AN ABSTRACT OF THE THESIS OF

Shengjun Lu for the degree of Doctor of Philosophy in Forest Science presented on April 4, 1994.

Title: Root Respiration of Douglas-fir and Western Hemlock Seedlings

Abstract approved_ Signature redacted for privacy.

Joe B. Zaerr

Most of the methods currently used to measure root respiration introduce various disturbances which may lead to biased results, and require destruction of roots which does not permit repeated measurements of root growth and respiration. A root box method was developed for measurement of root respiration, as well as root growth. Roots grew in the root box in two dimensions between a glass and a fabric sheet, and shoots grew in normal atmosphere. This method allowed simultaneous and repeated measurements of root growth and respiration over a three to four month period on undisturbed intact roots. With this method, the effects of the disturbances associated with some of the methods currently used, and effects of some of the controlling factors on root respiration were examined with three to six-month-old Douglas-fir and ten to twelve-month-old western hemlock seedlings. Root injury should be avoided for root respiration

measurements because root respiration increased immediately after root injury. The time elapsed from root injury to respiration recovery varied from species to species. For both species, root respiration decreased as soil water content decreased. Root respiration varied from day to day throughout the entire experimental period, and root respiration response to root temperature could be described as a regression of the logarithm of root respiration rate on the reciprocal of the absolute root temperature. The slope of these regressions did not vary under the various conditions tested. The determination coefficients of these regressions ranged between 0.3 and 0.5 for a group of seedlings grown at the same treatment, but ranged between 0.7 and 1.0 for individual seedlings. We can predict the root respiration rates of individual roots at other temperatures very well if we know the rate at a specific temperature. For Douglas-fir seedlings, the respiration rate of roots grown at 0.36 mM N (NH_4NO_3) was significantly lower than that of roots grown at 1.8 and 7.2 mM N level. Root respiration of western hemlock seedlings did not respond to nitrogen treatments. Root temperature, nitrogen level, soil water potential, root to root variation, and day to day variation need to be considered for prediction of root respiration for forest trees.

Root Respiration of Douglas-fir and Western Hemlock Seedlings

by

Shengjun Lu

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Completed April 4, 1994

Commencement June 1994

Doctor of Philosophy thesis of Shengjun Lu presented on April 4, 1994

APPROVED:

Signature redacted for privacy.

Major professor, representing Forest Science

Signature redacted for privacy.

Head of Department of Forest Science

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Signature redacted for privacy. 5-12-94

Shengjun Lu

ACKNOWLEDGEMENT

I would like to express my gratitude to my major professor Dr. J.B. Zaerr for his help in experimental design and idea development, providing financial support, making countless corrections on the English text of the earlier manuscript, and understanding all the difficulties I had in the foreign environment. Many thanks to Dr. Kim G. Mattson for providing funds for this dissertation research, for his guidance in experimental design, help in conducting research, and construction of the earlier manuscript. I am indebted to Dr. Tony H. H. Chen, Dr. Bart A. Thielges, Dr. Nan C. Vance, and Dr. John D. Marshall for their critical review and important suggestion on the earlier manuscript. I also want to thank Dr. Paul T. Rygiewicz, Dr. Iwan Ho, and Dr. Randy Molina for their contribution in the development of the root box, and Peter F. Meyer for building the root boxes. I would like to take this opportunity to thank professors, fellow graduate students in the Department of Forest Science at Oregon State University for their good suggestions, discussions, and encouragement. Special thanks to my family, my wife Xiaoying Shen, daughter Lihua Lu, and son Lishen Lu for their patience, sympathy, and love. This dissertation is part of the project "Scaling Root Respiration to Partition Autotrophic from Heterotrophic Respiration in a Forest Ecosystem" funded by The USDA Initiative Competitive Grants Program (No 91-37101-6856).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ROOT RESPIRATION OF DOUGLAS-FIR AND WESTERN HEMLOCK SEEDLINGS

INTRODUCTION

Root respiration is an integral component of the terrestrial carbon cycle. It is estimated that 10-30% of the carbon fixed daily in photosynthesis is respired in roots (Lambers *et al.* 1988, Van Veen *et al.* 1989). Root respiration provides energy for root growth, root maintenance, and nutrient uptake for the whole plant. However, we know less about plant respiration processes, especially about root respiration, than we know about photosynthesis. The lack of knowledge of below-ground processes is a limiting factor in understanding carbon cycles of plants and ecosystems.

The slow progress in our understanding of below-ground processes is partially due to the problems and limitations of the methods currently used for measurement of root respiration. Most of the methods currently used to measure root respiration introduce disturbances to the roots during measurements of root respiration and require destruction of roots after the measurements. These disturbances include excision of root tips (Boyer *et al.* 1971, Koncalova *et al.* 1989, Sowell and Spomer 1986, Tripepi and Mitchell 1984), excavation or separation of roots from the soil (Cropper and Gholz 1991, Edwards 1991, Mori and Hagihara 1991), trenching of plots (Bowden *et al.* 1993, Ewel *et al.* 1987b), and clear-

cutting entire forest stands (Nakane *et al.* 1983). The disturbances introduced during the separation of roots from the soil or cutting root tips from a root system may cause wounding of tissue. The respiration rates in most plant organs increase substantially after wounding. The proportion of increase in respiration and the time elapsed between injury and total recovery depend upon species and degree of injury (Evans 1972). Hence, bias of the results may be obtained if the injury is serious and if the measurement of root respiration is conducted shortly after injury. Repeated measurements of root growth and respiration on the same roots over time is not possible with most methods currently used because roots are destroyed after measurement in order to obtain root dry weights for calculation of root respiration rates. Repeated measurements on the same root over time would make it possible to evaluate the effects of controlling factors on root morphology, growth, and respiration rate measured before treatments and at various intervals after treatments. In addition, the sample size can be greatly reduced by using repeated measurements.

Soil respiration decreases as soil available N increases. This phenomenon of N depression in soil respiration has been reported by researchers in Sweden (Norhstedt *et al.* 1989), in Canada (Robarge 1976), and in Oregon (mattson 1994). However, whether the reduction in soil respiration after N addition is related to microbial activity

or root activity is not clear. It is currently difficult to separate root respiration from respiration of soil organisms because a standard method for measurement of root respiration does not exist. A comprehensive proposal funded by the USDA Initiative Competitive Grants Program (No 91-37101-6856) was designed to compare the results of five methods for measurement of root respiration and to suggest a best method for field use, and to identify processes influencing the root respiration rates of trees in a forest site in the Western Cascade Mountains near Scio, Oregon. The stand at this site is a 40-year old mixed Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] forest. This dissertation is part of the laboratory studies of this project.

The objectives of this dissertation were:

To develop, test, and evaluate the root box method for measurement of root respiration and growth over time repeatedly on intact root systems in an undisturbed condition;

Using the root box method, to characterize root respiration for Douglas-fir and western hemlock seedlings as a function of root temperature, N (NH_4NO_3) concentration in the nutrient solution, soil water content, time over a day, and time over a three month period; and

To examine consequences of the disturbances associated with some of the methods currently used for measurement of root respiration.

LITERATURE REVIEW

Root respiration research was initiated less than a century ago. Wollny (1897) linked respiration to plant roots by growing plants in sterilized soil. He measured soil respiration and assumed it was root respiration. Shortly afterward, Russell and Appleyard (1915), and Bizzel and Lyon (1918) found greater CO_2 production from cropped soil than from bare soil. They attributed this difference to respiration from plant roots. Lundegardh (1927) confirmed the root respiration phenomenon in forest soil. He realized that forest soil generally evolved much more CO_2 than agricultural soil or grassland due to greater porosity and more uniform humidity of the forest soil.

The number of studies in root respiration found in the literature increased rapidly following the initial efforts. Now we know that 10 to 30% of the carbohydrate fixed daily in photosynthesis is respired in roots (Lambers *et al.* 1988). Roots use the carbohydrates transported from leaves to provide energy for root growth and maintenance, and for nutrient uptake for the whole plant. Thus, plant productivity is directly affected by the root respiration processes. Some of the major aspects in our current understanding of root respiration, including root respiration process, factors controlling it, and methods for measuring it are reviewed in the following sections.

ROOT RESPIRATION PROCESS

Respiration is an oxidative process in which complex compounds are broken down into carbon dioxide and water with the release of energy. Some of the liberated energy is conserved in the form of adenosine triphosphate (ATP) molecules. The substrates for root respiration may be all the naturally occurring complex compounds or their constituent components. But sucrose is by far the most common substrate as most plants transport photosynthates from leaves to roots in this form (Goodwin and Mercer 1990).

The overall respiration process is complex and can be subdivided into a number of component reaction sequences. These reaction sequences include glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport chains, which include the cytochrome pathway and the alternative pathway. Glycolysis is a series of reactions in which one molecule of glucose is broken down into two molecules of pyruvate with the formation of two molecules of ATP and two molecules of reduced nicotinamide adenine dinucleotide (NADH), a high-energy reductant. Under anaerobic conditions, the pyruvate is reduced to ethanol and NAD^+ is regenerated. This is the fermentation process. Under aerobic conditions, pyruvate molecules diffuse into the mitochondria and are further broken down *via* the TCA cycle. Each pyruvate molecule entering the

TCA cycle produces three molecules of carbon dioxide, four molecules of NADH, one molecule of ATP, and one molecule of reduced flavine adenine dinucleotide (FADH). The reductant formed in the glycolysis and TCA cycle are used to reduce oxygen *via* the electron transport chains located in the inner membranes of mitochondria, with ATP production *via* oxidative phosphororylation coupled to the cytochrome electron transport chain (Goodwin and Mercer 1990).

There are two possible pathways for the terminal electron transport: the cytochrome pathway and the alternative pathway. The cytochrome pathway starts with internal or external NADH dehydrogenase, or succinate dehydrogenase located in the inner membrane of mitochondria. Electrons are passed to ubiquinol, then to cytochrome C *via* ubiquinol-cytochrome reductase, and finally to oxygen *via* cytochrome oxidase. The alternative pathway diverges at ubiquinol, where electrons are transported from ubiquinol to oxygen directly with alternative dehydrogenase (Lambers 1981, Lambers *et al.* 1991). For each electron entering the transport chain to oxygen, there are three molecules of ATPs formed *via* the cytochrome pathway, but only one molecule of ATP formed *via* the alternative pathway. In overall respiration, 36 molecules of ATP are formed when one molecule of glucose is oxidized in the respiration process *via* the

cytochrome pathway. Forty percent of the liberated free energy from glucose is conserved in the form of ATP molecules (Goodwin and Mercer 1990).

FACTORS CONTROLLING ROOT RESPIRATION

The root respiration process is a sequence of chemical reactions, so anything that affects the substrates, products, and activity of enzymes would be expected to affect the whole process. The root respiration process is also a regulated process between the cytochrome pathway and the alternative pathway. Factors influencing the activity of a specific pathway, and distribution between paths may affect the whole process. And the respiration process in roots may depend upon the substrate input from the above ground plant parts. Factors influencing the above ground photosynthesis, phloem loading, transporting, phloem unloading, and the permeability of cell membranes may affect the whole process also. Finding and characterizing the factors regulating the root respiration process is necessary to understand the whole process. Some of the factors regulating the root respiration processes are reviewed in the following sections.

Carbohydrates

Carbohydrates such as starch or sucrose are the major metabolic substrates when plants are grown under normal conditions. There is considerable evidence to support the hypothesis that light mainly affects root respiration through photosynthesis (Ledig *et al.* 1976, Singh and Gupta 1977), even though phytochrome in certain cases also controls respiratory activity by changing the size of the NAD^+ pool in the mitochondria (Morohashi *et al.* 1993). Using isotope techniques, Osman (1977) found that root respiration rate was higher when wheat shoots were put under light than in the dark. Hansen and Jensen (1977) also found a positive correlation between photosynthesis and root respiration in Italian rye grass. Even exogenous sucrose could stimulate root respiration rate in *Zea mays* (Saglio and Pradet 1980) and *Pisum sativum* (Bryce and ap Rees 1985). Farrar (1985) and Amthor (1989) also confirmed that an increase of carbohydrate concentration in roots affected root respiration positively.

On the other hand, some studies indicated that the rate of photosynthesis in the leaves did not influence root respiration because there was no diurnal trend of root respiration (Alm and Nobel 1991, Buwalda *et al.* 1992). Farrar (1981) and Hensen (1980) could not link root respiration change to the fluctuation of carbohydrate level. Lambers and associates (Lambers 1980, Lambers *et al.* 1991) generalized

that root respiration was not limited by carbohydrate level when plants were grown under high light intensity and long photoperiod; however, root respiration rate was regulated by carbohydrate input from leaves when plants were grown under low light conditions. These plants grown under low light increased root respiration during the day when the carbohydrate level increased, and decreased root respiration latter in the night when the carbohydrate level became depleted. The increase of root respiration during the day was predominantly due to an increase of respiration *via* the alternative pathway (Lambers *et al.* 1991). The alternative pathway serves as a kind of overflow mechanism in plant roots. The extra carbohydrates transported to roots from leaves goes to the alternative pathway as waste (Lambers 1980).

The mechanism by which available carbohydrates regulate the root respiration process is not well understood. We still do not understand how plants regulate the amount of carbohydrates transported to their root systems, how roots use the extra amount of carbohydrate besides in respiration *via* the alternative pathway as waste, what the capacity of the cytochrome pathway is and how it changes under various environmental conditions, what the significance of the alternative pathway is other than as an overflow mechanism, and how the concentration of available carbohydrate affects the activities of enzymes. To answer these questions, more research is required.

Oxygen

Unlike shoots, roots grow under a wide range of oxygen conditions. Oxygen concentration in the air phase of well aerated soil is generally close to that of the atmosphere, but it can be 1% or lower in flooded soils (Nobel and Palta 1989).

Considerable evidence suggests that oxygen concentration in the soil atmosphere affects root respiration. This information is mainly obtained from studies related to flooding (Carpenter and Mitchell 1980, Lee and Lee 1991, Tripepi and Mitchell 1984), which showed that oxygen consumption capacity of excised roots incubated under normal conditions declined after roots were kept under flooded condition for most species, although the proportion of this depression in respiration is smaller for tolerant species than for intolerant species.

When the concentration of ambient oxygen around the root was controlled more precisely, Korcak (1983) showed that root respiration did not change until the oxygen concentration was decreased to less than 10% for three blueberry progenies. Nobel and Palta (1989) and Palta and Nobel (1989a) also found for several desert shrubs that respiration of established roots remain constant until the oxygen concentration decreased to 16% followed by a steady decrease as oxygen concentration

was further decreased, but respiration of rain roots remained constant until the oxygen concentration decreased to as low as 5%, followed by a sharp decrease.

Leshuk and Saltveit (1991) measured the respiration rate change of carrot root disks continuously for four hours. They found that the respiration rate of root tissue decreased to a stable level two hours after transferring them from 21% to 8% or 4% oxygen in nitrogen gas mixture. Five to seven minutes after transferring the carrot disks from 21% to 2% or 1% oxygen, the respiration rate increased to a peak at about 50 minutes, then decreased to a stable level at three to four hours. The lower the oxygen concentration around the roots, the lower the respiration rate was after a stable level was reached. The authors attributed the respiration increase, which peaked at about 50 minutes, to anaerobic respiration. They suggested that there were two regulation mechanisms existing in plant roots. The first was a fast regulation response which occurred at very low oxygen concentrations (1 to 2%). This fast response was brought about by an increase in anaerobic respiration which leads to an increased ethanol or lactate production. The second mechanism hypothesized was a slow regulation response at low oxygen concentration (4 to 8%) and consisted of a reduction in activity of cytochrome oxidase.

Carbon dioxide

Carbon dioxide concentration has been reported to inhibit root respiration (Gifford *et al.* 1985). Plant root respiration remains unaffected when CO_2 concentration fluctuates over a relatively small range since roots are adapted to the soil environment (Amthor 1991). After studying several desert shrubs, Nobel and Palta (1989) and Palta and Nobel (1989a) found that root respiration did not change until CO_2 concentration increased from atmospheric concentration (approximately 350 ppm) to 2000 ppm, and root respiration decreased when the CO_2 concentration was further increased. In contrast to those results, Gifford *et al.* (1985) found that root respiration decreased sharply for several crop species when CO_2 concentration increased from 340 ppm to 680 ppm. Qi (1994) also found root respiration of Douglas-fir decreased exponentially as the CO_2 concentration in the root environment increased from atmospheric to 7000 ppm.

Carbon dioxide concentration has been shown to inhibit the phosphorylation enzymes (Monning 1983), and the alternative dehydrogen enzyme (Gifford *et al.* 1985). Thus the high CO_2 concentration inhibits the activity of both of the electron transport pathways. The high carbon dioxide concentration also reduces the permeability of cell membranes (Amthor 1991).

Temperature

Temperature effects on root respiration are commonly described as the equation proposed by Svante Arrhenius for chemical reactions (Earnshaw 1981, Sowell and Spomer 1986):

$$V = A\ e^{-Ea/RT}$$

Where V is the specific reaction rate, A is a constant for a particular reaction, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. When $Log_{10}(V)$ is plotted against (1/T), a straight line is obtained. This is called the Arrhenius plot. The activation energy can be obtained by multiplying the slope by 2.303R. The value of Q_{10} is also used to describe the response of root respiration to temperature, which is the ratio of the respiration rate at temperature T+10 to the respiration rate at temperature T. The activation energy and Q_{10} can be interconverted by the relationship:

$$E_a = \frac{2.303R \times T_1 T_2 \times Log_{10}(Q_{10})}{T_2 - T_1}$$

Where T_1 and T_2 are the two temperature levels at which respiration is measured. A typical response curve of root respiration to temperature is shown in Figure 1, which was redrawn from Palta and Nobel (1989bc). Root respiration rate increases in accordance with the Arrhenius equation when temperature is lower than point d. The curve may appear as an

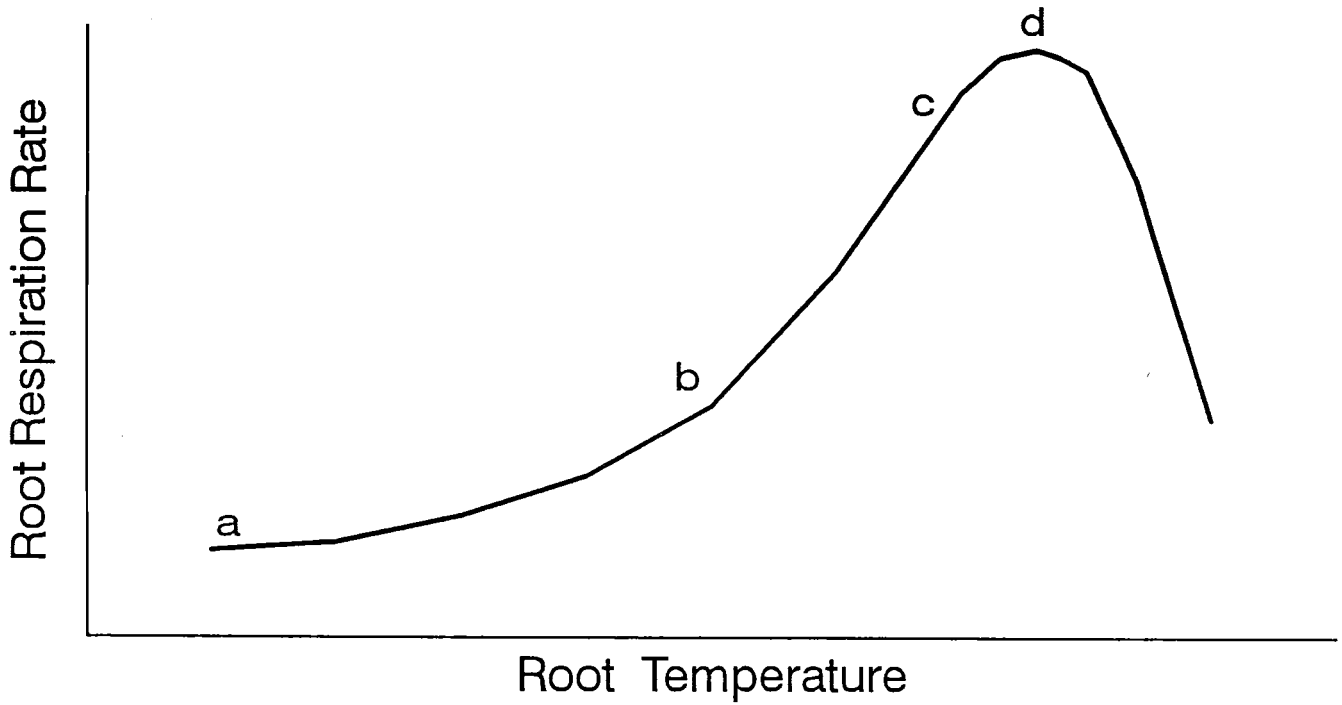

Figure 1. A typical response curve of root respiration to root temperature. The respiration from point a to d can be described by the Arrhenius Equation. Part of the line from point b to c may appear linear

exponential curve as temperature increases from a to b, as a near-linear line as temperature increases from b to c, and as a progressive saturation line from c to d. When the temperature is increased above point d, root respiration decreases. The temperature range from a to d described by the Arrhenius equation can be called the physiological temperature range. The physiological temperature range varies from species to species. For two cacti, *Ferocactus acanthodes*, and *Opuntia ficus-india*, the physiological temperature range of root respiration was from 5 to 50°C, and from 5 to 55°C respectively (Palta and Nobel 1989b). For *Agave deserti* (Palta and Nobel 1989ac), the physiological temperature range was from 2 to 42°C. The physiological temperature range tended to extend to higher temperatures with older roots.

For most plants, Q_{10} is approximately 2 (Ryan 1991b); that is, root respiration doubles when root temperature increases by 10°C. For example, Lawrence and Ochell (1983) found that the Q_{10} of root respiration for *Alnus crispa*, *Populus balsamifera*, *Populus tremuliodes*, and *Betula papyrifera* ranges from 1.98 to 2.33, 1.63 to 3.07, 1.46 to 2.0, and 1.53 to 2.58 respectively. Sowell and Spomer (1986) found that this value for *Abies losiocarpa* and *Picea engelmannii* was 1.93 to 2.03 and 1.98 to 2.00 respectively. Cropper and Gholz (1991) reported a value of 2.13 for *Pinus elliottii*.

Another interesting aspect of the effect of temperature on root respiration is the shift of Q_{10} values as temperature changes. This shift suggests that plants have the ability to adjust their root respiration process to their environment. Sisson (1983) reported that in *Yucca elata* plant roots, the Q_{10} decreased from 2.1 (12-22°C) to 1.7 (26-36°C) as temperature increased. Respiration ceased after five hours at 42°C. But others showed that plants did not adjust their root respiration response to root temperature when the root temperature regime changed. Earnshaw (1981) measured several arctic grass species under the same conditions, and found that species adapted originally to cold climatic conditions still have lower Q_{10} values, and higher respiration rates at low temperatures, but lower respiration rates at high temperatures than those species adapted to warmer temperature conditions. Sowell and Spomer (1986) reported that the differences in root respiration responses to temperature of plants transplanted from different elevations did not diminish two years after growing the plants under common conditions.

