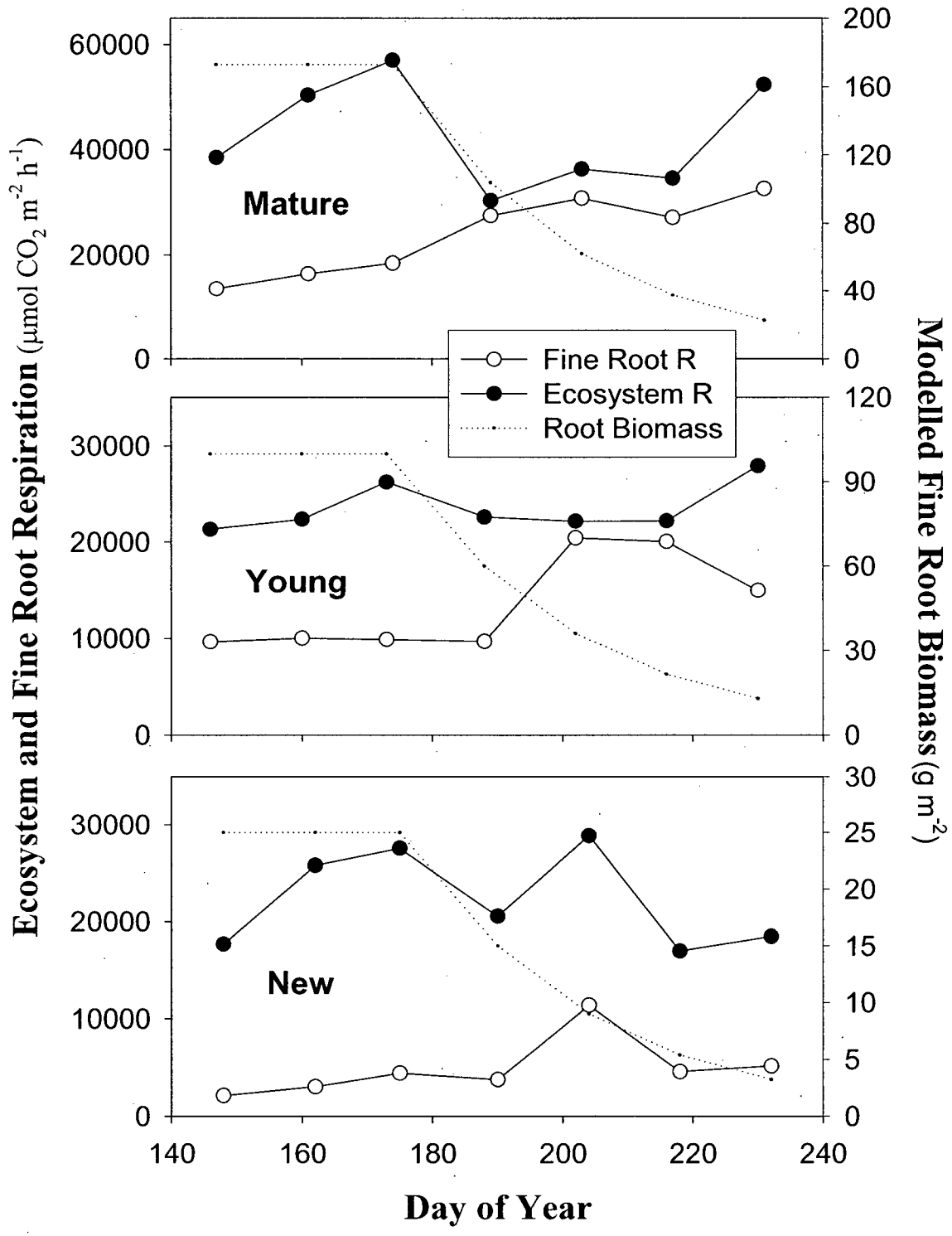


Figure 3.10. Average hourly ecosystem respiration (ER), scaled fine root respiration ($R_{FR}$) and modeled fine root biomass decline. Values of each are shown for days on which $R_{FR}$ was measured during the 2004 growing season, at all sites.