Activity of oxidative phosphorylation

The cytochrome electron chain is closely coupled with oxidative phosphorylation. Thus inactivation of the coupled reaction may limit the electron flow from reductant to oxygen

via the cytochrome electron chain. Low concentration of ADP and high ATP concentration may cause the reduction of this phosphorylation process. Lambers *et al.* (1991) defined this effect as the "adenylate regulation". Day and Lambers (1983) found that uncouplers stimulated cytochrome respiration for roots that had alternative respiration. They concluded that the activity of the cytochrome pathway was limited by the capacity of the oxidative phosphorylation process. Lambers *et al.* (1991) proposed that a certain concentration of ADP is essential for moving protons across the inner membrane. The membrane became leaky for protons when the ADP concentration was too low in the root cells, so the electron transport from various reductants to oxygen was restricted.

Root respiration tends to increase when plants are grown under such conditions that they need more energy which ultimately comes from the higher activity of oxidative phosphorylation. An observation is the "injury effect" described by Evans (1972), who reviewed seven papers and his own work, and concluded that respiration increased after injury of various plant tissues. Plant tissue requires more energy to reconstruct their damaged components. Other observations include an increase in respiration after plant roots were exposed to increased level of nitrogen fertilizer (Hansen 1980, Barneix *et al* 1984, Lambers 1987), infection of pathogens (Zacheo and Molinari 1987), when associated with nitrogen fixing symbiotic fungi (Tjepkema and Winship 1980),

or with mycorrhizal fungi (Baas *et al.* 1989, Anderson and Rygieweicz 1991). Plant cells need more energy in these cases.

Activity of the alternative pathway

The alternative pathway, which is also called the cyanide-resistant pathway, or non-phosphorylating pathway, is one of the two terminal electron transport pathways. In this pathway, alternative oxidase which resides in the inner membrane of mitochondria, passes the electron to O_2 from ubiquinone. Ubiquinone is the branching point between the cytochrome pathway and the alternative pathway. There is no proton extrusion, hence no energy conservation in the alternative pathway from the branching point (Lambers 1980).

The significance of the alternative pathway is not clearly understood. Lambers (1980) hypothesized that it serves as "an energy overflow and overcharge mechanism", i.e, the function of the alternative pathway in the roots of higher plants is to oxidize the extra sugars transported from the shoots, which exceeds that needed in roots for carbon skeleton and energy production for growth and maintenance processes. There is evidence supporting this hypothesis. Firstly, Lambers *et al.* (1991) found that electrons could be redirected to the alternative pathway when the cytochrome pathway was

blocked, but there was no redirection of electrons from the alternative pathway to the cytochrome pathway. Secondly, Lambers (1981) found that the activity of the alternative pathway decreased as photosynthesis decreased. Diurnal trends of root respiration for plants grown under low light intensity followed the activity pattern of the alternative pathway. Thirdly, alternative respiration decreased for plants under water and salinity stress. The amount of carbohydrates used in sorbitol synthesis for osmoregulation is the same as that saved in respiration due to the decreased activity of the alternative pathway (Lambers 1985). Fourthly, the decrease in root respiration of plants grown in the dark was mainly accounted for by the alternative respiration (Lambers 1980). Finally, alternative pathway respiration decreases together with increases in carbohydrates in roots which accumulates inulin, starch, and sugars (Steingrover 1981).

Soil water status

Under normal condition, roots partition available carbohydrates to intermediates for root growth or maintenance, cytochrome respiration, and alternative respiration as overflow (Lambers 1980). When roots are under water stress, available carbohydrates are used to synthesize solutes to maintain osmotic balance. This osmotic adjustment competes

with alternative respiration for available carbohydrates (Lambers *et al.* 1991). This competition may extend to the synthesis of intermediates for root growth and maintenance or even cytochrome respiration, depending on the severity of the stress. Exposure to dry soil leads to a gradual decline in root respiration in many plants (Vartanian and Chauveau 1986, Reekie and Redmann 1987). Nicolas *et al.* (1985) found that the decline in respiration in *Triticum aestivum* roots was correlated with the accumulation of organic solutes, and that the respiration reduction was predominantly due to the decrease of alternative respiration.

Palta and Nobel (1989bc) studied the water stress effect on three desert species: *Agave deserti, Ferocactus acanthodes*, and *Opuntia ficus-indica*, and found that root respiration decreased as the soil dried. Respiration in the rain roots decreased to zero and did not recover after a critical soil water potential was reached. Respiration of established roots also decreased at their critical soil water potential, but recovered when water was reapplied. Root respiration was not affected when soil water content decreased from field capacity to threshold values (-0.5 MPa and -0.3 MPa for established roots and rain roots respectively). These results suggest that during periods of soil drying, functionally different roots respond to water stress differently. Roots start to decrease their respiration when

a certain threshold value is reached, and continue to decrease to a critical value at which ability to recover may be lost permanently.

Under medium osmotic stress, the root respiration rate of *Arnica alpina* plants increased because of the additional ATP production to meet the metabolic demands. A decrease in root respiration rate when this plant was under higher osmotic stress could be due to the competition for the limited carbohydrates between respiration and synthesis of osmotic solutes (Collier and Cummins 1993).

Soil water potential may affect root respiration indirectly. Soil water content was reported to affect nitrogen absorption in Douglas-fir seedlings fertilized with ammonium nitrate. Uptake of ammonium ions was much higher in the wet soil than in the dry soil, whereas uptake of nitrate ions was not affected by soil water content (Gijsman 1991).

Nitrogen

Among the essential elements for plant growth, nitrogen is usually the most limiting in natural soils (Mengel and Kirby 1979). In physiological studies of crop plants, NO_3^- is often chosen as a measure of the total anion uptake because NO_3^- uptake comprises over 90% of the total anion uptake (Veen 1981).

Nitrogen fertilizer, whether in nitrate or ammonium form, needs to be absorbed in root cells, and needs to be incorporated at least partially into N-containing organic compounds in root cells. This assimilation process requires energy (Johnson 1983, Lambers 1987, Morris and Dacey 1984). The increase in respiration due to nutrient uptake has been called "nutrient uptake respiration" (Lambers 1985). Nutrient uptake respiration could amount to 10 to 60% of the total root respiration (Veen 1981). Increases in concentration of ammonium and nitrate have been shown to increase root respiration. Margolis and Waring (1986) studied nitrogen effects and found that ammonium nitrogen fertilizer caused an increase in root respiration and an increase in the amount of free amino acids in Douglas-fir seedlings. Hansen (1980) demonstrated that root respiration rate increases after N fertilization in the ammonium form and that this increase was enhanced by high light intensity in *Lolium multiforum*. Vessey and Layzell (1987) reported that roots of plants fed 10 mM NO_3^- displayed specific respiration activity 20% higher than plants fed 2 mM NH_4^+.

Nitrate nitrogen is favored over ammonium nitrogen in most plants (Mengel and Kirby 1979), except in boreal coniferous species (Lavoie *et al.* 1992). Plant roots have higher nitrate reductase activity than needles (Sarjala *et al* 1987), so nitrate N is readily absorbed and incorporated into amino acids in plant roots. For most plants, ammonium N is

toxic at high concentration especially when pH is high. The incipient concentration of NH_3 is 0.15 mM and the lethal concentration is 6.0 mM (Bennett 1974). Since nitrate N has to be reduced in the assimilation process by plants, while ammonium N is readily assimilated, the differential effects of these two forms of N fertilizer on root respiration rate may be anticipated. Indeed, Hansen (1980) found that plant roots have higher respiration rates when fertilized by nitrate than by ammonium.

Others

Other factors have been reported to affect root respiration either directly or indirectly. The biotic factors include species (Anderson *et al.* 1985, Ledig *et al.* 1976, Sowell and Spomer 1986), root age (Palta and Nobel 1989c, Singh and Gupta 1977), root activity (Lambers 1987, Veen 1981), root diameter (Mori and Hagihara 1991), root health condition (Evans 1972, Zacheo and Molinari 1987), root origin at various depth in the soil profile (Holthausen and Caldwell 1980), and association with Nitrogen fixing organisms (Tjepkema and Winship 1980) or mycorrhizal fungi (Anderson *et al.* 1985, Anderson and Rygeiweicz 1991, Baas *et al.* 1989, Marshall and Perry 1987, Nylund 1988). The abiotic factors include light, mainly through photosynthesis (Amthor 1991,

Farrar 1985, Ledig *et al.* 1976, Singh and Gupta 1977), time of day and time of season (Andre *et al.* 1981, Holthausen and Caldwell 1980), nutrient status (Hansen 1980, Radman and Shumaway 1985), soil pH (Yan *et al.* 1992), and pollutants (Edwards 1991, Nelson 1991).

METHODS FOR MEASUREMENT OF ROOT RESPIRATION

The various methods which have been developed for measuring root respiration can be categorized as indirect or direct methods. Indirect methods estimate root respiration based on some kind of model such as that proposed by Running and Coughlan (1988), and others (DeJong *et al.* 1979, Joslin and Henderson 1987, Kucera and Kirkham 1971, Marshall and Waring 1985, Mori and Hagihara 1991, Raich and Nadelhoffer 1989, Ryan 1991b). The focus of this review is on direct methods for measurement of root respiration. Direct methods monitor respiration of roots directly, and can be either destructive or nondestructive depending on the means of handling the roots. Destructive methods include excising root segments, digging root systems, trenching the soil, and clear-cutting entire stands. Nondestructive methods involve the use of intact root systems of seedlings or mature trees in a stand. The direct methods most often used are incubation of excised root tips, root exclusion, and intact root inclusion.

They are discussed respectively in the following sections. In addition, some other methods which are not commonly used but have their unique values, are also discussed at the end of this section.

Incubation of excised root tips

Perhaps the most commonly used method for root respiration measurement is incubation of excised root tips in specific solutions. After a period of incubation, change in O_2 or CO_2 concentration of the solution can be measured. The dry weight of these excised roots can then be determined after the measurement of root respiration. Boyer *et al.* (1971) put 6 cm long root tips of yellow poplar (*Liriodendron tulipifera*), loblolly pine (*Pinus taeda*), and river birch (*Betula nigra*) into air-saturated water. Root respiration rates were determined by comparing the O_2 concentrations in the air-saturated water with root tips before and after a 15 minute incubation. Tripepi and Mitchell (1984) studied several tree species using a Warburg manometer to determine respiration rate. They incubated 3-cm long root tips in a buffer solution inside a glass flask, and used a 20% KOH solution in the central vial inside the flask to trap the CO_2 released from root segments. The flask was connected to a manometer so that the O_2 absorbed by the root tips could be

monitored, and the respiration rate was determined based on the pressure change over time. Sowell and Spomer (1986) studied the root respiration of subalpine fir (*Abies lasiocarpa*), and Engelmann spruce (*Picea engelmannii*) by putting 1-cm long root tips into Hoagland solution. They measured the O_2 depletion after 30 minutes incubation time with a polarographic oxygen sensor. Koncalova *et al.* (1989) used an IRGA system connected to a sealed glass flask containing buffer solution. Before incubating the root segments in the solution, they passed CO_2-free air through the solution to bring out the CO_2 molecules dissolved in the solution. After incubating of roots in the solution for one hour, 1 ml 10 mM HCl was added to the solution to release the CO_2 produced by the root segments. A stream of N_2 carrier gas was passed through the container to flush the CO_2 from the container and carry it to an IRGA for measurement of CO_2 concentration.

This method of incubating excised roots is commonly used under laboratory conditions because of the ease in controlling the root environment. Temperature is easily controlled by putting the container with roots in solution into a temperature controlled chamber or water bath. Another advantage of this method is its adaptability for studying mechanisms of root respiration; e.g., inhibitors, promoters, and other chemicals can be added to the solution easily. But this method also has serious limitations. Cutting of the root

causes injury to root tips which may lead to a higher rate of respiration (Evans 1972). The measuring time for respiration is limited because depletion in available carbohydrates in the root tips during the lengthy incubation may occur. In addition, this method produces respiration values 10 to 100 times higher than those obtained with other methods because only root tips are sampled, and root tips are much more active than the average of all root tissues of root systems (Ledig *et al.* 1976).

Root exclusion method

Soil respiration is comprised of two components: root respiration and respiration from all the soil organisms other than roots. So root respiration can be calculated from the difference between soil respiration containing roots and soil respiration after removal of roots. The root exclusion method involves measuring the total soil respiration, extracting roots from the soil, remeasuring soil respiration, and calculating the difference between these two measurements. Soil respiration is easily measured by the alkaline solution absorption method (Lundergardh 1927), which involves putting a jar of alkaline solution into an inverted can which sits on

the soil surface, titrating the alkaline solution with acid solution after a specific time, and calculating the CO_2 released from the soil surface.

The root exclusion method has been used to measure root respiration of seedlings in pots. Lawrence and Oechel (1983) compared CO_2 production of one set of pots with roots, and another set of pots without roots. The results obtained by this method may be questionable because the populations of soil organisms could be different in the pots with and without roots because roots exude organic compounds which support soil organisms. Edwards (1991) studied the root respiration of loblolly pine (*Pinus taeda*) seedlings by growing them in pots. He first measured the total soil respiration by putting the pots (shoot not included) in a closed chamber which was connected to an IRGA system. He then extracted all the roots from the pots, and remeasured the soil respiration of the same pots two days later. The reason for waiting two days for soil respiration measurements after root extraction was because of the increased microbial activity during the two days after the root extraction. The results obtained by this method depend on the validity of the assumption that the heterotrophic respiration is the same before and after the soil was disturbed. This assumption may not be valid if soil microbial activities did change when soil O_2 and CO_2 concentrations were changed after soil disturbances.

Many researchers used the root exclusion method to measure root respiration in forests. Wiant (1967) measured the root respiration of a 29-year-old plantation (*Pinus strobus*, and *Tsuga canadensis*) by comparing plots with roots to plots in which all visible roots down to a 30 cm depth had been extracted. The air in the inverted can on the soil surface was circulated into an IRGA system. Behera *et al.* (1990) made soil respiration measurements with a jar of alkaline solution in the inverted can to trap the CO_2 released from soil. They then titrated the solution with acid solution to determine soil respiration. The root respiration was determined from the intercept of regression of soil respiration rates on root dry weight in the soil. Bowden *et al.* (1993) and Ewel *et al.*(1987ab) partitioned the soil respiration into litter, dead roots, organic matter, and root respiration by removal of litter layer, dead roots, and visible live roots in the soil, and measuring CO_2 evolved under various conditions. Nakane *et al.* (1983) modified the above method by clear-cutting a stand (*Pinus densiflora*) close to a control stand. They tried to create the same environmental conditions for the clear-cut area as in the control by providing shade. They then measured the CO_2 evolved from both sites after one year. They assumed that roots died in the clear cut area, taking into consideration CO_2 evolution due to the decomposition of roots which died as a results of the clear-cutting and the change of soil organic

carbon flows after clear-cutting, and concluded that the root respiration rate comprised about half of the total soil respiration.

The primary advantage of the root exclusion method over the excised root tip incubation method is that it can be used in the field, it measures the respiration of average root systems, it does not require expensive instrumentation, and it is easy to apply to a large area. The disadvantages are that mature trees, or at least roots are lost, and the soil is disturbed due to tree cutting or root extraction. This method generally yields lower values (Ledig *et al.* 1976) compared to other methods, and the disturbances introduced during root extraction clearly cause changes in soil structure, litter layer, microorganism and fauna distribution, and microclimate.

Intact root inclusion method

A recently developed method has made possible the measurement of root respiration in intact root systems. With this method, a portion of an intact root system is enclosed in a sealed chamber either with or without soil media, and air is circulated from the chamber to a CO_2 concentration detecting device. This method can be used under field conditions. Sisson (1983) used this method to measure root respiration for *Yucca elata*. He put a single intact root still connected to

the root system into a sealed chamber in which humid air was circulated. The CO_2 concentrations from the inlet and outlet were monitored by a gas chromatograph. The root respiration was computed from the difference between CO_2 concentrations of air at the inlet and the outlet, and the air flow rate. Vogt *et al.* (1989) designed a plexiglass chamber in which a portion of various diameters of roots can be clamped. The root respiration rate was calculated from the change of CO_2 concentration over time in the plexiglass chamber as a closed system. Palta and Nobel (1989ac) used a similar method to study root respiration of *Ferocactus acanthodes, Opuntia ficus-indica*, and *Agave deserti*. They enclosed various sizes of intact roots in a chamber and covered it with a layer of sterilized wet soil. Then they passed air of known CO_2 concentration through a wet cotton filter into the root chamber and monitored the CO_2 concentration of air at the inlet and outlet with an IRGA system. Cropper and Gholz (1991) modified the method that Palta and Nobel used by putting various sizes of roots of mature slash pine (*Pinus elliottii*) in a temperature controlled chamber, and including wet paper in the chamber to prevent water stress.

With the root inclusion method, we can measure respiration rate on intact roots. Disturbances of roots can be minimized by proper care. For most purposes, this method is the best of the methods currently used. Disadvantages of this method are that separating roots from soil particles

requires very careful work, destruction of roots is required if respiration is to be expressed on a weight basis, and repeated measurements of respiration and root growth cannot be performed.

Others

In addition to the commonly used methods described above, there are some other methods that may provide a basis for the development of new methods. These methods are discussed in this section.

Glosser and Tesarova (1978) took soil cores, hand separated the root segments from soil, laid the root segments into a chamber which was immersed in a temperature controlled water bath, and circulated humidified air through the chamber into an IRGA. The root respiration rate was calculated from the flow rate and CO_2 concentrations of inlet and outlet air. This method has the potential of being applied in the field, and various roots can be sampled. The disadvantages are that the injury of roots from coring cannot be avoided, and the respiration of nutrient uptake is missing.

Korcak (1983) used a nutrient solution incubation method. This method involves using a flask containing nutrient solution, putting the whole root system into the flask, sealing the flask, and circulating air through the

flask onto a CO_2 concentration device, and detecting the CO_2 concentration of inlet and outlet air. This method is basically a modified root tip incubation method, except intact roots are used. This method does not require expensive instruments, and the injury of roots can be minimized. The disadvantage is the long flushing time because of the dissolved carbon dioxide in the nutrient solution. If a lower pH in the solution is used, the flushing time can be shortened. Another disadvantage is that the water stress effect on root respiration cannot be assessed directly.

Helal and Sauerbeck (1991) measured respiration rates of non-sterile roots of maize during a short time of inhibition of microbial respiration with selected specific inhibitors. They found that 9 out of 11 microbicides tested either inhibited or stimulated root respiration. The inhibition ranged from 18 to 65%, and the stimulation ranged from 27 to 32%. Only two substances, namely streptomycin and p-hydroxymercuriphenylsulphonate, did not affect root respiration, presumably due to their slow uptake by roots. This method provides the potential to separate root respiration from microbial respiration in the soil. But the inhibitory effects of these chemicals on root respiration must be evaluated.

MATERIALS AND METHODS

ROOT BOX

The root box used in this study was developed based on descriptions of James *et al.* (1985) and Neufeld *et al.* (1989). The root box allowed the shoots of seedlings to grow in a normal environment while also constraining the root to grow in only two dimensions sandwiched between a micro-porous fabric and a glass plate for easy viewing (Figure 2). The modification to make the root box airtight (Anderson and Rygiewicz 1991, Rygiewicz *et al.* 1988) allowed the measurement of the amount of CO_2 released from the chamber, and the modification of lengthening the root box to 45 cm long extended the period of measurements of root growth and respiration. The root box was 15 cm wide, 45 cm long, and three cm thick with 570 cm^3 internal volume. The body of the root box was a black acrylic plastic block with a shallow cavity (42 x 10 x 1.3 cm) milled from the central portion, an O-ring groove around the cavity, a top opening for the plant shoot, and top and bottom hose fittings. Other components included a perforated plexiglass plate overlaid with fabric at the bottom of the cavity to retain the sand, a rubber (Buna A) O-ring to prevent air leakage, a 0.45 μm pore size Versapor membrane (Versapor, Gelman Sciences, New Jersey) to prevent root penetration into the sand, a six mm thick glass cover for

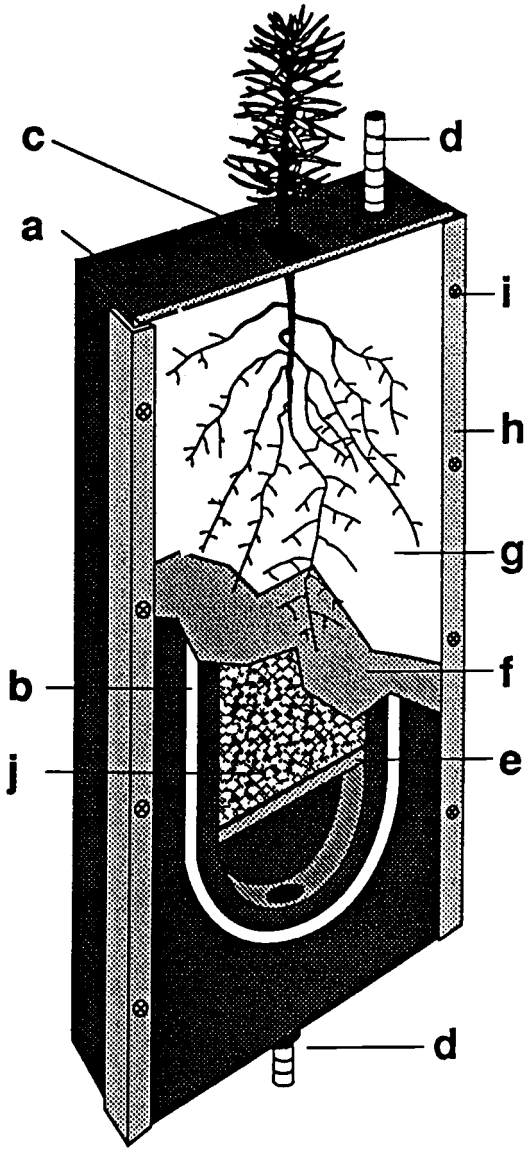

Figure 2. Three dimensional diagram of root box containing sand and a growing seedling. The main part of the root box is a black acrylic plastic block (a) with a rubber O-ring (b), a top opening (c), and top and bottom hose fittings (d). Other components include perforated plexiglass with a fabric layer (e), a Versapor membrane (f), a glass cover (g), aluminum frames (h), bolts (i), sand (j), and a removable black plastic sheet covering the glass (not shown)

easy observation of roots, aluminum angle brackets with bolts to secure the cover, and a removable black plastic sheet covering the glass. The 1.3 mm diameter silicon sand inside the root box was used as a soil medium to hold water and nutrients for the plants. the sand was ashed at 430°C for 24 hours in a muffle furnace to oxidize any organic matter.

The root boxes were assembled and packed with sand just before use. First, the O-rings, glass covers, and the black acrylic plastic blocks were washed with 70% ethanol, then with soap and sterilized water. The Versapor membranes were then cut to size. The membrane was wide enough so it could be pulled flat for good contact with the glass. Next, the perforated plexiglass plate with fabric layer, O-ring, and membrane were placed in position on the black acrylic plastic block. The glass cover was then placed over the cavity and aluminum brackets were bolted over the glass to hold it in place. After assembly, the root box was placed upright and sand was poured into the root box through the upper opening. An electric vibrator was used to settle the sand. A one-cm space above the sand was left at the top of the root box. After packing the sand, the top opening was sealed with rope caulking.

The sand was watered and allowed to drain overnight. Then the root box was opened, the cover glass was removed, and a seedling having a root length of about 15 cm was placed on the membrane with the shoot protruding through the opening at

the top of the box. The seedling shoot was sealed in place with rope caulking and the cover was reinstalled. When seedlings were placed into the boxes at the time the sand was installed, approximately one-third of the seedlings died. But when seedlings were planted after the sand had been packed and watered as described above, there was no seedling mortality.

SYSTEM FOR MEASUREMENT OF ROOT RESPIRATION

Measurements of root respiration and root growth was commenced when the root dry weight was 0.2 gram. The system for measuring root respiration included a root box, a tank of compressed standard air containing 1000 ppm CO_2, an IRGA (CI-301, CID Inc., Vancouver, Washington), a flushing line, a carrier line, and a reference line (Figure 3). All three lines originated at the same air tank. Air containing 1000 ppm CO_2 was chosen for measurement of root respiration because it is representative of the soil air at the top 10 cm of mineral soil in a forest at Scio, Oregon (Mattson, 1993), because CO_2 concentration has been shown to affect root respiration rates (Qi 1994), and because the level of CO_2 concentration of air drawn from the root boxes was within the measurement limit of the IRGA. Before measurement, the root boxes were flushed with the standard air. The purpose of the flushing was to bring the CO_2 concentration inside the root

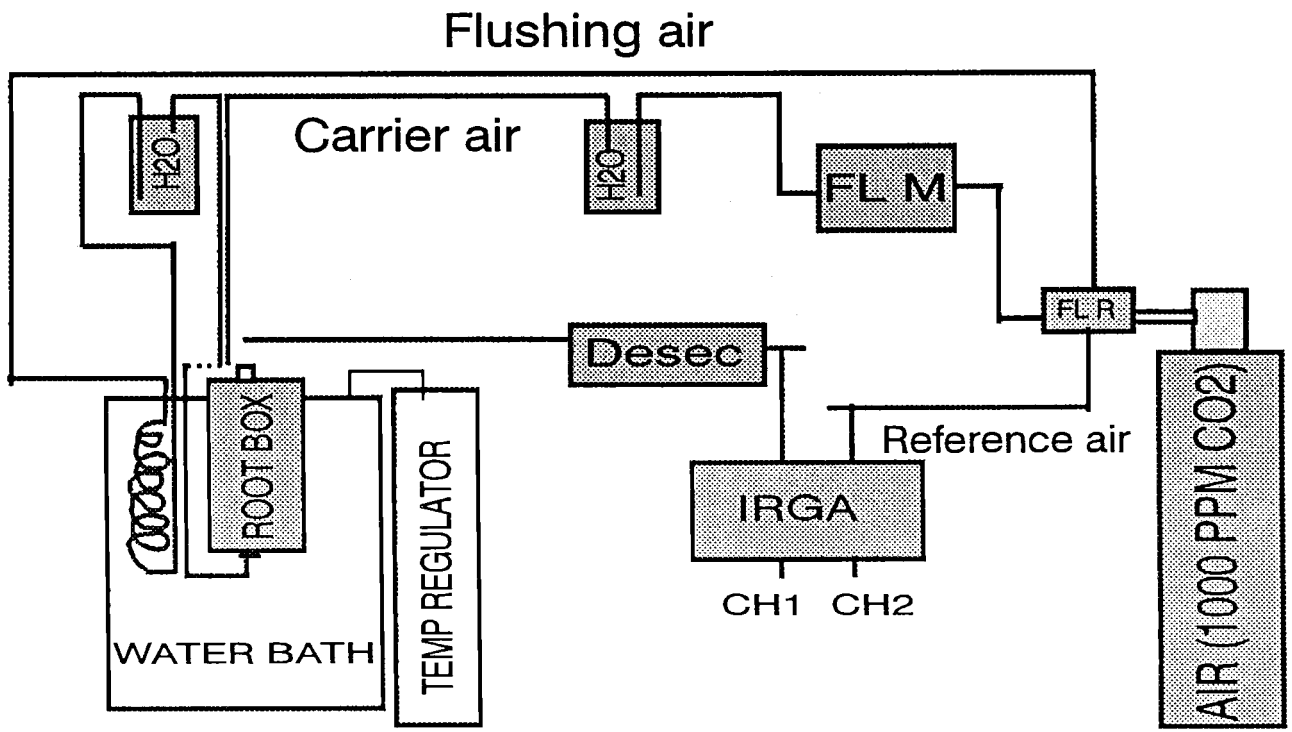

Figure 3. The system for measurement of root respiration

box to equilibrium so that the release of CO_2 dissolved in the nutrient solution inside the root box would be negligible. The flushing line directed the standard air through a water bubble device to humidify the air, then to a copper coil in the water bath for temperature adjustment, and finally to the bottom port of the root box. The effluent from the root box was vented to the atmosphere. The flow rate was about one liter per minute. At ten minute intervals during the 40 minutes before measurement, each root box was flushed for 20 seconds. Then the root boxes were subjected to a constant flush for seven minutes. The root boxes were ready to be connected to the carrier line after the constant flushing. The carrier line led the standard air through a flow meter, a water bubbling humidifier, the root box, a magnesium perchlorate dehumidifier, then to channel 1 in the IRGA. A temperature equilibrium coil was not included in the carrier air because the carrier air was found not to affect the root box temperature during the measurement of root respiration. A reference line led standard air directly to channel 2 in the IRGA. Two minutes after connecting the root box to the carrier line, the CO_2 concentrations for carrier air and reference air were recorded for five minutes using the two channel mode. The gap time between the channels was set at 25 seconds. The differences in CO_2 concentrations for carrier air and reference air were calculated from the last five recordings for both channels. The average of the five

differences between the two channels was used for the calculation of root respiration rate. The flow rate of the carrier air and the reference air was 0.4 L min^{-1}. The flow rate of carrier air was measured, while the flow rate for the flushing air and reference air was roughly set because they were not critical for calculation of root respiration rates.

SEEDLING PREPARATION

Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] seeds were collected from a 40-year-old stand in the western Cascade range near Scio, Oregon. The seeds were stratified at 2 to 5°C for three weeks in the dark, then germinated and grown at 25°C in sand in a growth room. Douglas-fir seedlings were three months old and western hemlock seedlings were nine months old, when they were transplanted into root boxes. The seedlings were put under low light intensity (200 μmol E $m^{-2}s^{-1}$) for recovery after transplanting. New root growth could be seen within two to three weeks. Then these seedlings were transferred to a greenhouse.

The greenhouse was illuminated with natural light supplemented with cold white fluorescent lights for 14 hours during the day. Lights were on at six in the morning and off at eight in the evening. The maximum light intensity was 200

and 750 μmol $m^{-2}s^{-1}$ for rainy days and sunny days respectively. During the experimental period, daily maximum air temperature ranged between 26 and 32°C and daily minimum air temperature ranged between 13 and 24°C. The temperature of shoots fluctuated with air temperature in the greenhouse, while the root temperature was controlled with temperature-regulated water baths. The root temperatures were 8, 13, and 18°C for Douglas-fir seedlings, and 15°C for western hemlock seedlings.

The seedlings were watered with nutrient solution modified from Rygiewicz (1983) containing 0.2 mM $K_2HPO_4 \cdot 3H_2O$, 0.2 mM $CaCl_2$, 0.04 mM $MgSO_4 \cdot 7H_2O$, 0.001 mM H_3BO_4, 0.001 mM $MnSO_4 \cdot H_2O$, 0.001 mM $ZnSO_4$, 0.0004 mM $CuSO_4$, 0.0004 mM MoO_3, and 0.04 mM Fe-EDTA in distilled water. NH_4NO_3 was the nitrogen source. The seedlings were watered with 1.8 mM N nutrient solution during the time before root respiration and N treatments. The pH was adjusted to 5.0 with 0.1 N HCl and 0.1 N NaOH solution.

Only nutrient solution was applied to the root boxes with or without seedlings. Sufficient nutrient solution was applied each time to cause it to drain from the bottom of the root boxes. Nutrient solution was applied to root boxes every two days routinely, except that it was applied in the evening when respiration measurements were to be made the following morning. In a preliminary experiment, wilting of Douglas-fir

seedlings was observed on the fourth day after watering. No sign of wilting was observed in western hemlock seedlings during five days after watering.

CALCULATION OF ROOT RESPIRATION RATE

Root respiration rates were calculated using the relationship:

$$RRR=\frac{(PPM_{sdl}-PPM_{blk})\times FR}{RDW\times VOLUME}$$

Where RRR is root respiration rate (μmol CO_2 $g^{-1}min^{-1}$), PPM_{sdl} is the difference in CO_2 concentration between the carrier and reference air from the root box with a seedling (μL L^{-1}), PPM_{blk} is the difference in CO_2 concentration between the carrier and reference air from the root box after removal of the seedling (μL L^{-1}) unless otherwise stated, FR is the flow rate of carrier air (L min^{-1}), RDW is the root dry weight (gram), and VOLUME is the volume that one mole of ideal gas occupies at the pressure and temperature at which the respiration measurement was conducted (L mol^{-1}).

DOUGLAS-FIR EXPERIMENTS

These experiments were conducted during the four month period from January to May 1993. Fifty-six seedlings were used in these experiments. Seedlings were randomly assigned to three groups. In the first group, there were 48 seedlings grown at three root temperatures (8, 13, and 18°C) and three nitrogen treatments (0.36, 1.8, and 7.2 mM N). The effects of root temperature and nitrogen concentration in the nutrient solution on root growth and respiration were examined. In the second group, there were four seedlings grown at greenhouse temperature and 1.8 mM N treatments. The effects of shoot and root disturbances on root respiration were examined. In the third group, there were four seedlings grown at greenhouse temperature and 1.8 mM N treatments. The effect of water stress on root respiration was examined.

Effects of temperature and N on root growth and respiration

The experiment was designed to: 1). determine how root respiration responds to root temperature, and develop a model to describe this relationship; 2). determine the N effect on root respiration, and identify possible interactions between temperature and N; 3). determine if the models describing the

response of root respiration to temperature are a function of root growth temperature; and 4). determine the variation in root respiration from day to day.

The 48 seedlings were randomly assigned to one of the three root temperature treatments: 8, 13, and 18°C. Within each temperature treatment, there were four seedlings treated with 0.36 mM N treatment, eight seedlings with 1.8 mM N, four seedlings with 7.2 mM N, and four blank root boxes bearing no seedlings with 1.8 mM N (table 1). The sample size of 1.8 mM N treatment was twice that of the other N treatments because one-half of the seedlings in 1.8 mM N treatment was originally planned to be infected with mycorrhizae. But inoculation with the fungus *Cenocuucum geophilum* Fr. failed and no roots were found to be infected. All seedlings under 1.8 mM N treatment were pooled as one treatment.

One month after the root temperature and nitrogen treatments began, respiration measurements including PPM_{sdl} from root boxes with seedlings, and PPM_{blk} from the blank root boxes bearing no seedlings were conducted at the temperature at which the roots were grown. After the respiration measurements, the root projection area for each seedling was measured by photocopying the roots onto transparent films and digitizing the images on the films with an area digitizing software program (Agimage, Decagon Devices Inc., Pullman, Washington). At two month after treatments, the same measurements were repeated on these same seedlings.

Table 1. Experimental design for the examination of the effects of root temperature and nitrogen treatments on root respiration of Douglas-fir seedlings. Root type s and b indicate root boxes with seedlings and without seedlings (blanks), respectively

Root temperature (°C)	Root box type	N concentration (mM)	Sample size
8	s	0.36	4
	s	1.8	8
	s	3.6	4
	b	1.8	4
13	s	0.36	4
	s	1.8	8
	s	3.6	4
	b	1.8	4
18	s	0.36	4
	s	1.8	8
	s	3.6	4
	b	1.8	4

At three-week intervals after two months of treatments, one of the three groups of seedlings grown at the three root temperatures was measured destructively. The first destructive measurements were conducted with the seedlings grown at 13°C root temperature. The PPM_{sdl} for all the root boxes with seedlings and PPM_{blk} for root boxes bearing no seedlings were measured on the first day at 13°C, then measured on the second day at 8°C and third day at 18°C. New root temperatures were normally reached before dark and maintained overnight before the respiration measurement to be conducted the following morning. The root projection area was measured on the fourth day. The seedlings were then harvested, and root and shoot dry weights were obtained by putting the seedlings into an oven at 70°C for 24 hours, and weighing them. After removal of seedlings, the root boxes were reassembled and put back into 13°C. The PPM_{blk} for the root boxes from which seedlings had been removed were measured for another three days at the three temperatures the same way as described for the root boxes with seedlings. The sequence of measurements took seven days. The second and third destructive measurements were conducted with seedlings grown at 18 and 8°C root temperature respectively. The measurements were performed as described above for the first destructive measurements except the order of root temperature changed from 18, 13, to 8°C, and from 8, 13, to 18°C for the second and third measurements respectively.

Due to temperature fluctuations within the three water baths, root box temperature fluctuated around the designed temperatures for the measurements conducted at one and two months after treatments (up to 2°C) and at the three destructive measurements (up to 1°C). Thus, PPM_{sdl} and PPM_{blk} values were recalculated to the designed root temperatures. The recalculation was based on the linear regression of Logarithm of PPM_{sdl} or PPM_{blk} on the reciprocal of the absolute temperature for each root box with or without a seedling.

There were two types of blanks in this experiment. The first type consisted of root boxes bearing no seedlings throughout the entire experiment. The second type consisted of root boxes after removal of roots. In the measurements conducted at one and two months after treatments, both types were considered for the PPM_{blk} calculation. The actual PPM_{blk} for the first type of root boxes could not be used because the variation in PPM_{blk} among root boxes was too large, and negative respiration values resulted for some roots. So an adjusted PPM_{blk} from the second type of root boxes was used. The adjustment was based on the equation:

$$PPM_{blk2} \times \frac{meanPPM_{blk1}}{meanPPM_{blk2}}$$

Where PPM_{blk1} is the individual values of PPM_{blk} from the first type of root boxes, and PPM_{blk2} is the individual values of PPM_{blk} from the second type of root boxes. In the three destructive

measurements, the second type of blanks was used directly. The PPM_{blk} values from both types of blanks measured at the same time, as in the destructive measurements, were not significantly different from each other ($P>0.05$).

In summary, for each seedling, there were three root projection area measurements (at one and two months after treatments, and at the destructive measurement), one dry weight measurement at the end of destructive measurements, and five measurements of root respiration rates (at one and two months after treatment at root growth temperature, and at three temperatures in the destructive measurements).

Root growth

A regression of root dry weights on projection area obtained from the three destructive measurements was conducted. This regression was then used to convert the projection area obtained from measurements conducted at one and two month after treatment into root dry weights. Root relative growth rate between one and two months after treatment was calculated as:

$$RGR = \frac{\ln\left(\frac{RDW_2}{RDW_1}\right)}{t_2 - t_1} \times 1000$$

Where RGR is relative growth rate (mg $g^{-1}day^{-1}$) of roots of each

individual seedlings, and RDW_1 and RDW_2 are root dry weights at t_1 (30 days) and t_2 (60 days) after treatments began. Mean relative growth rate (mg $g^{-1}day^{-1}$) of roots for individual seedlings during the entire experiment was obtained from the slope of the regression of root dry weight on time (days after treatments).

To examine the effects of root temperature and nitrogen treatments on root growth, two-way ANOVAs (α=0.05) were conducted for root dry weights at one and two months after treatments, for relative growth rates of roots between one and two months, and for mean relative growth rates of roots during the entire experiment. The LSD was used to separate the means.

Root respiration

The effects of root temperature and nitrogen on root respiration were examined with the data obtained from the three destructive measurements. A three-Factorial ANOVA (α=0.05) with a repeated measured variable was conducted. The root temperature at which roots of seedlings were grown, and nitrogen level were the two independent variables, and root temperature at which respiration was measured was the repeated measured variable. This analysis was conducted with the SAS program (SAS Institute Inc., Cary, NC). A MANOVA test was

used because the Sphericity test was significant (P=0.0001). The effects of the measurement temperature, the interactions between the measurement temperature and the growth temperature, and the interactions between the measurement temperature and the nitrogen level were examined in this analysis.

To further examine the measurement temperature effect, regressions of the logarithm of root respiration rates on the reciprocal of the absolute temperatures were conducted to obtain the intercept and slope values for each seedling, and for seedlings at each N level and root growth temperature. The intercepts, slopes, and significant levels of the regressions, along with determination coefficients were used to describe the root respiration response to root temperature. The Q_{10} values were also calculated from the slopes and temperatures (see the temperature section in literature review).

To examine the N treatment effect on root respiration, the root respiration data obtained from the three destructive measurements were used. Two-way ANOVA (α=0.05) was conducted at each measurement temperature. Nitrogen and the temperature at which root were grown were the two variables. The LSD was used to separate the means. To further examine if the N treatments affected the root respiration response to temperature (interaction between measurement temperature and nitrogen level), the slopes obtained from the destructive

measurements were compared by conducting two-way ANOVAs (α=0.05). Nitrogen level and growth temperature were the two variables.

A two-way ANOVA (α=0.05) for data obtained at one or two months after treatments was conducted to examine the effects of growth temperature, nitrogen level, and temperature and nitrogen interaction. The LSD was used to separate means.

Day-to-day variation of root respiration rates were examined with the data obtained from the two repeated measurements conducted one and two months after treatments began. The differences of root respiration rates for each seedling measured one and two months after treatments were calculated. The hypothesis that these differences were not significantly different from zero was tested (α=0.05).

Diurnal trend of root respiration

The diurnal effect on root respiration was examined on four of the seedlings (the same seedlings used to measure the effect of temperature and nitrogen level on root respiration and growth) grown at 13°C root temperature and 1.8 mM nitrogen treatment, along with the four blank root boxes containing no seedlings. The measurements of root respiration was conducted on a single day between one and two months after treatments. The diurnal measurements included PPM_{sdl} from each root box with

seedlings and PPM_{blk} from blank root boxes at different times during a 24-hour day and night cycle, and root projection area at the end of the day. The root area was converted to root dry weight according to the regression equation of root dry weight on root projection area. The average PPM_{blk} from blank root boxes at each measurement time was subtracted from PPM_{sdl} values for each of the root boxes with seedlings. The root respiration rate for each seedling at each measurement time was used in plotting the diurnal trends. A repeated measures analysis was conducted to examine the effect of diurnal time on root respiration.

Effects of disturbances

Effects of shoot and root disturbances on root respiration were examined with four Douglas-fir seedlings grown at greenhouse temperature and under 1.8 mM nitrogen treatment. One day before measurement of root respiration, the root boxes with seedlings, along with four blank root boxes without seedlings, were put into the 18°C root temperature treatment.

The effects of the following disturbances on root respiration were examined: (a). control: (b). shoot placed in the dark; (c). shoot excised: and (d). about 7% (by dry weight) of the five-cm long root tips severed from the main

root system, but left in the root box. All the measurements of root respiration were conducted when roots were in the root boxes. The PPM_{sdl} for all the root boxes with seedlings and PPM_{blk} from blank root boxes were measured before treatments and at different times thereafter for up to six days. Roots were harvested and dry weights were obtained at the end of these measurements. The average PPM_{blk} for the four blank root boxes at each measuring time was subtracted from the PPM_{sdl} of each root box with a seedling. The root respiration rates as percent of the respiration rates before treatments were used to represent the changes of root respiration rates over time. The effects of different disturbances on root respiration only provided preliminary results because there were no replicates for each treatment in this particular experiment.

Water stress

Water stress effects were examined with four seedlings grown at greenhouse temperature and under 1.8 mM nitrogen treatment. One day before the measurement, the four seedlings, along with four blank root boxes, were brought to 18°C root temperature. The root boxes were watered thoroughly in the evening and left to drain overnight. The PPM_{sdl} from root boxes with seedlings and PPM_{blk} from blank root boxes were first measured in the early morning (17 hours after watering),

and repeated at various intervals until 61 hours after watering. The root dry weights were then measured. Total root box weights were measured immediately after each respiration measurement. Water content was calculated as the percent of water weight to dry sand weight, which was determined at the time the root boxes were assembled. The average PPM_{blk} from the four blanks at each measuring time was subtracted from PPM_{sdl} for root boxes with seedlings. The root respiration rates were plotted against water content and hours after watering for each seedling. Regressions of logarithm of root respiration rates on water content for each seedling, as well as for all seedlings were conducted.

Maintenance respiration of roots

The maintenance respiration rate of roots are sometimes estimated from the intercepts of the regression of respiration rate of the roots on relative growth rate of the roots. The use of this method to obtain maintenance respiration rate of roots in this study failed because of the small sample size of seedlings under each treatment. The maintenance respiration rates were therefore obtained for both species from the measurements of root respiration rates over time after darkening the shoots.

The maintenance respiration rate of roots of Douglas-fir seedlings was estimated from the measurements of root respiration of those seedlings where the shoots were covered or darkened (as described in the section of effects of disturbances in the Douglas-fir experiment). Because the relatively stable respiration rate was never reached over time after treatment, the root respiration rates measured between 22 hours after treatment and 144 hours were used to indicate a rough range for maintenance root respiration rate.

WESTERN HEMLOCK EXPERIMENTS

Fifty-five seedlings were used in this study. Forty-eight of them were kept at 15°C root temperature and used for examination of temperature and nitrogen effects. The other seven seedlings were grown at greenhouse temperature and treated with 1.8 mM N in nutrient solution. Three of these seedlings were used for the water stress study, and the other four were used for measurement of maintenance root respiration.

Effects of temperature and N on root growth and respiration

All the 48 seedlings were grown at 15°C root temperature. There were four nitrogen levels each with 12 seedlings: 0.36, 1.8, 3.6, and 7.2 mM nitrogen in the nutrient solution, along with 12 blank root boxes treated with 1.8 mM nitrogen. Root respiration rates for all seedlings were measured at five root temperature levels: 8, 11, 14, 18, and 21°C. Shoot and root dry weights, and root respiration rates for all seedlings were conducted destructively at one, two, and three months after treatment began. The experimental design is summarized as:

Measurement date: 3 (1, 2, and 3 months after treatment)

Nitrogen level: 4 (0.36, 1.8, 3.6, and 7.2 mM N)

Root temperature: 5 (8, 11, 14, 18, and 21°C)

At the first destructive measurements, one-third of the seedlings for each nitrogen treatment, and blank root boxes were randomly selected and their root temperature was brought to 14°C one day before measurements. The PPM_{sdl} from root boxes with seedlings and PPM_{blk} from the blank root boxes were measured at 14°C root temperature on the first day, at 11°C on the second day, at 8°C on the third day, at 18°C on the fourth day, and at 21°C on the fifth day. The seedlings were then harvested to obtain shoot and root dry weights on the sixth day. The root boxes were reassembled after removal of seedlings and put into 14°C temperature treatment. PPM_{blk}

values from root boxes after removal of seedlings were then measured on the seventh day. At the second destructive measurement two months after treatments, half of the remaining seedlings for each nitrogen treatment and blank root boxes were randomly selected. The same measurements were conducted as described above except the order of temperature change was from 8, 11, 14, 18, to 21°C, and the PPM_{blk} from the root boxes after removal of seedlings were measured at 8°C. The third series of measurements were conducted similarly except the order of temperature change was 21, 18, 14, 11, and 8°C, and the PPM_{blk} from the boxes without roots were measured at 21°C.