Due to the current study's bias, only one element of ER was accounted for in this model; however, despite their low biomass contribution (up to 2% of total ecosystem), fine roots are considered to be the most active portion of the root system (Clark et al. 2001). The relationships depicted in Figure 3.10 demonstrate that efflux from other root system components and from the other components of ER, may have been quite limited in comparison to $R_{FR}$ in the summer of 2004. This indicates the potential for root activity to maintain ER during summer drought, while $R_H$ and aboveground $R$ are lower. Although likely overestimated given the large uncertainties about biomass and $R_{FR}$ contributions, this general pattern is feasible under the scenario that roots are the least drought sensitive component. Similar temporal variability for the relative contribution of $R_S$ / ER has been reported for a spruce dominated forest by Davidson et al. (2006b). These authors demonstrated that $R_S$ / ER began at 0.45 in the spring, increased to a plateau of 0.65 in the summer and then increased further during the fall to 0.8, despite falling temperatures. The relative contribution of $R_S$ to ER has also been shown to vary with stand age for the same stands studied in the present work (Humphreys 2004). In that study, yearly (2002) ratios of $R_S$ / ER were calculated for the Mature, Young and New stands, which revealed that $R_S$ accounted for 54%, 120% and 69% of ER, respectively.

The greatest problem with these scaling calculations was the lack of empirical data to confirm the activity of other ER components. Another potential error that was unavoidable during this exercise was the possible underestimation of ER by eddy covariance measurement (Law et al. 2001, Morgenstern et al. 2004); which would have influenced the projected contributions of $R_{FR}$ to ER. Another uncertainty that may have affected the model is the possibility that mycorrhizae were unintentionally included in the $R_{FR}$ results. Although obvious mycorrhizal roots were easily avoided in the present study, physiological effects from proximate or partial colonization may have been included in the measured rates. Regardless of these uncertainties, this exercise provides a useful example of incorporating a component displaying significant seasonal variability into the patterns of ER. Despite being simple in nature, this model provides a method of describing the high level of temporal heterogeneity that may be typical of root biological responses in some years (Körner 1995).

## 3.5 - Conclusions

The most significant result from these field studies was a considerable IAV in measured $R_{FR}$. Rates were much higher in the latter part of the 2004 season, at a magnitude that is not typical of other rates of $R_{FR}$ reported in the literature. A thorough review of the methodology however, revealed no possible artifacts that would indicate that this rate elevation was artificial. $R_{FR}$ in 2005 demonstrated a very different trend but higher rates in that spring suggest a carry-over effect from summer 2004. Site differences in both years were minimal, with the exception of the New stand, which had a more independent phenology of $R_{FR}$. It was also the only stand to demonstrate patterns consistent with down-regulating acclimation to warming soils, particularly in 2005. The IAV exhibited by Douglas-fir $R_{FR}$ at all sites may be explained by the significant differences in weather between these years, as 2004 experienced a hot, dry summer while 2005 had a more moderate temperature and moisture regime. However, there was autocorrelation between $R_{FR}$ and environmental variables, particularly in 2004, which complicates any conclusions about the dominant controls and may have masked the indirect influences that different factors could have had on ecosystem function. In particular, GEP decline may have been relevant during the summer drought of 2004 and any resulting changes in allocation would have contributed to the observed pattern of $R_{FR}$.

Mortality of fine root biomass was not quantified but a significant, visually observed decline during the summer of 2004 is believed to have been the consequence of a general stress response by these trees to the harsh conditions. This decline is also thought to have been a reason behind the rate increase in that summer; due to a combination between environmental stress and a concomitant alteration of endogenous root dynamics. A scaling effort to place the observed rates within the trend of ER was undertaken using standing biomass for May 2004 and a hypothetical biomass decline scenario based on the visual estimation of fine, white root decline. This effort was intended to serve as a heuristic model and portrays a scenario in which fine roots could play a key role in maintaining ER during summer drought, at a time when other contributors may be less active.