PPM_{blk} values of root boxes after removal of seedlings were measured at one temperature level only, and values at other temperature levels were calculated. A regression of the Logarithm of PPM_{blk} values of the blank root boxes on the reciprocal of the absolute temperature was conducted for each of the three destructive measurements. Using this regression, PPM_{blk} of root boxes after removal of seedlings at all the root temperatures were then calculated from the one point measurement for each root box.

Root growth

To examine the nitrogen effect on root growth, a two-way ANOVA (α=0.05) was conducted for root dry weights, shoot

dry weights, and shoot-to-root ratios obtained destructively at one, two and three months after treatments began. Measuring dates and nitrogen treatments were the two variables. The mean relative growth rates of roots were obtained from the slope of the regression of the logarithm of root dry weights of seedlings under each nitrogen treatment on time of measurement (days after treatment).

Root respiration

A three-factorial ANOVA (α=0.05) with one repeated measured variable was conducted to examine the root temperature effect on root respiration. Nitrogen and measurement date were the two independent variables, and temperature at which respiration was measured was the repeated measured variable. A MANOVA test was used since the sphericity test was significant (P=0.0001). Root temperature effect, interactions between temperature and measurement date, and between temperature and nitrogen were examined. To further describe the root temperature effect, a regression of the logarithm of root respiration rate for each seedling on the reciprocal of the absolute root temperature was conducted.

To examine the N effect on root respiration, the root respiration rates of seedlings measured at the same root temperature on three dates were compared by conducting two-way

ANOVA (α=0.05). Nitrogen and measurement dates were the two variables, and the LSD was used to separate the means.

Effects of root damage

The effects of root damage were examined with 12 western hemlock seedlings grown at 15°C root temperature. These seedlings were from the third destructive measurement. These seedlings were put under 18°C treatment one day before measurements. The experiment was a two-factorial experiment. There was only one seedling per treatment cell. There were three nitrogen levels: 0.36, 1.8, and 7.2 mM, and four root damage treatments: (a). control, normal seedlings in the root boxes; (b). light root damage in the root box produced by opening the root box, lifting the root systems from the Versapor membrane, and replanting the root systems back into the root box; (c). light root damage in nutrient solution [same as (b) except the respiration was measured with the solution incubation method (described in the next paragraph)]; and (d). heavy root damage in nutrient solution [same as (c) except root systems were also cut into five-cm length segments]. The PPM_{sdl} was measured immediately after the treatments were applied. Because the root respiration rates kept decreasing for the heavy root damage treatment, root respiration data was recorded 10 to 20 minutes and 40 to 60

minutes repeatedly after treatment. After all the measurements, the seedlings were harvested, and root dry weights obtained. After removal of seedlings, the root boxes in treatments a and b were put back into the 18°C treatment, and PPM_{blk} were remeasured. The PPM_{blk} was zero for all the seedlings in treatments c and d, because root respiration was measured with the nutrient solution incubation method.

This experiment permitted examination of the effects of root damage on root respiration, and a comparison of the root box method and the nutrient solution incubation method as used by Korcak (1983) for measuring root respiration. The measurement system for solution incubation method is the same as the root box method (Figure 3) except for the substitution of a flask containing nutrient solution for the root box.

One-way ANOVA (α=0.05) was conducted to examine the effect of treatments a, b, c, and d (at 10 to 20 minutes after treatments). The N treatment served as blocks. Because of the repeated measurements under treatment d at different times after treatment, another ANOVA was conducted to examine the effect of treatment a, b, c, and d at 40 to 60 minutes. The difference of respiration between treatment d at 10 to 20 minutes and 40 to 60 minutes was used to test the hypothesis that the differences of respiration rates between these two times was not significantly different from zero. The LSD analysis was used to separate means of root respiration of seedlings under different disturbance treatments.

Water stress

The effect of water stress was examined with three of the seven seedlings grown at greenhouse temperature and 1.8 mM nitrogen treatment, along with four blank root boxes. The PPM_{sdl} from each root box with seedlings and PPM_{blk} from blank root boxes were measured at 15°C root temperature three times during the three day period after watering with nutrient solution. The average PPM_{sdl} from the four blank root boxes at each time of measurement were subtracted from PPM_{sdl} from each of the root boxes with seedlings. Root dry weights were measured destructively after all the respiration measurements were completed. Water contents were obtained as described for the Douglas-fir seedlings.

Maintenance respiration of roots

Maintenance respiration rate of root of western hemlock seedlings was measured using four of the seven seedlings kept at greenhouse temperature and 1.8 mM nitrogen treatments, along with four blank root boxes. The PPM_{sdl} from each root box with seedlings and PPM_{blk} from blank root boxes were measured once before covering the shoots, and continued for up to six days after treatment. The average PPM_{blk} from blank root boxes

at each measurement time was subtracted from the PPM_{sdl} of root boxes with seedlings. Relatively stable root respiration rates commenced four days after darkening the shoots. Therefore the overall mean of the root respiration rates four days after darkening the shoots was used as an estimation of maintenance respiration rate of roots.

RESULTS

THE ROOT BOX METHOD

The seedlings grew normally in the root boxes for four months for Douglas-fir seedlings and three months for western hemlock seedlings. There was no sign of abnormality of shoots in either species. Roots grew progressively downwards in the available space in the root boxes for both species. A typical root system of a Douglas-fir seedling grown at 13°C root temperature and 1.8 mM N concentration is shown in Figure 4. Growth data can be found in detail in the shoot and root growth section. Some roots tips tended to be congregated as they approached the bottom of the root box, probably due to increased resistance to rooting at the bottom of the root box. This could be improved by mixing sand with other soil media, such as Perlite, to reduce soil settling.

Several simple tests about how well the root box method worked were conducted. The pressure that root boxes could sustain was first determined, since leakage from the root box during root respiration measurement in the measurement system used here would result in an underestimate of the root respiration rate. Since the pressurized gas tank was used to force air through the root boxes, a slightly elevated pressure inside the root boxes would be expected. The root boxes were found to sustain a pressure of 1400 kg m^{-2} without leaking.

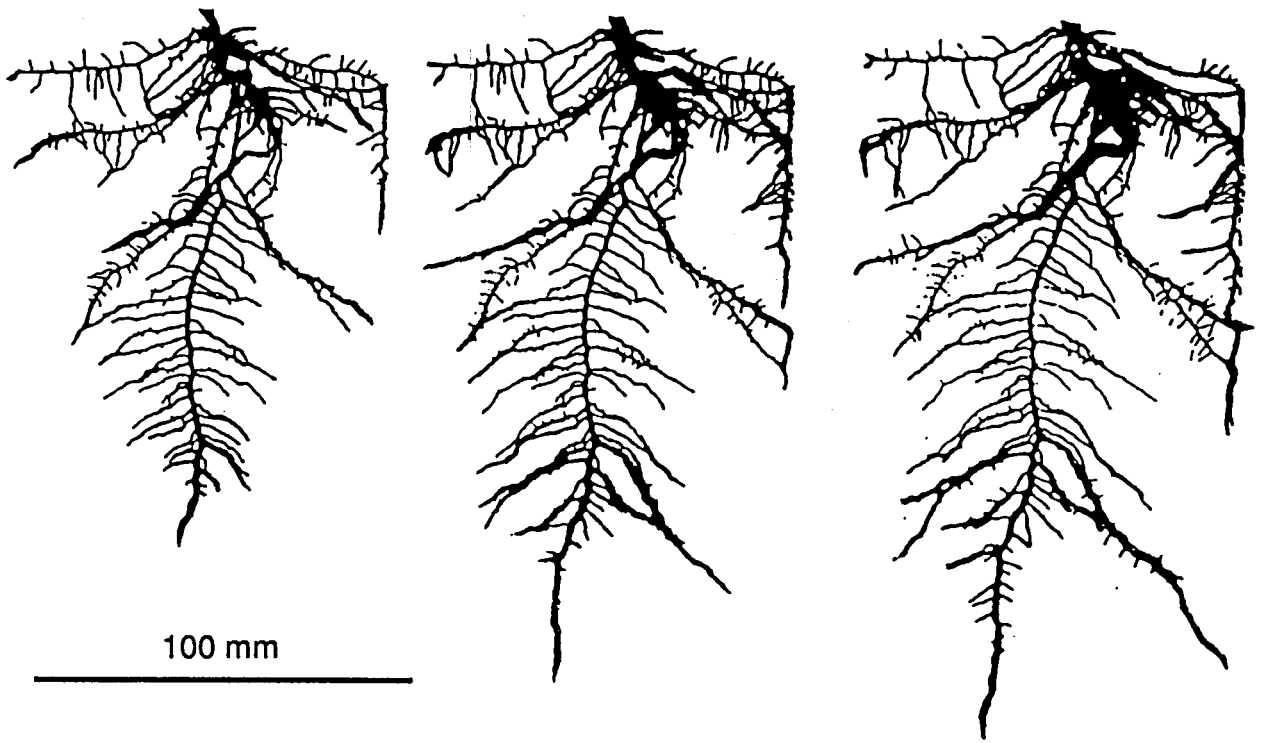

Figure 4. A typical root system of a Douglas-fir seedling grown in a root box. The seedling was under 13°C root temperature and 1.8 mM N treatments at 8, 11, and 14 weeks in the root box

The highest pressure encountered in this experiment was 480 kg m^{-2} during the flush of root boxes before root respiration measurements.

The second test determined the water holding capacity of the sand used in the root boxes. After thoroughly watering the sand in the root boxes and draining them overnight (17 hours after drainage), the water content of the sand averaged 11%, then decreased gradually (Figure 5).

The third test measured the respiration of blank root boxes. In a preliminary experiment, commercial sand was used, and the blank root box respiration was almost the same as respiration of root boxes with seedlings. The commercial sand was found to contain 0.09% organic matter. Several root boxes were sent to Soil Microbial Biomass Service, Botany department, Oregon State University for microbial analysis. It was found that the commercial sand support growth of bacteria, fungi, and several protozoa species. After ashing the sand, the respiration of blank root boxes averaged 20±7% of the total respiration from root boxes with seedlings. Protozoa species were not observed by naked eyes after ashing the sand during the experiment. Bacteria and fungi could be present as blank root boxes produced carbon dioxide.

The fourth test measured CO_2 concentration of the air inside the root boxes. The average CO_2 concentration of air inside the root boxes with Douglas-fir seedlings kept at 18°C root temperature was 5,500 ppm and ranged from 1,200 to 10,000

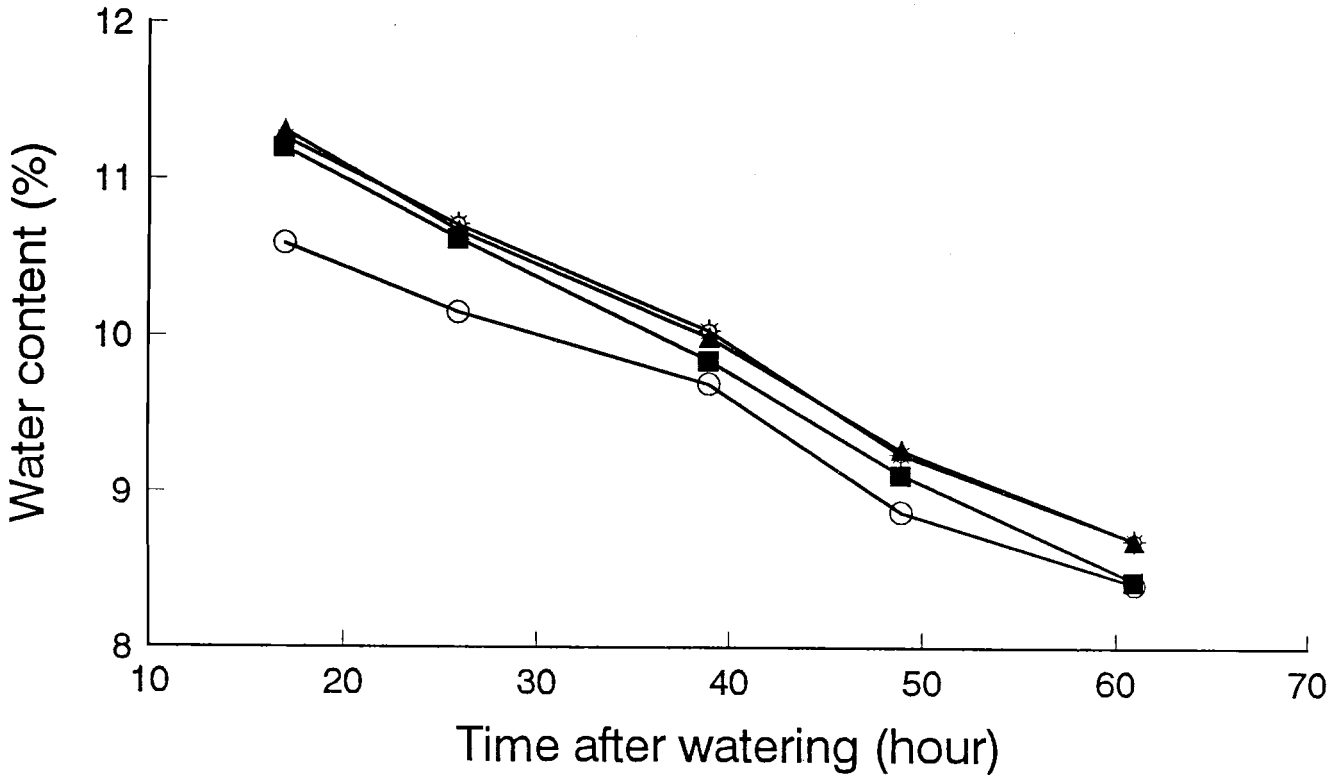

Figure 5. Changes of water contents of sand in four root boxes over time after watering. The four-month old Douglas-fir seedlings were grown at 18°C root temperature

ppm. The CO_2 concentration of air inside the root boxes increased as temperature increased from 8 to 18°C. The average CO_2 concentrations of air inside the root boxes with western hemlock seedlings grown at 15°C was 16,000 ppm and ranged from 3,700 to 35,000 ppm.

The last measurement evaluated the effect of flushing the root boxes before measurement of respiration. The CO_2 molecules dissolved in the nutrient solution inside the root boxes was slowly released to the carrying air when passing air containing lower CO_2 concentration (1000 ppm was used in this study) through the root boxes. Because of this effect, the root boxes were flushed with the standard air until the CO_2 release from the solution was negligible before any measurements of root respiration were taken. At least 30 to 40 minutes were required to reach the equilibrium point at which only CO_2 from the root system was released to the carrier air. The time needed to reach the equilibrium point depends on the size of cuvette, the flow rate, the CO_2 concentration of the carrier air, and the amount of CO_2 dissolved in the nutrient solution, which , in turn, depends on the pH and temperature of the solution. Because the size of cuvette and CO_2 concentration of the carrier air were preselected, only flow rate of flushing air and pH of the solution could be modified. After increasing the flow rate of the flushing air from 0.4 to 1.0 L min^{-1}, decreasing the pH of the nutrient solution from 7.0 to 5.0, and flushing the root

boxes with four brief flushes at 10 minute interval during the 40 minutes before measurement, five to seven minutes were required to reach the equilibrium level.

EFFECTS OF DISTURBANCES

All the three disturbances caused root respiration rates of Douglas-fir seedlings to decrease after a certain time (Figure 6). The root respiration rate oscillated for seedlings treated by either darkening or severing the shoot within the first eight hours after treatments, but respiration rate decreased thereafter. Severing the shoot had a greater effect on root respiration than simply darkening the shoot after 22 hours after treatments. Cutting off of 7% of the fine root tips and leaving them in the root box caused root respiration to increase to 6% higher than control values at one and half hours, then decreased to 15% below control values after 22 hours after treatments.

Root respiration rates of western hemlock seedlings increased immediately after cutting the root systems into five-cm segments, and recovered to normal respiration values at about one hour after treatment (Table 2). There were no significant differences between root respiration rates measured with the root box method and those measured with the solution incubation method. Simply lifting the root systems

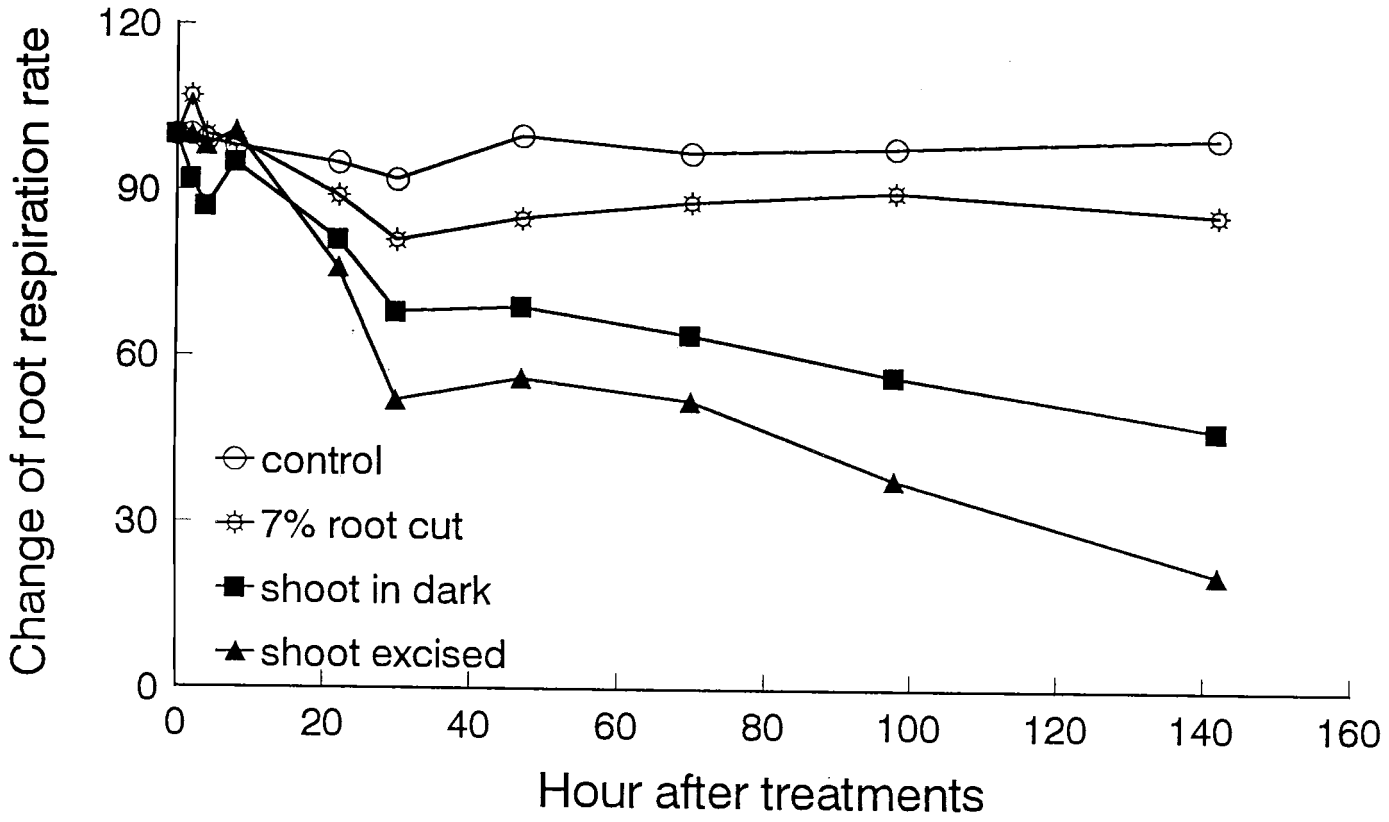

Figure 6. Changes of root respiration rates of Douglas-fir seedlings after various disturbances. The seedlings were grown at 18°C root temperature and 1.8 mM N treatments. The change of root respiration rate is percentage of the respiration rate at different times after treatments to respiration rates before treatments

Table 2. Root respiration rates of western hemlock seedlings under various root treatments. Root treatment: (A). control; (B). Root systems were lifted from fabric sheet in the root boxes, and replanted into root boxes. Respiration was measured in root box; (C). Treatment was same as (b) except respiration was measured in nutrient solution bottle; (D). Treatment was same as (c). In addition, root systems were cut in 5-cm segments. Respiration was measured at 10-20 minutes (D1) and 40-60 minutes (D2)after treatment.

N treatment (mM)	A	B	C	D1	D2
0.36	** 0.30	0.20	0.32	0.81	0.45
1.8	0.39	0.40	0.38	0.45	0.31
7.2	0.27	0.29	0.31	0.52	0.26
sign. test *	a	a	a	b	a

* The root respiration rates (μmol CO_2 $g^{-1}min^{-1}$) between columns with the same letters are not significantly different ($P<0.05$)

** From a single measurement of root respiration

from the Versapor membrane and replacing did not cause significant change of root respiration rate. Root respiration rates decreased back to normal value at 40 to 60 minutes after treatment for seedling under root cutting treatments.

SHOOT AND ROOT GROWTH

Root dry weights were highly correlated with root projection area (Figure 7). The root projection area obtained at one and two months after treatment, and obtained from the diurnal measurements were converted to root dry weight according to the regression:

$$RDW = 9.2 \times 10^{-5} \times A_r$$

Where RDW is root dry weight (gram), and A_r is the root projection area (mm^2). The P-value is 0.0001, and the determination coefficient (r^2) is 0.85 for the equation.

Douglas-fir

Two-way ANOVA on root dry weights showed both root temperature and nitrogen treatments significantly affected root dry weight of seedlings at one and two months after treatments. The interaction between temperature and nitrogen treatments was significant at one month after treatment

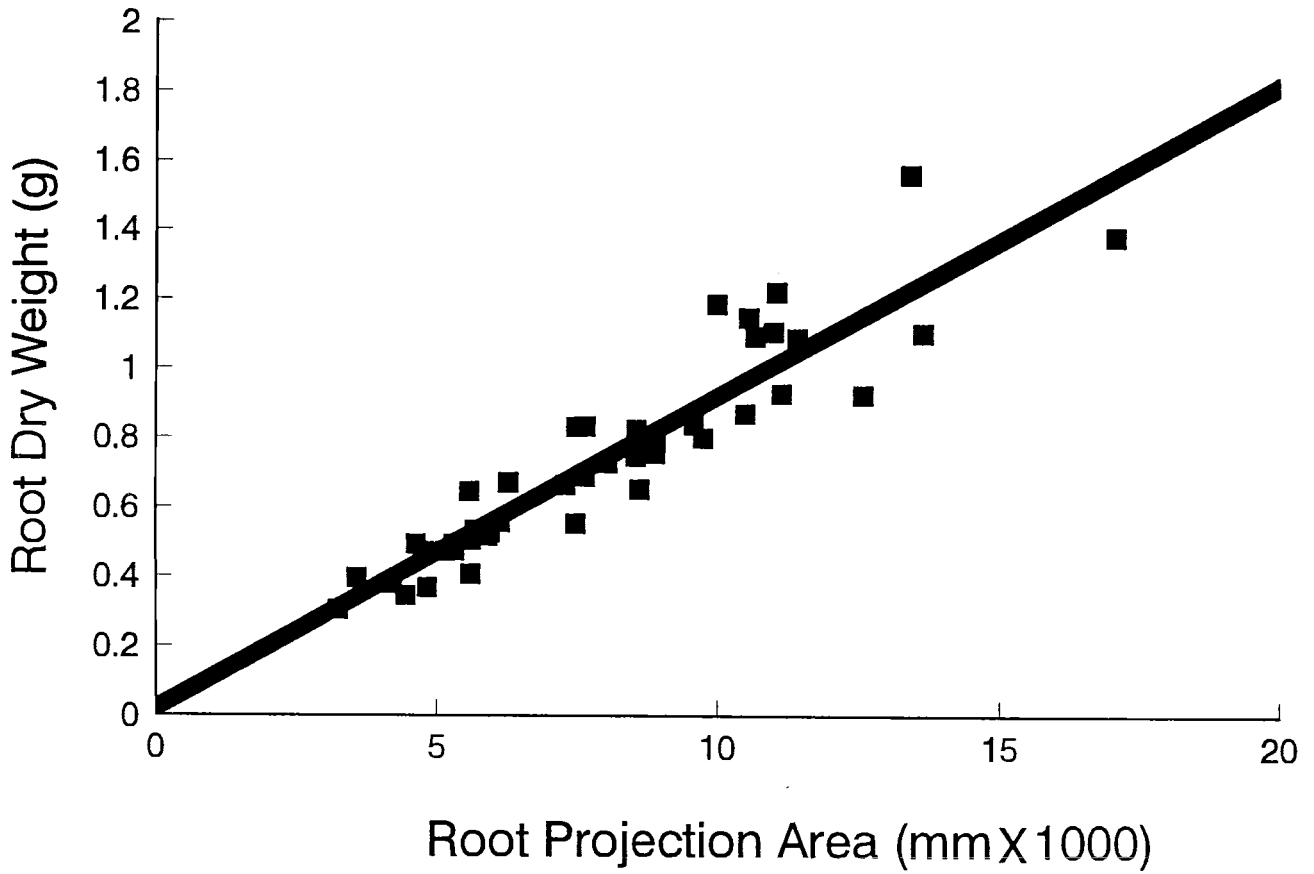

Figure 7. Relation between root dry weight (RDW) and root projection area (RPA). The line in the diagram represents the regression: RDW(g) = 9.2×10^{-5} RPA(mm^2)

(P=0.02) but not significant (P=0.10) at two month after treatment. Two-way ANOVA on relative growth rate obtained between one and two months after treatments showed significant effects of temperature (P<0.0001) and nitrogen (P=0.03) treatments. There was no significant interaction between temperature and nitrogen treatments. Two-way ANOVA on mean relative growth rate calculated for the entire experiment showed significant effect of nitrogen treatment. The effect of root temperature cannot be examined because the last root dry weights of seedlings grown at different temperatures were measured at different times. Generally speaking, both root temperature and nitrogen treatments affected root dry weight and relative growth of roots (P<0.05), and the interaction between temperature and nitrogen was not significant.

Root dry weight increased significantly (P<0.05) as root temperature was increased at one month after treatments (table 3). At two months after treatments, root dry weight of seedlings grown at 13 and 18°C root temperature were not significantly different from each other, but both were significantly higher than those of seedlings grown at 8°C root temperature. The relative growth rates of roots grown at different root temperatures between one and two months after treatments were significantly different, with the highest for roots grown at 13°C root temperature, intermediate for roots grown at 18°C root temperature, and lowest for roots grown at 8°C root temperature. This suggests that roots of seedlings

Table 3. Root dry weights and relative growth rates of Douglas-fir seedlings grown at three root temperatures. Root dry weight (g) at one month (RDW1) and two month (RDW2) after treatments, when seedlings were four and five months old. Relative growth rates (RGR1-2, mg $g^{-1}day^{-1}$) were calculated from one to two months after treatments.

Temperature (°C)	n	RDW1	RDW2	RGR1-2
8	16	* 0.242 a	0.272 a	6.7 a
13	16	0.344 b	0.498 b	18.5 c
18	16	0.431 c	0.544 b	11.7 b

* Means within each column with the same letters are not significantly different ($P<0.05$)

grown at 18°C grew faster within the first month after treatment began, but slowed down thereafter, compared to root of seedlings grown at 13°C.

Nitrogen treatment significantly ($P<0.05$) affected root dry weights and root relative growth rates of seedlings (Table 4). The root dry weights of seedlings grown under the lowest N level was significantly greater than those of seedlings grown under the two higher nitrogen levels at either one or two months after treatment began. At the end of the experiment, the root dry weights of seedlings at highest N level was significantly lower than those of seedlings at the two lower N levels. Root relative growth rate of seedlings grown under 1.8 mM N treatment between one and two months after treatments was significantly higher than that of seedlings grown under 7.2 mM N treatment, but not significantly different from that of seedlings grown under 0.36 mM N treatment. There was no significant difference between seedlings under 0.36 mM N and 7.2 mM N treatment. The mean relative growth rate of seedlings grown under 1.8 mM N treatment during the entire experiment was significantly higher than that of seedlings grown under the other two N treatments. There was no significant difference between the mean relative growth rate of seedlings under 0.36 mM N and that of seedlings under 7.2 mM N treatment. These results suggests that roots of seedlings at lowest N level grew fast in the first month after treatment, but slowed down to the

Table 4. Root dry weights, relative growth rates, and mean relative growth rates of roots of Douglas-fir seedlings grown at three root temperatures each with 3 N treatments. RDW1, RDW2, and RDWend are root dry weights (g) at 1 or 2 months after treatments and at the end of experiment respectively. RGR1-2 is the relative growth rate (mg $g^{-1}day^{-1}$) between 1 and 2 months after treatments.

Root Temp. (°C)	N Level (mM)	n		RDW1	RDW2	RGR1-2	RDWend *	MRGR
8	0.36	4	**	0.32(0.03)	0.35(0.05)	7.05(2.06)	0.87(0.08)	13.55(1.18)
	1.8	4		0.23(0.03)	0.28(0.04)	8.00(1.78)	0.75(0.08)	15.95(1.18)
	7.2	3		0.18(0.04)	0.18(0.05)	5.01(2.06)	0.48(0.09)	13.67(1.36)
13	0.36	4		0.38(0.03)	0.58(0.04)	20.18(1.78)	0.66(0.08)	13.78(1.18)
	1.8	6		0.27(0.03)	0.40(0.04)	20.08(1.46)	0.50(0.07)	15.52(0.96)
	7.2	3		0.38(0.04)	0.52(0.05)	15.09(2.06)	0.58(0.09)	10.47(1.36)
18	0.36	4		0.57(0.03)	0.70(0.04)	10.65(1.78)	1.08(0.08)	10.48(1.18)
	1.8	7		0.37(0.02)	0.49(0.03)	13.98(1.35)	1.12(0.06)	17.94(0.89)
	7.2	4		0.35(0.03)	0.43(0.04)	10.45(1.79)	0.77(0.08)	12.93(1.18)

* Seedlings grown at 13, 18, and 8°C were harvested at 11, 14, and 17 weeks after treatments

** Mean (standard error)

same or even lower rate during the later part of the experiment, compared to that of seedlings at the other two N levels.

At the end of the experiment, only Nitrogen effect could be examined because the root and shoot dry weights of seedlings grown at different root temperature were measured at different times. The two-way ANOVA, root temperature as block, on root dry weights showed that nitrogen significantly affected root dry weight (P=0.003). Seedlings grown at the highest N level had significantly small roots than seedlings grown at the two lower N levels (Table 5). Nitrogen did not affect shoot dry weights of seedlings (P=0.3). Seedlings grown at the lowest N level had smaller shoot-to-root ratios than seedlings grown at the two higher N levels (P=0.05).

It seems clear that seedlings grown at the two lower N levels had larger roots than seedlings grown at the highest N level. The roots of seedlings at the lowest N level grew fast at the beginning of the experiment, but slowed down later compared to that of seedlings at medium N level. This is probably the reason that the seedlings at medium N level had the highest mean relative growth rate of roots obtained for the entire experiment.

Table 5. Root and shoot dry weights, and shoot/root ratios of Douglas-fir seedlings grown at 3 temperatures and 3 N levels

Root Temp. (°C)	N Level (mM)	n	RDW (g)	SDW (g)	S/R ratio (g/g)
*	0.36	4	** 0.87 (0.08)	1.61 (0.22)	1.81
8	1.8	4	0.75 (0.08)	1.41 (0.22)	1.99
	3.6	3	0.48 (0.09)	0.98 (0.25)	2.14
	0.36	4	0.66 (0.08)	1.06 (0.22)	1.63
13	1.8	6	0.50 (0.07)	1.26 (0.18)	2.58
	3.6	3	0.58 (0.09)	1.46 (0.25)	2.56
	0.36	4	1.08 (0.08)	1.27 (0.22)	1.23
18	1.8	7	1.12 (0.06)	1.84 (0.17)	1.67
	3.6	4	0.77 (0.08)	1.35 (0.22)	1.76

* Seedlings grown at 13, 18, and 8°C were harvested at 11, 14, and 17 weeks after treatment respectively

** Mean (standard error)

Western hemlock

All the western hemlock seedlings were grown at 15°C root temperature under four N treatments. Only nitrogen effects on root and shoot growth could be examined (Table 6). Root dry weight of seedlings treated with 0.36 and 7.2 mM N was significantly ($P=0.007$) lower than that of seedlings treated with the two medium N level at all times after treatment. There was no nitrogen effect on shoot dry weight and shoot-to-root ratio of seedlings ($P>0.05$). Shoot-to-root ratio averaged 1.08 over the period from one to three months after treatments began. Nitrogen treatment did not affect mean relative growth rate of roots. The overall mean relative growth rate of roots for all the seedlings grown under different N treatments was 11.36 mg $g^{-1}day^{-1}$.

EFFECTS OF CONTROLLING FACTORS ON ROOT RESPIRATION

Effects of root temperature and N treatments

For Douglas-fir seedlings, the three-factorial ANOVA with one repeated measured variable showed significant effect of measurement temperature ($P=0.00001$) and nitrogen treatments ($P<0.05$) on root respiration rate. There was no interactions between root measurement temperature and root growth

Table 6. Root (RDW) and shoot (SDW) dry weights (g), shoot/root ratios (S/R), and mean relative growth rate of roots of western hemlock seedlings at four N treatments. Mean relative growth rates (mg $g^{-1}day^{-1}$) of roots (MRGR) was obtained from regression of root dry weights on time after treatment (days)

Measurement time		n	0.36	1.8	3.6	7.2
1 month	RDW	4	* 0.48(0.05)	0.70(0.17)	0.71(0.10)	0.55(0.02)
	SDW	4	0.59(0.17)	0.92(0.19)	0.83(0.18)	0.67(0.07)
	S/R	4	1.20(0.24)	1.37(0.11)	1.14(0.14)	1.23(0.13)
2 month	RDW	4	0.72(0.11)	0.94(0.19)	0.98(0.09)	0.88(0.25)
	SDW	4	0.71(0.12)	0.78(0.08)	0.89(0.10)	0.98(0.17)
	S/R	4	0.99(0.09)	0.86(0.07)	0.96(0.17)	1.22(0.20)
3 month	RDW	4	1.07(0.07)	1.37(0.26)	1.55(0.31)	1.17(0.20)
	SDW	4	0.87(0.15)	1.42(0.13)	1.40(0.26)	1.55(0.34)
	S/R	4	0.80(0.10)	1.31(0.15)	0.93(0.17)	1.36(0.17)
MRGR		1	11.77	10.36	12.09	11.22

* Mean (standard error)

temperature. There was no significant interaction between root measurement temperature and nitrogen treatment.

For western hemlock seedlings, The three-factorial ANOVA with one repeated measured variable showed that root temperature significantly affected root respiration rate ($P<0.00001$). There was no nitrogen effect, and interaction between nitrogen and root temperature treatments. However, the root respiration response to root temperature did depend on measurement dates and the order of root temperature change before respiration measurements. The effects of root temperatures and nitrogen treatments on root respiration rates for both Douglas-fir and western hemlock seedlings are reported in the following sections.

Root temperature

Root temperature significantly ($P=0.0001$) affected root respiration rates for both species. Root respiration rate of seedlings increased as root temperature was increased from 8 to 18°C for Douglas-fir seedlings (Figure 8) and from 8 to 21°C for western hemlock seedlings (Figure 9). Response of root respiration to root temperature for both species could be described as a linear equation:

$$LOG(RRR) = a - b \times \frac{1}{T}$$

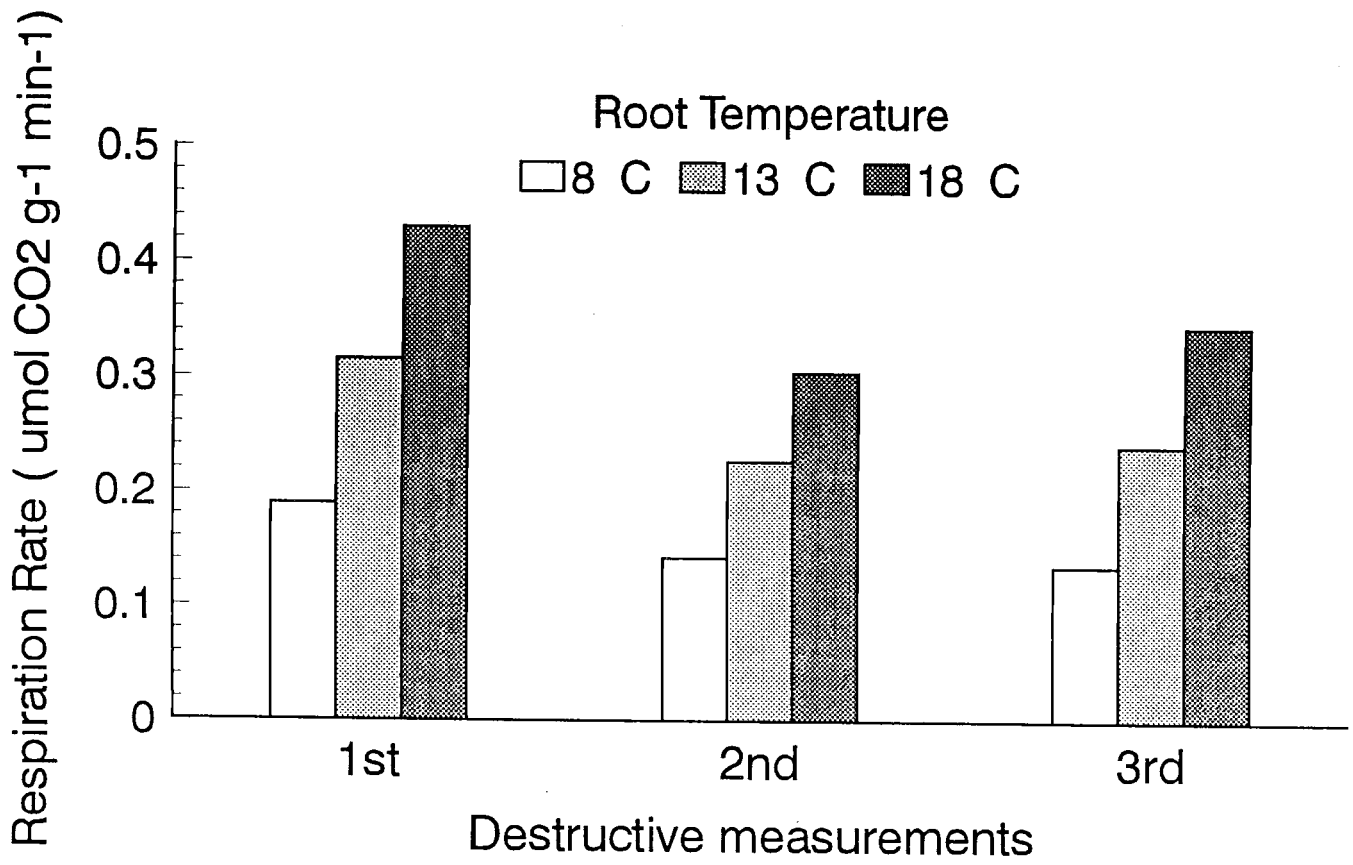

Figure 8. Root respiration rates of Douglas-fir seedlings measured at three temperatures destructively on three dates. The seedlings were grown at 13, 18, and 8°C root temperature for the 1st, 2nd, and 3rd destructive measurements. Root respiration rates of seedlings under different N treatments were pooled at each temperature level. Standard error bars were not included in the diagram because of the repeated measurements of respiration rates at the three temperatures on each date

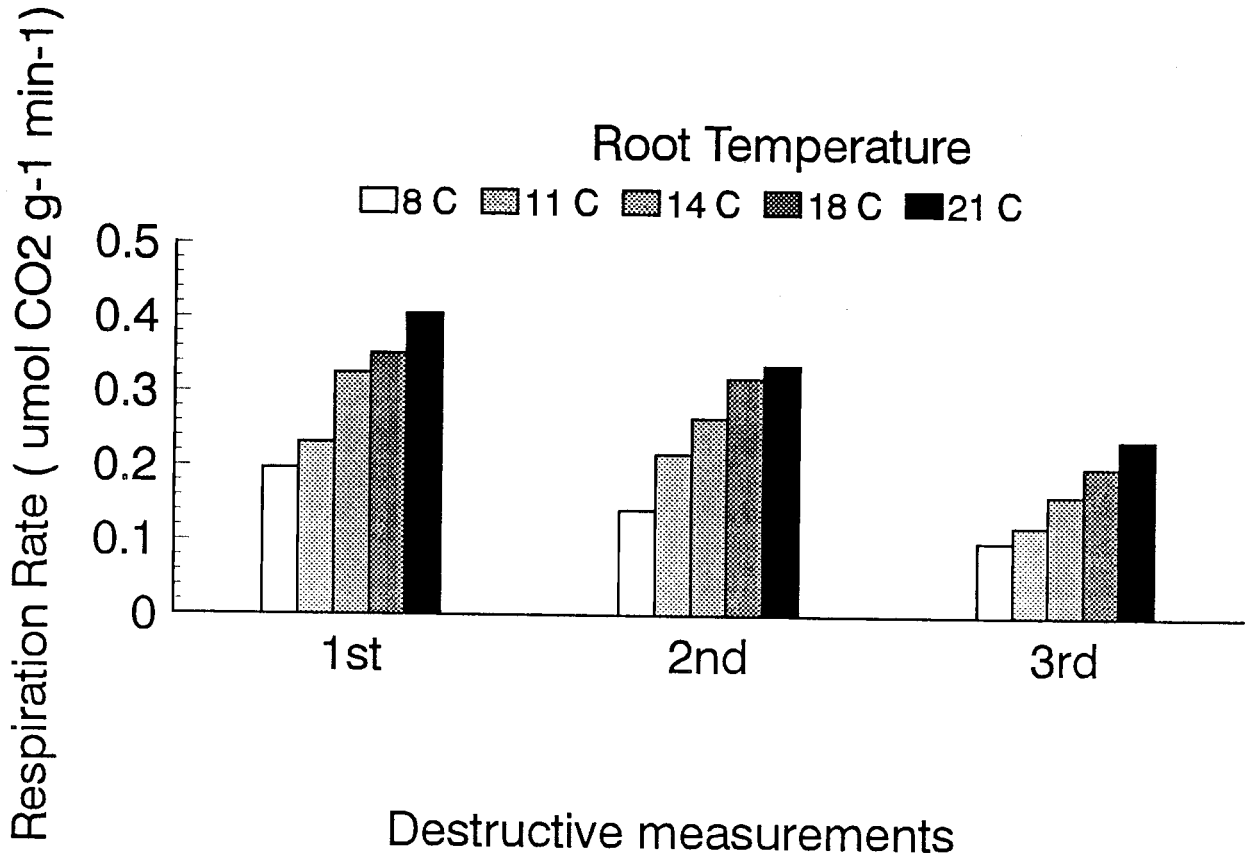

Figure 9. Root respiration rates of western hemlock seedlings measured at five root temperatures destructively on three dates. All the seedlings were grown at 15°C root temperature. Root respiration rates of seedlings under different N treatments were pooled at each temperature level. Standard error bars were not included in the diagram because of the repeated measurements of respiration rates at the five temperatures on each date

Where RRR is root respiration rate, a is the intercept, b is the slope, and T is the absolute temperature at which respiration rate was measured. The coefficients of these regression equations for individual Douglas-fir seedlings were:

a ranged between 6.07 and 11.70

b ranged between 1970 and 3572

r^2 ranged between 0.70 and 1.00

P-values ranged between 0.00004 and 0.1

Because there was no significant difference between respiration rate of seedlings at 1.8 mM N treatment and that of seedlings at 7.2 mM N treatment, these two groups were combined. This regression for all the seedings at 1.8 and 7.2 mM N treatment measured during the experiment was:

a 9.10

b 2771

r^2 0.34

P 0.00001

This regression for all seedlings at 0.36 mM N treatment during the experiment was:

a 9.69

b 2988

r^2 0.49

P 0.00001

The coefficients of these regression equations for individual western hemlock seedlings were:

a ranged between 3.23 and 9.84

b ranged between 1108 and 3151

r^2 ranged between 0.73 and 1.00

P-values ranged between 0.00003 and 0.09

Since there was no nitrogen treatment effect on root respiration found, all the groups were combined. This regression was:

a 7.25

b 2276

r^2 0.37

P 0.00001

The Q_{10} values ranged between 1.7 and 3.7 with an average of 2.23 for all the Douglas-fir seedlings, and ranged between 1.52 and 3.39 with an average of 2.35 for all the western hemlock seedlings.

The root respiration rates of Douglas-fir seedlings measured on the first destructive period were significantly ($p=0.0002$) higher than those measured on the second and third destructive period (Figure 8). There was no significant difference between root respiration rates measured on the second and third destructive period. The root respiration rates of western hemlock seedlings decreased significantly ($P<0.0001$) from the first, to the second, and to the third destructive measurement (Figure 9).

Root respiration responses to root temperature for Douglas-fir seedlings were not significantly different,

whether measured at the root growth temperature at which the roots of seedlings were grown (as measured at one and two months after treatments), or at measurement temperatures shortly increased or decreased from the root growth temperatures one day before measurement (as in the three destructive measurements). The slopes and intercepts from the regression equations obtained from both the two repeated measurements and the three destructive measurements were not significantly different for Douglas-fir seedlings ($P>0.05$).

For Douglas-fir seedlings, the three destructive measurements were conducted on three dates, the order of root temperature increase or decrease were different from one destructive measurement to another, and the root growth temperature were different for seedlings used on different dates. There is no way to separate these three factors. Using the slopes from the temperature response curve of root respiration for each seedling of the three destructive measurements, I conducted a two-way ANOVA to compare the effects of dates and N levels. There was no significant effect of either date or N treatment on the slopes found. The only difference in respiration rates of roots measured on three dates was the higher rates on the first date than the other two. Since respiration did change from day to day (see time section for detail), we can thus say root growth

temperature (the preconditioning root temperature), and the order of root temperature change one day before measurement did not alter the respiration response curve to temperature.