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# 4. Conclusions on the role of Coastal Douglas-fir fine root respiration in the carbon budget

Characterizing and modeling the response of ecosystems to changes in climate is a research field in rapid development. Building understanding and improving the ability to project potential changes to the carbon (**C**) cycle are two of the main facets of this research. Currently, advancing the accurate prediction of plant respiration (***R***) responses to temperature change is a particularly active area of research. A major uncertainty at this point is the significant inter-annual variability (**IAV**) that *R* can display. The role of stand age in trends of ecosystem respiration (**ER**) is also only partially understood, although there is greater recognition that it should be a necessary component of scaled-up C models (Chen et al. 2002, Coursolle et al. 2006). The present study was carried out to investigate seasonality, IAV and stand age effects on fine root respiration ($R_{FR}$) of Coastal Douglas-fir, a dominant and widespread tree species of the Pacific Northwest region. The primary aim of this research was to further understanding of temperature response in general, while also trying to clarify whether acclimation of $R_{FR}$ is relevant in this species.

Currently, there is insufficient empirical basis to thoroughly account for the above factors in carbon-climate models (Hui et al. 2003). It is therefore the role of projects like the present one to provide modelers with insights from measured representations of ecosystem dynamics. The results from this project provide valuable information on Coastal Douglas-fir $R_{FR}$ from both the laboratory and field perspectives. Presented in Chapter 2 were findings from a laboratory experiment, carried out using the reciprocal transplant of Douglas-fir seedlings, whose seed originated from an eastern Vancouver Island provenance. The goal was to create the necessary conditions for either a cold or warm acclimation response to occur in fine roots, if they indeed had the capability to do so. Over the course of this experiment, there were no clear signs of an acclimation response by $R_{FR}$; there are however, a few factors which limit the conclusiveness of this test. To begin with, the interpretation of seedling response was partially confounded by the presence of a 'transfer effect'; this appeared to be transient, however, and therefore

did not invalidate the experiment. The subsequent trend of $R_{FR}$ for all treatments only demonstrated differences for the separate measurement temperatures (**MTs**). Despite the opportunity for both warm and cold acclimation, the seedlings were completely unaffected by treatment. In addition to the transfer effect, a further uncertainty with these results is whether the selected temperature range thoroughly covered conditions that seedling roots may experience in the field. Given the hot, dry summers typical to the eastern coast of Vancouver Island, it is possible for soil temperatures in a clearcut to reach 30°C. With the lack of significant winter temperature stress, extreme summer conditions may be the only scenario under which these trees would benefit from acclimation. Further studies using multiple provenances and experimental conditions replicating clearcut temperatures, would help to characterize the potential for Coastal Douglas-fir tree roots to compensate in this manner. Nevertheless, with no signs of acclimation, it appears that trees from this provenance may lack the capability to adjust their rate of $R_{FR}$ following long-term temperature change.

Due to the complicated nature of field studies, temperature response is not isolated as easily and therefore this investigation was more descriptive. In addition to providing a multi-year account of seasonal $R_{FR}$ at three stands of differing maturity, the results were also interpreted for evidence of acclimation. This field based work was presented in Chapter 3 and yielded findings that were in part unexpected but provided evidence for a strong IAV in the seasonality of $R_{FR}$ capacity and sensitivity, at all of the Fluxnet sites. The most notable result was in the 2004 season when a surprising and unexpected summer increase in $R_{FR}$ occurred at all sites. Although seasonal increases in root and rhizosphere $R$ have been previously reported, the magnitude of increase was large compared to other studies. Despite thorough investigation, there were no apparent artifacts that could have caused this occurrence and in 2005, the trend was not repeated. This suggests that a combination between the environmental conditions in 2004 and the accompanying endogenous responses were behind the rate increase. Higher rates of $R_{FR}$ in 2005 compared to the spring of 2004 support this explanation, indicating the presence of a sustained, 'carry-over effect' linking the two seasons. Sensitivity was coupled to changes in respiratory capacity for both seasons, which further points to a difference in