For western hemlock seedlings, the three destructive measurements were conducted the same way as Douglas-fir except the root growth temperature was 15°C before measurement. Root respiration rates of seedlings did change significantly from day to day (figure 9). The effects of growth temperature were of no concern since all the seedlings grew at 15°C. The ANOVA analysis on slopes for the logarithm of respiration rate on the reciprocal of the absolute temperature for individual seedlings on three destructive measurements showed that there was no effect of the order of temperature change before measurement on respiration. However, the interactions between measurement temperature and measurement date was significant. This could be due to the lower respiration rate at 21°C at the second destructive measurement (Figure 9).

Nitrogen

Nitrogen effects on root respiration rates of Douglas-fir seedlings measured at different root temperatures and N treatments at different times are shown in Figure 10. At all three temperature levels on all measuring dates, root respiration rates of seedlings increased as N level increased

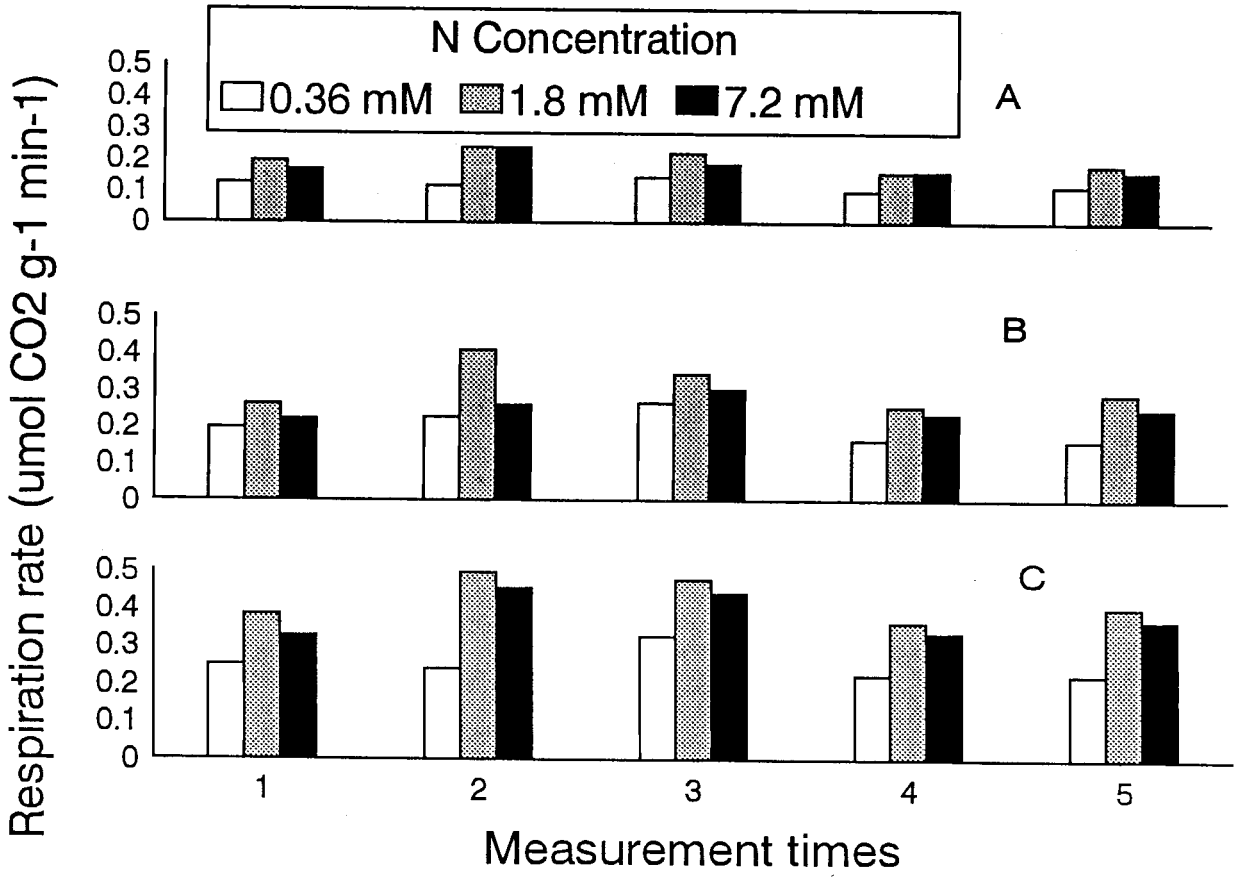

Figure 10. Root respiration rates of Douglas-fir seedlings at three temperatures each with three N levels. Root respiration rates were measured at one and two months after treatments began (1 and 2 in the diagram) at the temperatures at which roots were grown, and at 11, 14, and 17 weeks destructively after treatments began (3, 4, and 5 in the diagram). Standard error bars were not included in the diagram because of the repeated measurements of respiration rates at the three temperatures for the three destructive measurements (3, 4, and 5 in the diagram)

from 0.36 mM to 1.8 mM, and most of the respiration rates decreased as N level further increased from 1.8 mM to 7.2 mM. Analysis of variance showed that the root respiration rates of seedlings grown under 1.8 and 7.2 mM N treatment were not significantly different, but both were significantly higher than that of seedlings grown under 0.36 mM N treatment for all the root temperature levels measured on all dates, repeatedly or destructively. Nitrogen treatment did not affect the slope of the regression of the logarithm of respiration rate of roots on the reciprocal of the absolute temperature. The Q_{10} for all the seedlings under different N treatments was the same (2.23).

Generally speaking, nitrogen treatment did not affect root respiration rates of western hemlock seedlings, except the root respiration rates of seedlings under 7.2 mM N treatment was significantly higher than that of seedlings grown under other N treatments at the lowest root temperature level (table 7). The level of N treatments did not affect the slopes of the regression of the logarithm of root respiration rate on the reciprocal of the absolute temperature. All the seedlings under different N treatments had a common Q_{10} which was 2.35.

Table 7. Mean root respiration rates (μmol CO_2 $g^{-1}min^{-1}$) of western hemlock seedlings grown at four N treatments and measured at five root temperatures

Measurement dates (after treatment)	Root temp. (°C)	N concentration (mM N) 0.36	1.8	3.6	7.2
	*				
1 month	8	0.18(0.05)	0.19(0.05)	0.16(0.05)	0.26(0.05)
	11	0.21(0.05)	0.23(0.05)	0.19(0.05)	0.29(0.05)
	14	0.29(0.05)	0.32(0.05)	0.28(0.05)	0.42(0.05)
	18	0.34(0.05)	0.33(0.05)	0.29(0.05)	0.44(0.05)
	21	0.36(0.05)	0.41(0.05)	0.33(0.05)	0.52(0.05)
2 month	8	0.13(0.03)	0.15(0.03)	0.14(0.03)	0.14(0.03)
	11	0.22(0.03)	0.23(0.03)	0.21(0.03)	0.22(0.03)
	14	0.28(0.03)	0.26(0.03)	0.26(0.03)	0.26(0.03)
	18	0.36(0.03)	0.32(0.03)	0.30(0.03)	0.30(0.03)
	21	0.38(0.03)	0.33(0.03)	0.31(0.03)	0.32(0.03)
3 month	8	0.09(0.02)	0.12(0.02)	0.08(0.02)	0.11(0.02)
	11	0.11(0.02)	0.15(0.02)	0.10(0.02)	0.13(0.02)
	14	0.14(0.02)	0.20(0.02)	0.13(0.02)	0.18(0.02)
	18	0.17(0.02)	0.24(0.02)	0.17(0.02)	0.22(0.02)
	21	0.20(0.02)	0.29(0.02)	0.21(0.02)	0.25(0.02)

* All the means were from four measurements, mean (standard error)

Species

Root respiration rates across N levels for the two species were not significantly different (P=0.11) at any of the root temperature levels over time (table 8). However, the variation in root respiration rates of western hemlock seedlings was larger than that of Douglas-fir seedlings. The overall mean standard error for root respiration rates of western hemlock and Douglas-fir seedlings was 0.018 and 0.014 respectively. The minimum root respiration rate of Douglas-fir seedlings measured at 8 to 18°C root temperature was 0.07 μmol CO_2 $g^{-1}min^{-1}$, and the maximum was 0.69 μmol CO_2 $g^{-1}min^{-1}$. The minimum and maximum root respiration rate for western hemlock seedlings measured at the same root temperature range was 0.06 and 0.75 μmol CO_2 $g^{-1}min^{-1}$ respectively.

Time

There was no diurnal trend of root respiration rate change during the 24-hour day and night cycle for four Douglas-fir seedlings (Figure 11). One-way ANOVA of repeated measures design showed that the measurement time did not significantly affect root respiration rate (P=0.1). This suggests that the root respiration rate fluctuated randomly around a mean during the 24-hour day/night cycle.

Table 8. Means of root respiration rates of Douglas-fir and western hemlock seedlings measured at three root temperatures. There was no significant differences between root respiration rates (μmol CO_2 $g^{-1}min^{-1}$) of Douglas-fir and that of western hemlock seedlings (P=0.11)

Species	Root temp. (°C)	n		Destructive measurement 1	2	3
Douglas-fir	8	12	*	0.189	0.143	0.135
	13	15		0.315	0.226	0.241
	18	16		0.430	0.304	0.344
Western hemlock	8	16		0.196	0.141	0.100
	13	16	**	0.325	0.265	0.162
	18	16		0.351	0.318	0.201

* The significant test can not be performed on means within each column due to repeated measurements at different temperatures, but the regressions of the logarithm of the means on the reciprocal of the absolute temperatures were significant ($P < 0.05$).

** Calculated from respiration rates measured at 14°C, based on the regression: LOG_{10}(respiration rate) = a - b 1/T

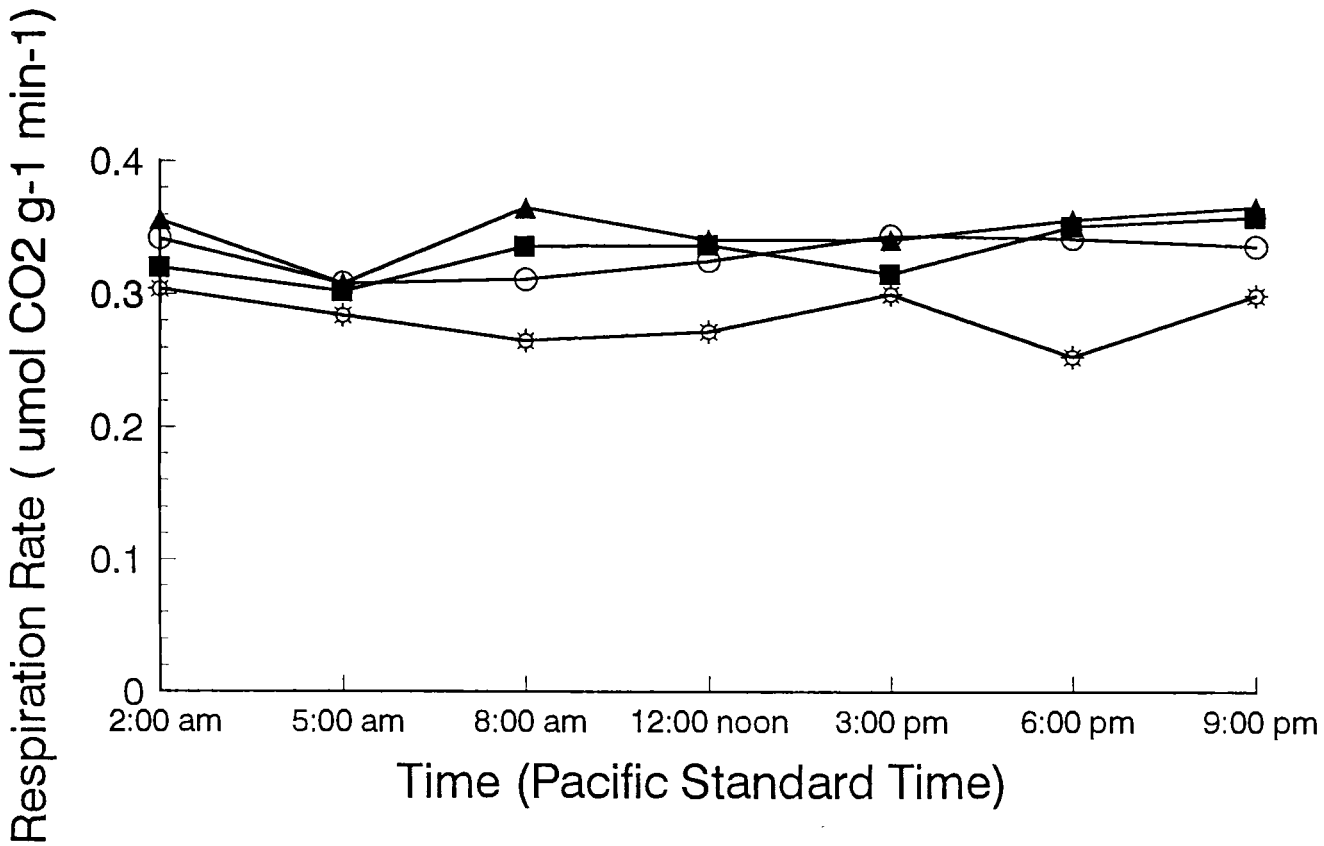

Figure 11. Root respiration rates of four Douglas-fir seedlings measured during a 24-hr day/night cycle. Root temperature fluctuated between 14.3 and 14.8°C. Artificial lights were on at 6:00 am and off at 8:00 pm. It was cloudy in the morning and became clear after 2:00 pm.

In contrast to the constant root respiration rate during the 24-hour day, the variation from day to day during the three-month period is large. Root respiration rates of Douglas-fir seedlings under different treatments measured one month after treatments were lower than those measured two months after treatments (Table 9). The differences of root respiration rates of the same seedlings measured at one and two months after treatments was significantly different from zero (P=0.0005). Root respiration rates of seedlings measured at two months after treatment averaged 0.066 μmol CO_2 $g^{-1}min^{-1}$ higher than those measured at one month after treatment. This suggests that root respiration varies from day to day.

Water stress

Root respiration rate decreased as soil water content decreased for Douglas-fir seedlings (Figure 12). The root respiration rates of western hemlock seedlings was measured three times only and showed the same response to water stress (table 10) as that of Douglas-fir seedlings. For Douglas-fir seedlings, the relation between respiration rate and water stress could be described as a regression of the natural logarithm of root respiration rates on soil water content for individual seedlings. The intercepts ranged from -1.8685 to -2.7386, the slope ranged from 0.1072 to 0.1748, and the

Table 9. Mean root respiration rates (μmol CO_2 $g^{-1}min^{-1}$) of Douglas-fir seedlings under different root temperature and nitrogen treatments measured repeatedly on two dates after treatments

N treatment	Root temperature (°C)							
(mM)	at one month			Sig. Test	at two month			Sig. Test
	8	13	18	*	8	13	18	*
0.36	0.134	0.196	0.250	a	0.115	0.239	0.226	a
1.8	0.201	0.261	0.383	b	0.235	0.406	0.476	b
7.2	0.167	0.220	0.327	b	0.238	0.279	0.453	b

* Root respiration rates with the same letters at various N levels measured on each date are not significantly different ($P<0.05$). The ANOVA tests were conducted on each temperature level on each date.

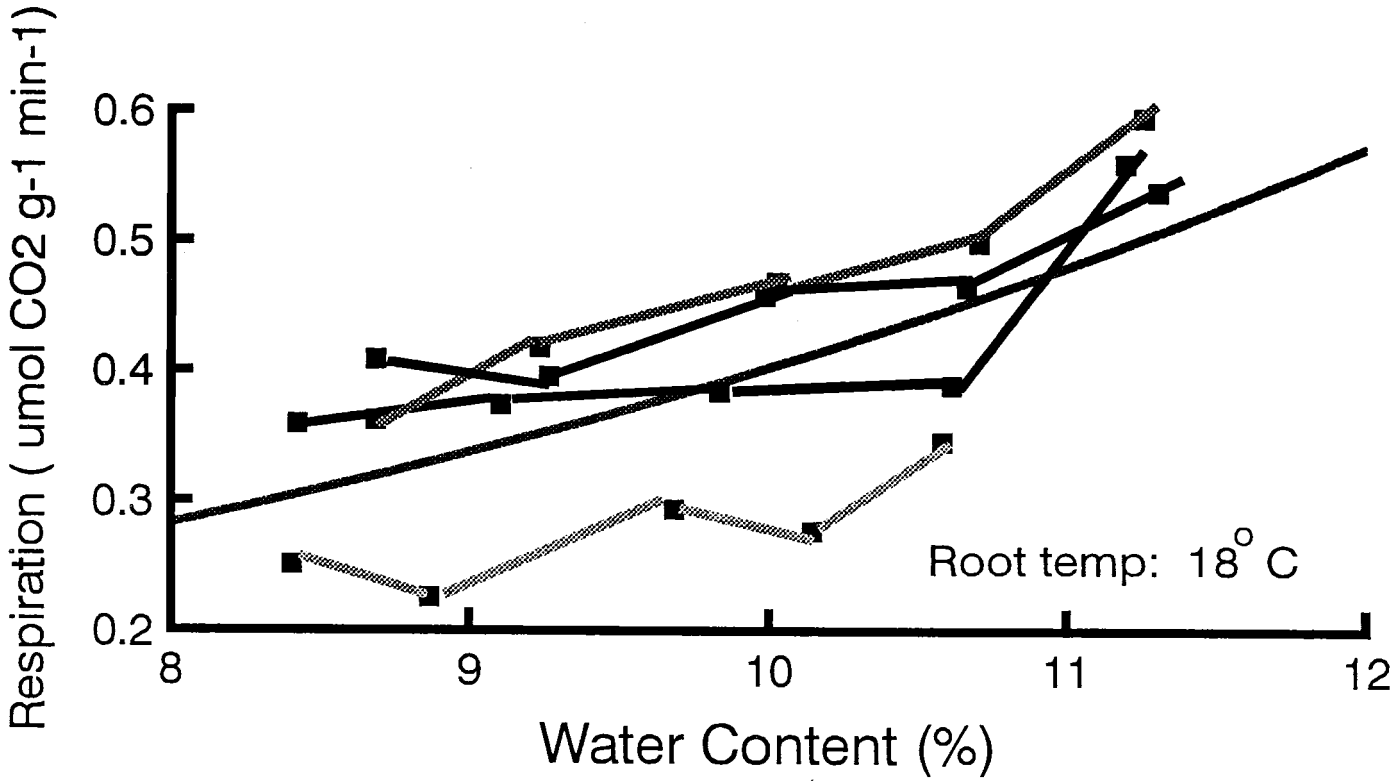

Figure 12. Relation between root respiration rates of Douglas-fir seedlings and soil water content. Measurements of root respiration were conducted at 18°C from 17 to 61 hours after watering

Table 10. Root respiration rates of western hemlock seedlings under a soil drying phase

Box#	Hours after watering					
	17		61		85	
	* RR	* WC	RR	WC	RR	WC
44	0.12	11.8	0.12	9.3	0.09	8.6
48	0.17	12.8	0.13	9.8	0.10	8.9
80	0.17	11.8	0.16	9.8	0.15	9.2

* RR is root respiration rate (μmol CO_2 $g^{-1}min^{-1}$) measured at 15°C, and WC is water content (percent of water weight to dry sand weight in the root box)

determination coefficients (r^2) ranged from 0.63 to 0.97. The overall regression of the natural logarithm of root respiration rate on soil water content was:

$$\ln (RRR) = - 2.6741 + 0.1764 \times (WC)$$

$$P\text{-value}=0.0018 \quad r^2=0.43$$

Where RRR is root respiration rate (μmol CO_2 $g^{-1}min^{-1}$), and WC is the water content expressed as percent of weight of water to weight of dry sand.

MAINTENANCE RESPIRATION OF ROOTS

The change of root respiration rates of Douglas-fir seedlings grown under 1.8 mM N treatment measured at 18°C root temperature is shown in Figure 6. The maintenance root respiration rate can be estimated from the two seedlings whose shoot was covered or severed. Root respiration rate decreased after 22 hours after treatments. The maintenance root respiration rate estimated from the root respiration values measured between 22 hours and at the end of the experiment at 144 hours ranged between 0.15 and 0.21 μmol CO_2 $g^{-1}min^{-1}$. This is about 40% to 56% of the total root respiration rate.

Figure 13 shows the change of root respiration rate after covering the shoots of western hemlock seedlings grown at 15°C root temperature and 1.8 mM N treatments. Root respiration rate decreased to a relatively stable level four

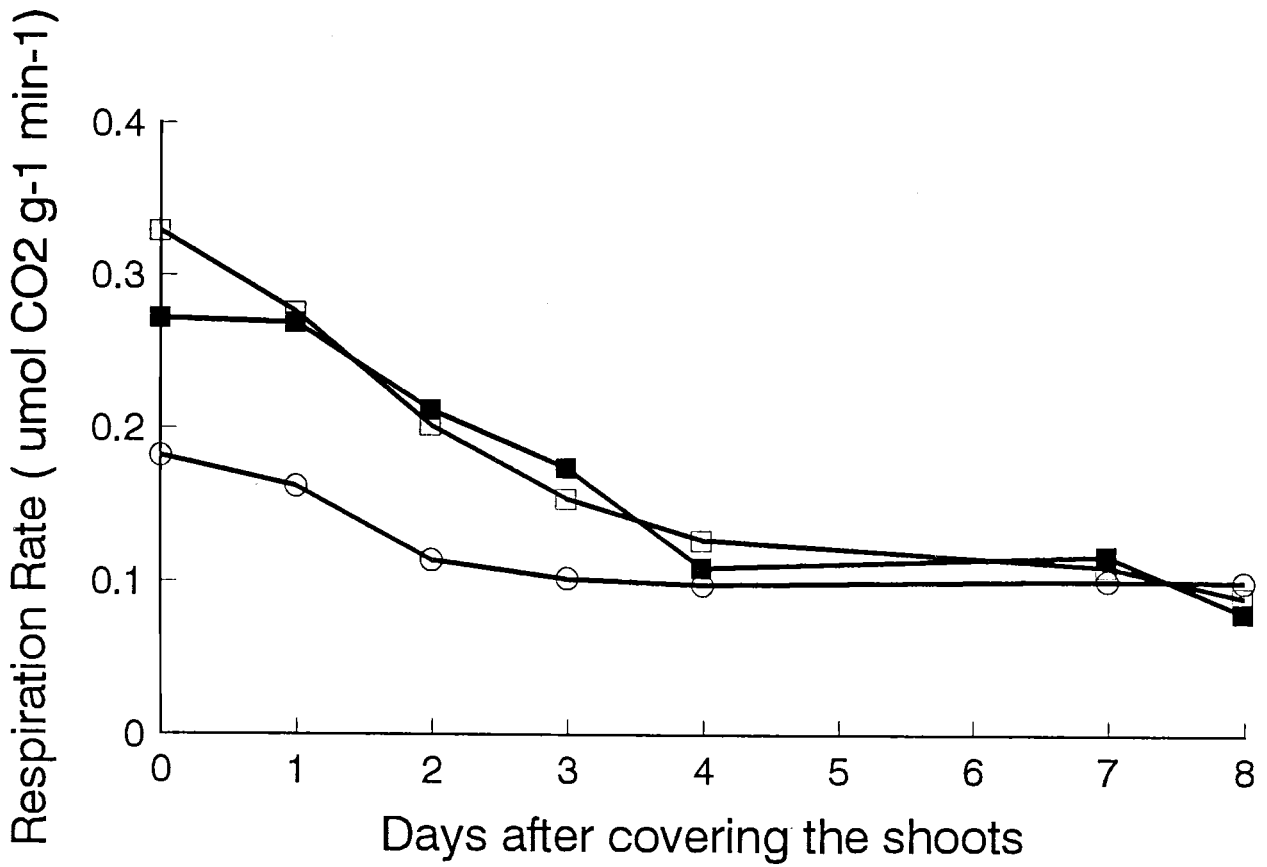

Figure 13. Change of root respiration rates of western hemlock seedlings over time after darkening the shoots. Measurements of root respiration were conducted at 15°C.

days after darkening the shoots. The root respiration rates after this time averaged 0.104 μmol CO_2 $g^{-1}min^{-1}$, which is about 40% of the root respiration rate of normal seedlings under light.

DISCUSSION

THE ROOT BOX METHOD AND COMPARISON WITH OTHERS

Seedlings grew normally in the root boxes for four months for Douglas-fir and for three months for western hemlock seedlings. At the time of planting into the root boxes, the Douglas-fir seedlings were three months old and western hemlock seedlings were nine months old. There was no seedling mortality during the period when seedlings were grown in the root boxes. The mean relative root growth rates of 14.3 mg $g^{-1}day^{-1}$ for Douglas-fir seedlings and 11.4 mg $g^{-1}day^{-1}$ for western hemlock seedlings were in the range of 3 to 178 mg $g^{-1}day^{-1}$ for various species as suggested by Poorter (1991). The shoot to root ratios for Douglas-fir and western hemlock seedlings were in the same range found in the literature under field conditions (Burgess 1991, McCreary and Zaerr 1987).

Compared to methods currently used in the literature, the root box method generally produced root respiration values similar to those obtained with the intact root inclusion method, higher values than the root exclusion method, and lower values than the excised root tip incubation method. The root respiration rate obtained with the root box method for Douglas-fir seedlings ranged from 0.07 to 0.69 μ mol CO_2 $g^{-1}min^{-1}$ at 8 to 18°C root temperature, and 0.06 to 0.75 μmol CO_2 $g^{-1}min^{-1}$ at 8 to 21°C root temperature for western hemlock seedlings.

The respiration rate obtained with the intact root inclusion method ranged from 0.06 to 0.42 μmol CO_2 $g^{-1}min^{-1}$ for *Alnus crispa, Populus balsamifera, Populus tremuloides*, and *Betula papyrifera* at 2 to 25°C root temperature (Lawrence and Oechel 1983), and 0.04 to 0.45 μmol CO_2 $g^{-1}min^{-1}$ for *Pinus elliottii* at 5 to 40°C root temperature (Cropper and Gholz 1991). Thus, the root inclusion method produces similar results to those produced by the root box method. Root respiration values obtained with the excised root tip incubation method ranged from 1.2 to 2.1 μmol CO_2 $g^{-1}min^{-1}$ for *Acer rubrum, Betula nitra* at 26°C temperature (Tripepi and Mitchel 1984), from 1.0 to 4.8 μmol CO_2 $g^{-1}min^{-1}$ for *Abies lasiocarpa, Picea engelmannii* at 5 to 25°C temperatures (Sowell and Spomer 1986), and from 0.6 to 2.7 μmol CO_2 $g^{-1}min^{-1}$ for *Pinus glauca* at 25°C temperature (Johnson-Flanagan and Owens 1986). Generally speaking, the excised root tip incubation method produces excessively high root respiration values because only root tips are sampled. The root tips are much more active than the average of roots (Ledig *et al.* 1976). Furthermore, excision of roots causes wounding which may lead to a higher respiration rate (Evans 1972). The root respiration rate obtained with the root exclusion method ranged from 0.05 to 0.06 μmol CO_2 $g^{-1}min^{-1}$ for a natural stand of *Pinus densiflora* at 4 to 25°C root temperature (Nakane *et al.* 1983), and from 0.02 to 0.08 μmol CO_2 $g^{-1}min^{-1}$ for *Pinus elliottii* at 20°C root temperature (Ewel *et al.* 1987ab). This method would be expected to produce low

respiration values because of higher heterotrophic respiration in the soil after root extraction (Lawrence and Oechel 1983). In addition, CO_2 concentration of air in the natural soil could be higher than that of the above-ground atmosphere. The inhibiting effect of high CO_2 concentration in the soil on root respiration was reported by Qi (1994).

The root box provided normal conditions for root growth. Nutrient solution was applied every two days and the water content was never lower than 9%, which is about 80% of field capacity. The highest CO_2 concentration of air in the root boxes containing Douglas-fir seedlings was 1%. The CO_2 concentration increased as root temperature increased. At the highest root temperature, the CO_2 concentration of air in the root boxes with Douglas-fir seedlings averaged 4885 ppm.

The chamber flushing time was greatly reduced by increasing the flow rate of the flushing air, by decreasing the pH of the nutrient solution, and by including four brief flushes before the constant flushing for the measurement of root respiration. Bawalda *et al.* (1992) found it took two to three hours to flush the CO_2 dissolved inside a 10 liter pot containing nutrient solution to reach the equilibrium point. As pH decreased, the amount of CO_2 dissolved in the nutrient solution decreased. For respiration measurements, Koncalova *et al.* (1989) added 1 ml 10 mM HCl to the incubation solution containing root tissue to release the CO_2 to the ambient air

after one hour of incubation. Hari *et al*. (1991) claimed that the CO_2 dissolved in the solution was negligible when the pH of the nutrient solution was lower than 5.0.

The root box method requires more labor and equipment cost than the excised root tip incubation method, root exclusion method, or the intact root inclusion method. Each root box cost about $70 US dollars. In this experiment, 70 root boxes were used. Although the initial investment is high, the root box can be used many times. The labor required with the root box method includes cleaning the root boxes, assembling the root boxes, installing sand into the root boxes, planting seedlings into the root boxes, culturing seedlings, and photocopying and digitizing the root images. If repeated measurements over a relatively long period of time are not required, the root inclusion method and the solution incubation method are probably the preferred methods. The solution incubation method involves placing intact roots into a solution in a sealed bottle and measuring root respiration. Both the root inclusion and solution incubation methods involve digging and cleaning the roots, putting them into a cuvette or solution bottle, and measuring respiration. Both methods introduce minimal disturbances if proper care is taken, require lower labor cost and initial instrumental investment, and yield the same results as the root box method.

One disadvantage of the root box method is its higher initial investment and higher labor cost. A major advantage

is that measurements are free from disturbances, which should lead to a more reliable result. In fact, the results obtained with the root box method agreed very well with those obtained with the intact root inclusion method, which is the best of the methods currently used. The second advantage is direct observation of roots, which gives an indication of root pattern, morphology, root color, and new root growth. The third advantage is the possibility of making repeated measurements of root respiration and growth on the same roots, which allows long term monitoring, versatility of treatments, and reduced sample size. The fourth advantage is the root box allowed environmental control, expecially water status, which cannot be controlled easily with either the root inclusion or the solution incubation methods.

EFFECTS OF DISTURBANCES

Either darkening the shoot or excising the shoot of Douglas-fir seedling resulted in a reduction in root respiration 22 hours after treatments (Figure 6). This effect may be due to carbohydrate depletion. Tschaplinski and Blake (1994) reported that root starch and sucrose concentration were reduced four days after decapitation in hybrid poplar

root cuttings. The greater reduction in respiration of decapitated roots may be because the source of carbohydrate input from shoots was eliminated.

Immediately after injury, root respiration increased for a short time, then decreased to the original value. In western hemlock seedlings, cutting the root systems into five-cm segments caused an increase in root respiration rate within 10 to 20 minutes, followed by a decrease to the normal respiration level within 40 to 60 minutes. For Douglas-fir seedlings, cutting 7% of the root tips off the main root system caused an increase in root respiration rate one and a half hours later, and a decrease of respiration rate to a constant value 22 hours later. However, only one Douglas-fir seedling was used. This increase in respiration at one and a half hour after injury could be caused by other factors also. The injury effect on respiration has been reported for other plant organs. Evans (1972) reviewed seven papers and found the respiration rate ranged from 26% to six fold higher after gentle handling, squeezing, cutting, or stripping of plant leaves, tubers, branches, and roots of several tree, herbs, and bamboo species. The time elapsed from injury to the time respiration rate recovered to normal ranged from several hours to as long as 10 days. The magnitude of respiration increase and length of the elapsed time depends on species, organs, and severity of injury. The increase in respiration rate after injury of plant tissues was probably caused by reconstruction

of damaged cell components. Alternatively, the increase could be caused by a sudden change in O_2 or CO_2 concentrations in the interior of cells due to leaky cell membranes, and subsequent leakage of certain compounds such as oxidative enzymes caused by cell distortion.

For accurate measurement of root respiration, care should be taken to avoid wounding roots. If roots must be cut or damaged by excavating or separating roots from the soil, then the injury effect and the period from time of injury to respiration rate recovery must be evaluated.

SHOOT AND ROOT GROWTH

The regression used for estimation of root dry weight from root projection area was strong. The coefficient of slope was significant (P=0.0001), and the determination coefficient was 0.85. The dry weight estimate for Douglas-fir seedlings from the two repeated root projection area measurements yields valid data.

The relative growth rate of roots for Douglas-fir seedlings increased as root temperature was increased from 8 to 13°C, then decreased as root temperature was further increased from 13 to 18°C. These results agreed well with the typical temperature response curve suggested by Kaspar and Bland (1992) for many temperate region species, even though

optimal root temperature ranges vary from species to species. It has been reported that root growth for Douglas-fir seedlings increase at first, then decrease as root zone temperature increases (Lopushinsky and Max 1990). The relative growth rates and the shoot-to-root ratios for both species fit the range suggested for various species by Poorter (1991).

Nitrogen fertilizer affected root growth of Douglas-fir seedlings, but did not affect root growth of western hemlock seedlings. It is reported that Douglas-fir was more efficient than western hemlock in N uptake (Burgess 1991). The lack of response to N fertilizer was previously reported for western hemlock (Gill and Lavender 1983). This lack of response to N fertilizers could not be improved by applying different sources of N and their combinations (Radwan *et al.* 1984).

Root dry weight of Douglas-fir seedlings grown under 0.36 mM N treatment was significantly higher than that of seedlings grown under the two higher N treatments during the first part of the experiment, and there was no significant difference between root dry weights of seedlings grown under 0.38 and 1.8 mM N treatments at the end of the experiment. The mean relative growth rate of seedlings in the 1.8 mM N treatment during the experimental period was significantly greater than that of seedlings grown under the other two N treatments. This result could be because the seedlings grown in the 0.36 mM N treatment grew rapidly at first, then slowed

down later in the experiment, compared to seedlings grown under other N treatments during the experimental period. On the average, roots under 1.8 mM N treatment grew faster than roots under other N treatments. These results - faster root growth of seedlings grown under optimal N concentration - agreed with those reported by Bloom *et al.* (1993), who found that roots of seedlings under optimal N concentration grew faster than those of seedlings grown under too low or too high concentrations of both NH_4^+ and NO_3^- N.

ROOT RESPIRATION RATE

Root respiration rates of all Douglas-fir seedlings measured at 18°C root temperature ranged from 0.16 to 0.69 μmol CO_2 $g^{-1}min^{-1}$ in the present study. This range is higher than that reported by Marshall and Waring (1985) or by McCreary and Zaerr (1987), and about the same as that reported by Marshall and Perry (1986). Marshall and Waring reported that the maintenance respiration rate for Douglas-fir at 20°C root temperature was 0.04 μmol CO_2 $g^{-1}min^{-1}$. They claimed that the results should be one third of the actual value because growth of seedlings was completely stopped, and seedlings lost weight. If we also assume that maintenance respiration was about 50% of the total respiration rate, the correct root respiration rate should be 0.24 μmol CO_2 $g^{-1}min^{-1}$, which falls

within the range of the result obtained in this study. McCreary and Zaerr (1987) reported that root respiration rates for Douglas-fir seedlings grown at 20°C root temperature were 0.006 to 0.01 μmol CO_2 $g^{-1}min^{-1}$. The results from McCreary and Zaerr were lower, probably due to storage of seedlings in a cold room for one month followed by damage treatments such as freezing, warm storage, soaking in hot water, and drying. Another reason they measured lower respiration rates may be the result of pruning seedling roots to a length of 25 cm. The average root in their study was thus much older and larger in diameter than the roots of the present study. It is known that older roots respire less than younger roots (Ledig *et al* 1976), and that larger diameter roots respire less than smaller diameter roots (Behera *et al*. 1990). The maintenance respiration rate for Douglas-fir grown at 20°C temperature reported by Marshall and Perry (1986) was 0.2 to 0.32 μmol CO_2 $g^{-1}min^{-1}$. Again assuming that maintenance respiration is 50% of total respiration, then the root respiration in Marshall and Perry's study would have been 0.4 to 0.64 μmol CO_2 $g^{-1}min^{-1}$. The rate estimated in the present study corrected to a 20°C root temperature, based on the regression of the logarithm of respiration rate on the reciprocal of absolute temperature, ranged from 0.18 to 0.79 μmol CO_2 $g^{-1}min^{-1}$. This estimated value agrees very well with the measured values reported by Marshall and Perry (1986).

It was earlier reported that stem respiration rates were underestimated because a portion of the respiratory CO_2 became dissolved in the transpiration stream and was carried away from the site of measurement during periods of high transpiration (Martin *et al.* 1992). The amount of CO_2 dissolved in the transpiration stream solution depends positively on temperature, and partial pressure of CO_2 in the ambient air of tissue under investigation, and depends negatively on the hydrogen ion concentration in the transpiration stream solution (Hari *et al.* 1991). At pH 6.0, 15°C temperature, and 20,000 ppm CO_2 concentration in the air, the calculated amount of CO_2 carried by the transpiration stream was 2% of the amount of photosynthetically fixed carbon. If we assume root respiration rate was 10% of photosynthesis, the amount of CO_2 carried away by the transpiration stream was 0.01% of the root respiration rate when pH of the transpiration stream was 6.0, under the conditions of the present study, at which the CO_2 concentration in the air around the roots was 1,000 ppm. The effect of possible underestimation of root respiration rate was negligible. In addition, the diameter of roots in this study was much smaller compared to that of the stems of shoots used by Martin *et al.* (1992). The CO_2 diffusion from roots to ambient air in this study should therefore have been faster than from stems in the study by Martin *et al.* (1992). It

seems safe to conclude that the underestimation of root respiration due to circulation from root to shoot in the present study is negligible.

EFFECTS OF CONTROLLING FACTORS

Species

Root respiration rates of western hemlock seedlings were not significantly different from those of Douglas-fir seedlings in the present study. But the variation in root respiration rate for western hemlock seedlings was greater than for Douglas-fir seedlings. This inherent species difference persists even when seedlings are grown under identical environmental conditions (Poorter 1990). The differences of variation in root respiration may due either to differences in the rates of processes requiring energy (the maintenance respiration and the proportion of the alternative respiration), or to the specific respiratory energy costs of these processes (Van der Werf 1990).

The maintenance respiration rate for western hemlock seedlings was 40% of the total root respiration rate. And the maintenance root respiration rate for Douglas-fir seedlings was about 40% to 56% of the total respiration rate. The maintenance respiration rate is a relatively fixed value for

seedlings grown at a specific root temperature with a given chemical composition. Other components of total respiration vary as other controlling factors vary. The proportion of maintenance respiration for western hemlock seedlings is smaller than for Douglas-fir seedlings. This difference could account for greater variation in root respiration obtained in western hemlock seedlings than in Douglas-fir.

Time

The root respiration rate of Douglas-fir seedlings grown at 14.3 to 14.8°C root temperature remained constant throughout a 24 hour light and dark period (Figure 6). This relatively constant respiration rate over a short time period indicates that the root respiration measuring system employed in this study is stable and reliable. The error attributable to the measuring system was small compared to the effects of the other variables.

Root respiration has been shown to respond positively to shoot photosynthesis in some studies (Huck *et al.* 1962, Neales and Davies 1966), but not others (Alm and Nobel 1991, Buwalda *et al.* 1992). Lambers *et al.* (1991) generalized that root respiration would not show a light response when plants were grown under a constant root temperature, high light intensity, and long photoperiod because the substrate level

for root respiration would not become depleted during the short dark periods. The plants used in this study were grown under constant root temperature levels, relatively high light intensity, and a long photoperiod. Therefore, as expected, little diurnal change was observed in this study.

There was greater variation in root respiration rate for Douglas-fir seedlings than for western hemlock seedlings from time to time during the four-month period despite the constant root temperature, and controlled N levels and water content. The variation in root respiration may be due partially to the differential relative growth rate of roots. Total root respiration has been partitioned into maintenance respiration and growth respiration. The seedlings with a higher growth rate may have higher total respiration as well, because, maintenance respiration is a relatively fixed value. In fact, maintenance respiration has been estimated from total respiration when growth respiration is zero (Amthor 1984). Another reason for the highly varied respiration rates from time to time during the four-month period may be due to differences in available root carbohydrate levels (Farrar 1985, and Figure 5), since light condition has been shown to affect root respiration rate of plants under low light intensity. Prolonged cloudy periods prior to respiration measurements may thus lead to lower carbohydrate levels compared to sunny periods.

Root temperature

Root temperature is probably the most well-known factor regulating root respiration. Root respiration response to root temperature follows the relation suggested by Svante Arrhenius in 1889, i.e., reaction rate increases exponentially as temperature increases. Root respiration rate would thus be expected to increase as temperature increases because the root respiration process is a series of chemical reactions. This relationship between root respiration rate and temperature has been verified in many studies of various species grown under their physiological temperature range (Earnshaw 1981, Foster *et al.* 1991, Lawrence and Oechell 1983, Palta and Nobel 1989bc, Ryan 1991a, Sission 1983, Sowell and Spomer 1986, Young *et al.* 1987). The root respiration - temperature relationship in both species of this study followed the Arrehnius postulation. Regression of the logarithm of root respiration rate on the reciprocal of the absolute root temperature for individual seedlings was very strong; the determination coefficient was never lower than 0.7, and the average for both species was above 0.9. This result indicates that root temperature was a very good predictor of root respiration rate over a range of temperatures.

The Q_{10}, the ratio of respiration rate at T+10°C to respiration rate at T°C, for most species is about 2.0 (Earnshaw 1981, Grossman and DeJong 1994, Ryan 1991). This

means the root respiration rate is doubled when root temperature is increased by 10°C. The Q_{10} obtained here for Douglas-fir seedlings (2.23) was not significantly different from that obtained for western hemlock seedlings (2.35).

McCreary and Zaerr (1987) reported a Q_{10} of 1.77, and Marshall and Waring (1985) reported a value of 1.98 for Douglas-fir seedlings. The value obtained for Douglas-fir in this study was higher than that obtained in either of those two previous reports. The reason for the higher Q_{10} values obtained in the present study is not clear. The seedlings used in this study were from four to six months old. The seedlings used by Marshall and Waring were older than six months, and the seedlings used by McCreary and Zaerr were more than two years old. The Q_{10} is known to vary with species and type of root (Lambers 1985, Curran *et al.* 1986), which could explain some of the differences. Another explanation may be differences in temperature range; 8 to 18°C in this study, 20 to 30°C in McCreary and Zaerr's study, and 10 to 30°C in Marshall and Waring' study. A change in the Q_{10} at certain temperatures was reported by Minchin and Raison (1973). The differences in temperature range used could help explain the different Q_{10} values reported in the various studies.

The preconditioning effect of root growth temperature on root respiration was examined in this study by comparing respiration rates of roots measured at the temperatures at which they were grown with respiration rates of roots measured

at the temperatures which were shortly increased or decreased from root growth temperatures. The differences in these two types of respiration rate at the same temperature and N levels were not significant. This suggests that seedlings originated from a common population, but grown at different root temperatures for three months, respond to temperature similarly. The inherent nature of roots in respiration response to temperature does not change over a short period of time. The result in this study is consistent with that reported by Sowell and Spomer (1986), who collected seedlings from different elevation levels and grew them under the same conditions for two years. They found that the inter-population differences in root respiration rate did not diminish during the two year period, and continued to reflect their inherited adaptive characteristics. The seedlings thus did not have the ability to modify their respiration rate to their new temperature environment during the two year period.

Nitrogen

The root respiration rate of Douglas-fir seedlings increased when N concentration increased from 0.36 mM to 1.8 mM, and remained unchanged when N concentration was further increased from 1.8 to 7.2 mM. This increase in respiration rate as N concentration increases is mainly due to NO_3^-

reduction and NH_4^+ incorporation into amino acids, which requires energy (Johnson 1983, Morris and Dacey 1984, Lambers 1987, Vessey and Layzel 1987), and maintenance of higher N content in root tissue (Margolis and Waring 1986, Ryan 1991b). The reduction of NO_3^- to form NH_4^+, incorporation of ammonium ions into amino acids, and transport of amino acids from roots to above ground parts require energy (Goodwin and Mercer 1990, Poorter *et al.* 1991, Van der Werf *et al.* 1988). Lovett and Tobiessen (1993) reported that the N absorbed was mostly reduced in roots. The root respiration increase when N concentration increases from 0.36 to 1.8 mM can be explained by the energy requirement of N assimilation. However, as N concentration was further increased, root respiration remained constant. There are two possible explanations for this result: 1). Limited capacity for N absorption, nitrate reduction, and ammonium ion incorporation into amino acids in the roots. Like all the enzyme catalyzed reactions, the reaction capacity of N assimilation can be saturated when N concentration increases to a certain level in the nutrient solution (Goodwin and Mercer 1990). 2). The root tissue can process as much N ion as absorbed, but the NH_4^+ absorbed from the nutrient solution and NH_3 dissociated from NH_4^+ are toxic to root respiration. The respiration increase due to ion uptake and respiration decrease due to the toxic effect of NH_4^+ and NH_3 may offset each other. The toxic effect of NH_4^+ and NH_3 has been reported for many plants (Bennet 1974, Vines

and Wedding 1960). It has been reported that Douglas-fir preferred to absorb NH_4^+ to NO_3^- when both were present in the nutrient solution (Wijk and Prins 1993). In the 7.2 mM N nutrient solution, the NH_3 concentration dissociated from NH_4^+ would be close to 0.15 mM, which is the toxic level suggested by Bennet (1974). When NH_4^+ concentration is too high in the nutrient solution, it can enter the roots along with the mass flow of water, and both NH_3 and NH_4^+ ions are inhibitory to the respiration chain.