the physiological state of these trees between the two years. The observed trends in $Q_{10}$ also support the view that the sensitivity of $R_{FR}$ to temperature may be more dynamic than is often assumed. When short-term $Q_{10}$ values are calculated frequently over a season as in the current work, it becomes apparent that assuming either a $Q_{10}$ of 2.0 or some other static value, could lead to inaccurate estimation of temperature sensitivity and resulting fluxes. Modelers and researchers should continue to be aware of the limitations of this descriptor, particularly when long-term $Q_{10}$ values are used for describing multi-component fluxes like soil respiration ($\boldsymbol{R_S}$). Further study on the temperature response of the different ER components and the temporal variability of temperature sensitivity is required to clarify the benefits and drawbacks of modeling with $Q_{10}$.

Although all sites generally followed the same trend in both seasons, the New stand displayed a more chaotic seasonal change in $R_{FR}$ and was the only site to exhibit a pattern consistent with an acclimation response. This was most apparent in 2005; however, there are other factors which could account for the decline in $R_{FR}$, such as the influence of drought and/or substrate limitation. New stands are known to act as net sources of $CO_2$ for a period of time following establishment. With the advent of intensive silviculture, the age of canopy closure in conifer forests is being reduced to as low as 20 years (Cohen et al. 1996), which indicates that establishing stands may become sinks sooner in the future. In order to accurately represent how root activity in new stands contributes to or delays progression to a net sink, it should be acknowledged that seasonal $R_{FR}$ in new Douglas-fir stands may have a chaotic nature, especially during dry years. The possibility of acclimation responses in new stands could also be of importance in modeling; however, given the results from the present study, the dynamic variability demonstrated by Douglas-fir $R_{FR}$ irrespective of stand age, may greatly outweigh any potential influence that acclimation could have on the C balance.

The IAV exhibited by $R_{FR}$ may have come about because of the significantly different weather conditions between the two seasons; however, given the autocorrelation between the trends of $R_{FR}$ and environmental variables, particularly in 2004, it is difficult to conclude on the most important factors. Indirect influences of temperature and drought

in 2004 could have been just as influential as direct effects on metabolism; i.e. through potential changes in C allocation belowground and influences on nutrient cycling. Primarily, the trend of $R_{FR}$ in 2004 is suggestive of a general stress response by these trees to the hot and dry conditions. Such an occurrence would help to explain the apparent carry-over effect in 2005 and the lack of a rate elevation in that year, which was characterized by moderate weather. A combination of stress response and environmental conditions could also help to explain the significant and rapid decrease in the number of fine, white roots observed in 2004, compared to a moderate decline in 2005. It has been hypothesized herein, that this visually observed fine root mortality and the endogenous changes that are believed to have accompanied it, led to the large respiratory increase in remaining fine, white roots that were sampled during the summer of 2004. Using standing biomass data for May 2004 and a hypothetical mortality scenario, the 2004 $R_{FR}$ results were used in a scaling exercise to place this increase within an ecosystem context. The modeled trends reveal that during a year typified by more extreme weather, as in 2004, $R_{FR}$ may play an important role in maintaining levels of ER while other components have reduced activity.

To summarize, this research project contributed to the understanding of acclimation in Coastal Douglas-fir and presented evidence that $R_{FR}$ in the field can be dynamic and exhibit significant IAV. Given that 80-90% of global vegetative biomass is stored in forests (Körner 1995), studies on the role of $R$ in forest C dynamics must continue. Research networks like Fluxnet-Canada are leading the way to a more thorough understanding of C movement at the local and landscape level and given an uncertain future under what is now being collectively labeled as 'Global Change', the foresight that these efforts should yield will enhance our ability to adapt and keep informed in the days ahead.

## 4.1 - Chapter 4 references

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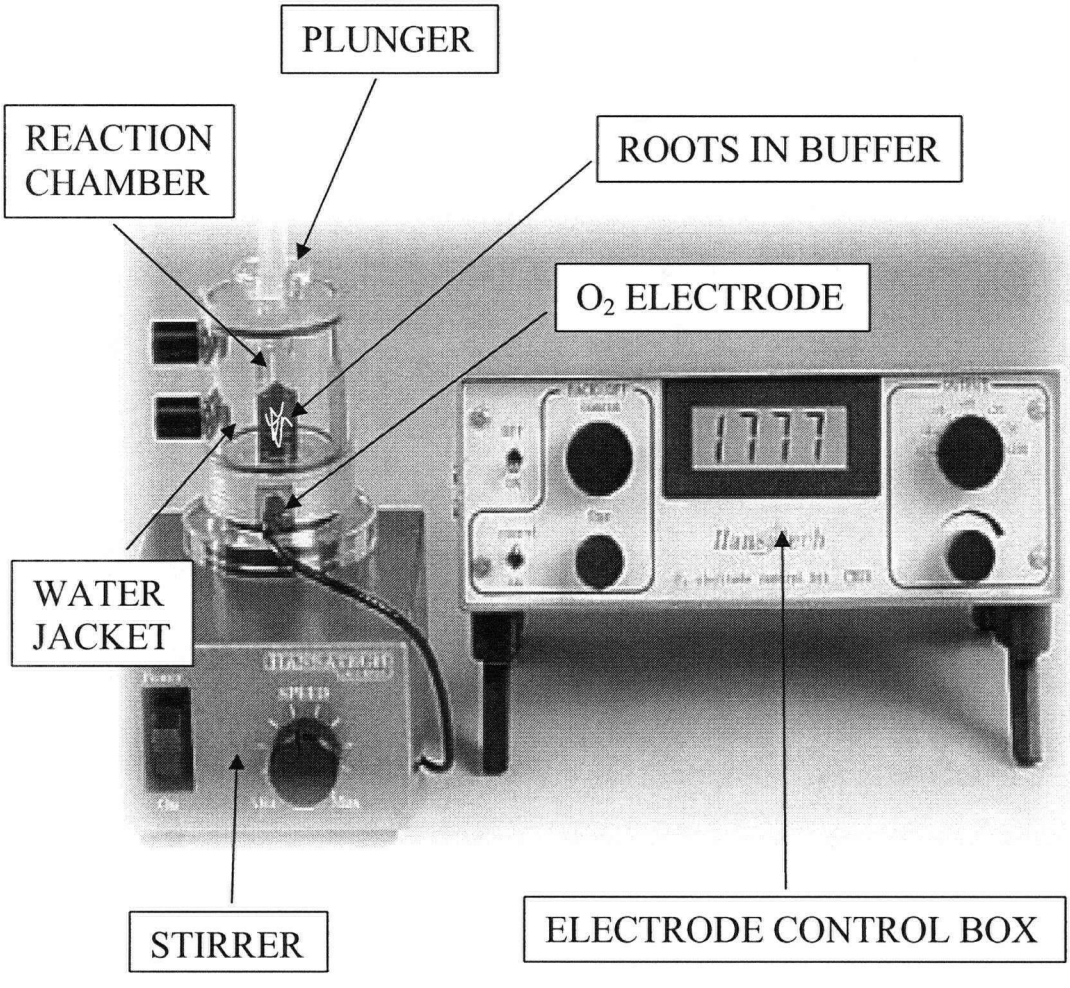
**Appendix A: Reaction chamber with water jacket, plunger, $O_2$ electrode, stirrer and electrode control box**




Source: www.hansatech-instruments.com

**Appendix B: Example of calculations for fine root respiration rates**

Rate = slope of calibration curve * slope of $O_2$ depletion curve / root biomass * time correction

= µmol $O_2$ (in 2 ml buffer) / mv * mv / s * 1/g DW * 3600s / 1h

= µmol $O_2$ / g DW h = µmol $O_2$ g $DW^{-1}$ $h^{-1}$

## Appendix C: Fine root N and C Content in 2004 and 2005 for all sites

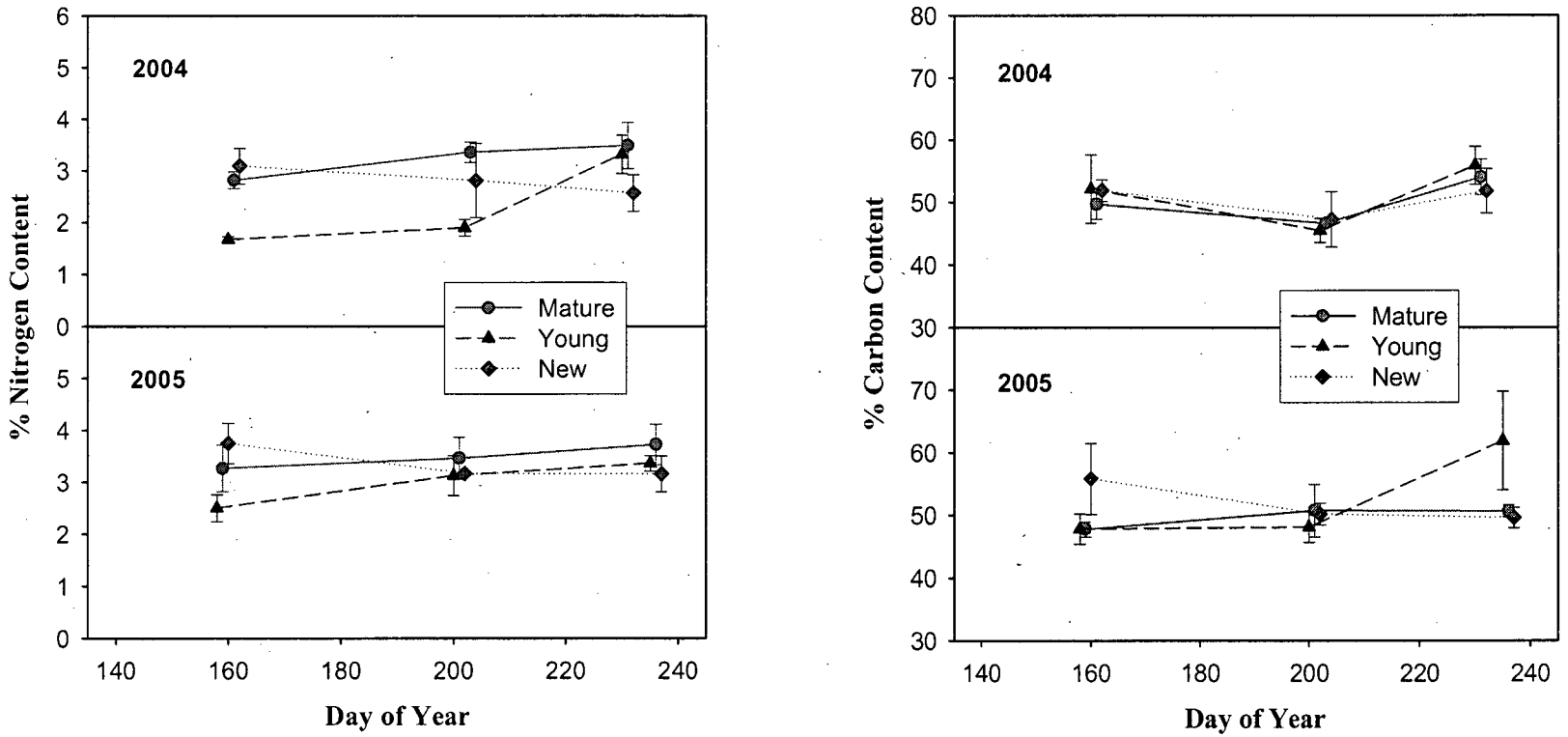



Figure 3.11. Percent (%) nitrogen (N) and carbon (C) content (n=3) of fine, white roots across all sites and for both years during early-June, late-July and late-August. Error bars represent standard error of mean.