Root respiration of western hemlock seedlings remained unchanged when subjected to different N treatments, but the reason for this lack of response remains unclear. The lack of response to various N fertilizers has been noticed previously (Radwan *et al.* 1984, Radwan *et al.* 1991). One possible explanation could be insufficient activities of enzymes responsible for the reduction of nitrate and for the incorporation of ammonium ions into amino acids in hemlock roots, as species are known to vary in those enzyme activities in their roots (Goodwin and Mercer 1990). It is unlikely that western hemlock seedlings require insignificant amount of nitrogen because the shoots did show positive response to higher N levels (Table 6). Both NO_3^- and NH_4^+ ions absorbed in the roots, therefore were, most likely, transferred to shoots along with xylem sap and assimilated in shoots.

Water stress

The decrease in root respiration rates for both species as water content decreased is consistent with reports in the literature. Decreased root respiration during a soil drying phase was found in *Agave deserti* (Alm and Nobel 1991), *Ferocactus acanthodes* and *Opuntia ficus-indica* (Palta and Nobel 1989bc), and *Triticum aestivum* (Nicolas *et al.* 1985). One explanation for this observation is that when plants are under water stress, osmotic regulation may occur and it competes with respiration for available root carbohydrates (Lambers *et al.* 1991). Part of the carbohydrate transported from shoots to roots is used to synthesize solutes for this osmotic adjustment. Another explanation for the observed reduction in respiration might be the reduced root growth. Since total root respiration is comprised of maintenance respiration and growth respiration, total root respiration is reduced when growth respiration is decreased.

From 0 to 17 hours after watering, root respiration rates of seedlings were not measured, but probably remained constant. The water content of the soil was unlikely to change significantly because the seedlings were in the dark period and plant stomata have been shown to be closed (Lu 1989). It has been reported that plant root respiration does not change until soil water content is reduced to a certain threshold level (Palta and Nobel 1989bc). Root respiration

rate was constant while the soil dried to -0.3 MPa for rain roots and to -0.5 MPa for established roots of several desert species (Palta and Nobel 1989bc). It is likely that Douglas-fir and western hemlock seedlings responded similarly in the present study.

MAINTENANCE RESPIRATION OF ROOTS

One major component of total root respiration rate is maintenance respiration rate. A major part of the maintenance energy cost is supposed to be associated with the maintenance of ionic gradients across membranes (Bouma and Visser 1993) and with protein turnover (Ryan 1991b).

Maintenance respiration rate of roots has sometimes been estimated by measuring root respiration for several days after covering or severing the shoots. In those studies, the respiration rate decreased to a relatively stable level which was called the maintenance respiration rate (48 Hours for Douglas-fir, Marshall and Perry 1987). From the results of the present experiment (Figure 5), it appears that the two methods can lead to different results for Douglas-fir. Severing the shoot and measuring maintenance respiration could underestimate the actual maintenance respiration rate because the root respiration rate after severing the shoot was consistently lower than the root respiration rate after

covering the shoot. This result is probably due to the relatively rapid depletion of available carbohydrates in the severed root system which was no longer supplied by the shoot. A root still connected with the shoot, on the other hand, could be expected to get extra carbohydrates from the shoot. A relatively stable value was not observed for Douglas-fir seedlings with either darkened or excised shoots as it was for western hemlock in the present study (Figure 8). Thus, for the measurement of maintenance respiration in Douglas-fir roots, other method or methods should be used.

The maintenance root respiration rate for western hemlock seedlings grown at 15°C root temperature was 0.104 μmol CO_2 $g^{-1}min^{-1}$. There is no report of maintenance root respiration found in the literature for western hemlock. This value is lower than that reported by Marshall and Perry (1986) and higher than that reported by Marshall and Waring (1985) for Douglas-fir seedlings.

CONCLUSIONS

Douglas-fir and western hemlock seedlings grew normally in the root boxes. The root box method for root respiration measurement produced results similar to those obtained with the root inclusion method and solution incubation method, which are considered to be the best methods currently used. The root box method allowed control of water content, and allowed repeated measurements of root growth and respiration on undisturbed roots over a relatively long period of time. However, this method requires higher initial investment and higher labor cost compared to other methods. If repeated measurements are not critical, and water potential is not the concern, the nutrient solution incubation method and intact root inclusion method are recommended.

Root injury should be avoided for measurement of root respiration. Root respiration increased immediately after roots were injured, then decreased to normal after a certain period of time. The magnitude of this increase, and the time elapsed from injury to the time of recovery varied from species to species. If the injury could not be avoided, then the respiration measurement can only be measured after recovery. The time elapsed from injury to recovery needs also to be determined.

The temperature at which root respiration measurements are made is probably the most important single factor in

controlling root respiration of the variables tested in this study. The root respiration response to root temperature can be very well described as a regression of the logarithm of root respiration rates on the reciprocal of the absolute root temperatures. Since the temperature at which roots were grown, and the order of increasing or decreasing root measurement temperature did not affect this regression, and because measuring date or nitrogen concentration in the soil did not affect the slope of this regression, we can predict root respiration rates of individual roots very well (r^2=0.9) at other root temperatures in the same condition if we know the root respiration rate at a specific temperature.

To model the root respiration rates under various environment conditions, the following factors, besides root temperature, need to be considered based on the present study: (1). Larger variation in western hemlock seedlings than in Douglas-fir seedlings was observed; (2). Root respiration rates increased first as N concentration in the nutrient solution increased, but leveled off at higher N levels for Douglas-fir. Western hemlock seedlings did not respond to N fertilizer; (3). Root respiration rates varied from date to date for both Douglas-fir and western hemlock seedlings. But there was no diurnal trend found for Douglas-fir root respiration. Root respiration rates of western hemlock seedlings decreased from one to three months after treatment. This decrease may be attributed to decreased respiration rate

for older roots; and (4). Root respiration rates increased exponentially as water content was increased in the range tested in the present study for both species.

Western hemlock seedlings are quite different from Douglas-fir seedlings. Root growth and respiration of western hemlock seedlings did not respond to N treatments. Addition of N fertilizer for western hemlock may not be as efficient as for Douglas-fir. The variation in root respiration rates for western hemlock seedlings are larger than for Douglas-fir seedlings, which could be due to lower root maintenance respiration for western hemlock than for Douglas-fir.

Darkening or severing shoots and measuring total respiration through time is not a good method for measuring maintenance respiration rate for Douglas-fir roots. The root respiration rates of Douglas-fir seedlings obtained by either darkening or severing the shoots decreased steadily after treatments. Relatively stable respiration rates were not observed for 6 days following treatment. In addition, the method of severing shoots and then measuring root respiration rate underestimated maintenance respiration rates for Douglas-fir.

BIBLIOGRAPHY

Alm, D.M., and P.S. Nobel 1991 Root system water uptake and respiration for *Agave deserti*: Observations and predictions using a model based on individual roots. Ann. Bot. 67:59-65.

Amthor, J.S. 1984 The role of maintenance respiration in plant growth. Plant, Cell and Environment 7:561-569.

Amthor, J.S. 1989 Respiration and crop productivity. Springer-Vertag, Berlin. 215p.

Amthor, J.S. 1991 Respiration in a future, higher-CO_2 world. Plant, Cell and Environment 14:13-20.

Anderson, P.C., J.M. Montano, and P.B. Lombard 1985 Root anaerobiosis, root respiration, and leaf conductance of peach, willow, quince, and several pear species. HortScience 20(2):248- 250.

Anderson, C.P. and P.T. Rygiewicz 1991 Stress interactions and mycorrhizal plant response: Understanding carbon allocation priorities. Environ. Pollution 73:217-244.

Andre, M., C. Richaud, A. Dagunet, J. Messiminod, and J. Vovoli 1981 The effect of a day at low radiance on a maize crop. I. Root respiration and uptake of N. P. K. Physiol. Plant. 51:150- 155.

Baas, R., A. Van der Werf, and H. Lambers 1989 Analysis of growth and root respiration in *Plantago major ssp. pleiosperma*: effects of VA mycorrhizal infection and P addition. Plant Physiol. 91:227-232.

Barneix, A.J., H. Breteler, and S.C. Van de Geijin 1984 Gas and ion exchanges in wheat roots after nitrogen supply. Physiol. Plant. 61:357-362.

Behera, N., S.K. Joshi, and D.P. Pati 1990 Root contribution to total soil metabolism in a tropical forest soil from Orissa, India. For. Ecol. Manage. 36:125-134.

Bennett, A.C. 1974 Toxic effects of aqueous ammonia, copper, zinc, lead, boron, and manganese on root growth. In: The plant root and its environment. pp669-683. ED: E.W. Carson. University of Virginia, Charlottesville.

Bizzell, J.A., and T.L. Lyon 1918 The effect of certain factors on the carbon dioxide content of soil air. J. Am. Soc. Agron. 10:97-112.

Bloom, A.J., L.E. Jackson, and D.R. Smart 1993 Root growth as a function of ammonium and nitrate in the root zone. Plant, Cell and Environment 16:199-206.

Bouma, T.J., and R.D. Visser 1993 Energy requirements for maintenance of ion concentration in roots. Phsiol. Plant. 89:133-142.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo, and J.B. Garrison 1993 Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. Can. J. For. Res. 23:1402-1407.

Boyer, W.D., R.M. Romancier, and C.W. Ralston 1971 Root respiration rates of four tree species grown in the field. Forest Sci. 17(4):492-493.

Bryce, J.H., and T. Rees 1985 Effect of sucrose on the rate of respiration of the roots of *Pisum satrium*. J. Plant Physiol. 120:363-367.

Burgess, D. 1991 Western hemlock and Douglas-fir seedling development with exponential rate of nutrient addition. Forest Sci. 37(1):54-67.

Buwalda, J.G., M. Fossen, and F. Lenz 1992 Carbon dioxide efflux from roots of calamodian and apple. Tree Physiol. 10:391-401.

Carpenter, J.R., and C.A. Mitchell 1980 Root respiration characteristics of flood-tolerant and intolerant tree species. J. Amer. Soc. Hort. Sci. 105(5):684-687.

Cheng, Weixing, D.C. Coleman, C.R. Carroll, and C.A. Hoffman 1993 *In situ* measurement of root respiration and soluble C concentrations in the rhizosphere. Soil. Biol. Biochem. 25(9):1189-1196.

Collier, D.E., and W.R. Cummins 1993 Effects of osmotic stress on the respiratory properties of shoots and roots of *Arpica alpina*. Can J. Bot. 71:1102-1108.

Conlin, T.S.S., and V.J. Lieffers 1993 Anaerobic and aerobic CO_2 efflux rates from boreal forest conifer roots at low temperature. Can. J. For. Res. 23:767-771.

Cropper, W.P., Jr., and H.L. Gholz 1991 *In situ* needle and fine root respiration in mature slash pine (*Pinus elliottii*) trees. Can. J. For. Res. 21:1589-1595.

Davies, D.D, S. Grego, and P. Kenworthy 1974 The control of the production of lactate and ethanol by higher plants. Planta. 118:297-310.

Day, D.A., and H. Lambers 1983 The regulation of glycolysis and electron transport in roots. Physiol. Plant. 58:155-160.

Earnshaw, M.J. 1981 Arrhenius plots of root respiration in some arctic plants. Arctic and Alpine Research 13(4):425-430.

Edwards, N.T. 1991 Root and soil respiration responses to ozone in *Pinus taeda* L. seedlings. New Physiol. 118:315-321.

Evans, G.C. 1972 The quantitative analysis of plant growth. University of California Press. Berkeley and Los Angeles 734p.

Ewel, K.C., W.P. Cropper, Jr., and H.L. Gholz 1987a Soil CO_2 evolution in Florida slash pine plantations. I. Changes through time. Can. J. For. Res. 17:325-329.

Ewel, K.C., W.P. Cropper, Jr., and H.L. Gholz 1987b Soil CO_2 evolution in Florida slash pine plantations. II. Importance of root respiration. Can. J. For. Res. 17:330-333.

Farrar, J.F. 1981 Respiration rate of Barley roots: its relation to growth, substrate supply and the illumination of the shoot. Ann. Bot. 48:53-63.

Farrar, J.F. 1985 Fluxes of carbon in roots of barley plants. New Phytol. 99:57-59.

Gifford, R.M., H. Lambers, and J.I.L. Morison 1985 Respiration of crop species under CO_2 enrichment. Physiol. Plant. 63:351-356.

Gijsman. A.J. 1991 Soil water content as a key determining the source of nitrogen (NH_4^+ or NO_3^-) absorbed by Douglas-fir (*Pseudotsuga menziesii*) and the pattern of rhizosphere pH along its roots. Can. J. For. Res. 21:616-625.

Gill, R., and D.P. Lavender 1983 Urea fertilization: Effects on primary root mortality and mycorrhizal development of young-growth western hemlock. Forest Sci. 29(4):751-760.

Gloser, J., and M. Tesarova 1978 Liter, soil, and root respiration measurement. An improved compartmental analysis method. Pedobiologia 18:76-81.

Goodwin, T.W., and E.I. Mercer 1990 Introduction to plant biochemistry. Second edition. Pergamon Press, BPCC Wheatons Ltd., Exeter, Great Britain.

Grossman, Y.L., and T.M. DeJong 1994 Carbohydrate requirements for dark respiration by peach vegetative organs. Tree Physiol. 14:37-48.

Hansen. G.K. 1980 Diurnal variation of root respiration rates and nitrate uptake as influenced by nitrogen supply. Physiol. Plant. 48:421-427.

Hansen, G.K., and C.R. Jensen 1977 Growth and maintenance respiration in whole plants, tops, and roots of *Lolium multiforum*. Physiol. Plant. 39:155-164.

Hari, P., P. Nygren, and E. Korpilahti 1991 Internal circulation of carbon within a tree. Can. J. For. Res. 21:514-515.

Helal. H.M., and D. Sauerbeck 1991 Short term determination of the actual respiration rate of intact plant roots. IN: Plant roots and their environment. pp88-92. ED: B.L. McMichane and H. Person. Elsevier Science Publishers B.V.

Hermann, R.K. and D.P. Lavender 1990 Douglas-fir, *Pseudotsuga menziesii*. (Mirb) Franco: Douglas-fir. In: Bunrns, R.M., and R.H. Honkala. Tech. Coords. Silvics of North America: Volume 1, Conifers. pp527-540. Agric. Handb. 654. Washington DC: Forest Service, USDA.

Holthausen, R.S., and M.M. Caldwell 1980 Seasonal dynamics of root system respiration in *Atriplex confertifolia*. Plant and Soil 55:307-317.

Huck, M. G., R.H. Hageman, and J.B. Hansaon 1962 Diurnal variation in root respiration. Plant Physiol. 37:371-375.

James, B.R., R.J. Bartlett, and J.F. Amadon 1985 A root observation and sampling chamber (rhizotron). Plant and Soil 85:291-293.

Johnson, I.R. 1983 Nitrate uptake and respiration in roots and shoots: A model. Physiol. Plant. 58:145-147.

Johnson-Flanagan, A.M., and J.N. Owens 1986 Root respiration in white spruce (*Picea glauca* [Moench] Voss) seedlings in relation to morphology and environment. Plant Physiol. 81:21-25.

Joslin, J.D., and G.S. Henderson 1987 Organic matter and nutrient associated with fine root turnover in a white oak stand. For. Sci. 33:330-346.

Kaspar, T.C., and W.L. Bland 1992 Soil temperature and root growth. Soil Science 154(4):290-299.

Koncalova, H., G. Albrecht, J. Pokorny, and E.-M. Wiederoth 1989 Measurement of respiratory CO_2 production of roots in an aquatic medium. Biol. Plant. 31(1):1-7.

Korcak, R.F. 1983 Root respiration and soil variation status of blueberries. J. Plant Nutrition 6(4):283-289.

Kucera, C.L., and D.R. Kirkham 1971 Soil respiration studies in tallgrass prairie in Missouri. Ecol. 52:912-915.

Lambers. H. 1980 The physiological significance of cyanide-resistant respiration in higher plants. Plant, Cell and Environment 3:293-302.

Lambers. H. 1981 The efficiency of root respiration in different environments. IN: Structure and function of plant root. pp281-285. ED: R. Brouwer *et al*.. Martinus Nijhoff/Dr W. Junk Publishers.

Lambers, H. 1985 Respiration in intact plants and tissue: its regulation and dependence on environmental factors, metabolism and invaded organisms. In: Encyclopedia of Plant Physiol. pp418-473. Springer-Verlag, Berlin.

Lambers, H. 1987 Growth respiration, exudation and symbiotic associations: The fate of carbon translocated to the roots. IN: Root development and function. pp125-145. ED: P.J. Gregory, J.V. Lake, and D.A. Rose. Cambridge University Press.

Lambers, H., and A. Van Der Werf 1988 Variation in the rate of root respiration of two *Carex* species: A comparison of four related methods to determine the energy requirements for growth, maintenance and ion uptake. Plant and Soil 111:207-211.

Lambers, H., and A. Van der Werf 1991 Respiratory pattern in roots in relation to their functioning. IN: Plant roots, the hidden half. pp229-263. ED: Y. Waisel, A. Eshel, and U. Kafkafi. Marcel Dekker, Inc..

Lavoie, N., L.Vezina, and H.A. Margolis 1992 Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. Tree Physiol. 11:171-183.

Lawrence, W.T., and W.C. Oechel 1983 Effects of soil temperature on the carbon exchange of taiga seedling. I. Root respiration. Can. J. For. Res. 13:840-849.

Ledig, F.T., A.P. Drew, and J.G. Clark 1976 Maintenance and constructive respiration, photosynthesis, and net assimilation rate in seedlings of Pitch pine (*Pinus rigida* Mill.). Ann. Bot. 40:289-300.

Lee, D.K., and J.C. Lee 1991 Studies on the flooding tolerance and its physiological aspects in fruit plants: II. Physiological changes associated with flooding. J. Korean Soc. Hort. Sci. 32(1):97-101.

Leshuk, J.A., and M.E. Saltveit 1991 Effects of rapid changes in oxygen concentration on the respiration of carrot roots. Physiol Plant. 82:559-568.

Lopushinsky, W., and T.A. Max 1990 Effect of soil temperature on root and shoot growth and on bud burst timing in conifer seedling transplants. New Forests 4:107-124.

Lu, Shengjun 1989 Seasonal and diurnal trends of leaf water potential and stomatal conductance of red alder (*Alnus rubra* Bong) growing along a density gradient in western Oregon. M.S. thesis. Oregon State University, Corvallis, Oregon. 95p.

Lundegardh., H. 1927 Carbon dioxide evolution of soil and crop growth. Soil Sci. 23:417-454.

Mamaev, V.V. 1987 Respiration of Scots pine roots in different forest types. Lesovedenie 4:46-50.

Margolis, H.A. and R.H. Waring 1986 Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Can. J. For. Res. 16:897-902.

Marshall, J.D., and R.H. Waring 1985 Predicting fine root production and turnover by monitoring root starch and soil temperature. Can. J. For. Res. 15:791-800.

Marshall. J.D., and D.A. Perry 1987 Basal and maintenance respiration of mycorrhizal and nonmycorrhizal root systems of conifers. Can. J. For. Res. 17:872-877.

Martin, T.A., R.O. Teskey, and D.B. Warnell 1992. Causes of temperature-independent diurnal fluctuations in CO_2 efflux from loblolly pine (*Pinus taeda* L) stems. Proceedings of the Twelfth North American Forest Biology Workshop. pp103. August 17-20, 1992. Saul Ste. Marie, Ontario, Canada.

Mattson, K.G. 1993 CO_2 efflux from coniferous soils: comparison of measurement methods and effects of added nitrogen. Advances in Soil Science (in press)

McCreary, D.D., and J.B. Zaerr 1987 Root respiration has limited values for assessing Douglas-fir seedlings quality. Can. J. For. Res. 17:1144-1147.

Mengel, K. and E.A. Kirkby 1979 Principles of plant nutrition. International Potash Institute. Second edition. Worblaufen-Bern, Switzerland.

Monning, A. 1983 Studies on the reaction of Krebs cycle enzymes from apple tissue (*cv Cox orange*) to increase levels of CO_2. Acta Horticulture 138:113-119.

Mori, S., and A. Hagihara 1991 Root respiration in *Chamaecyparis obtusa* trees. Tree Physiol. 8:217-225.

Morohashi, Y., S. Sasanuma, and T. Seto 1993 Phytochrome-mediated control of respiratory activities of mitochondria in cotyledons of dark grown cucumber seedlings. Physiol. Plant. 89:295-300.

Morris, J.T., and J.W. Dacey 1984 Effects of O_2 on ammonium uptake and root respiration by *spartina alterniflora*. Amer. J. Bot. 71(7):979-985.

Nakane, K., M. Yamamoto, and H. Tsubota 1983 Estimation of root respiration rate in mature forest ecosystem. Jap. J. Ecol. 33:397-408.

Neales, T.F., and J.A. Davies 1966 The effect of photoperiod duration upon the respiration activity of the roots of wheat seedlings. Aust. J. Bot. Sci. 19:471-480.

Nelson, T.E. 1991 Root and soil respiration responses to ozone in *Pinus taeda* L. seedlings. New Physiol. 118:315-321.

Neufeld, H.S., D.M. Durall, P.M. Rich, and D.T. Tingey 1989 A rootbox for quantitative observations on intact entire root systems. Plant and Soil 117:295-298.

Newton, M. 1978 Site preparation requirements for western hemlock. In: Atkinson, W.A. and R.J. Zasoski. Western hemlock management. University of Washington.

Nicolas, M.E., H. Lambers, R.J. Simpson, and M.J. Dalling 1985 Effect of drought on metabolism and partitioning of carbon in two varieties of wheat differing in drought tolerance. Ann. Bot. 55:727-742.

Nobel, P.S., J.A. Palta 1989 Soil O_2 and CO_2 effects on root respiration of Cacti. Plant and Soil 120:263-271.

Nylund, J.-E. 1988 The regulation of mycorrhiza formation-carbohydrate and hormone theories reviewed. Scand. J. For. Res.

Osman, A.M. 1977 Root respiration of wheat plants as influenced by age, temperature and irradiation of shoot. Photosysnthetica 5:107-112.

Palta, J.A., and P.S. Nobel 1989a Influence of soil O_2 and CO_2 on root respiration for *Agave deserti*. Physiol. Plant. 76:187-192.

Palta, J.A., and P.S. Nobel 1989b Influences of water status, temperature, and root age on daily patterns of root respiration for two cactus species. Ann. Bot. 63:651-662.

Palta, J.A., and P.S. Nobel 1989c Root respiration for *Agave deserti*: Influence of temperature, water status and root age on daily patterns. J. Exper. Bot. 40(211):181-186.

Poorter, H. 1990 Interspecific variation in relative growth rate: on ecological causes and physiological consequences. ED: H. Lambers *et al*. Causes and consequences of variation in growth rate and productivity of higher plants. pp45-68. SPB Academic Publishing bv, The Hague, The Netherlands.

Qi, Jingen 1994 High ambient soil carbon dioxide concentration inhibit root respiration of one-year-old seedlings of Douglas-fir (*Pseudotsuga menziesii*). M.S. thesis. Idaho University. Moscow, Idaho pp75.

Radwan, M.A., D.S. DeBell, and S.P. Gessel 1984 Different nitrogen sources for fertilizing western hemlock in western Washington. Can. J. For. Res. 14:155-162.

Radwan, M.A., and J.S. Shumway 1985 Response of Douglas-fir seedlings to nitrogen, sulfur, and phosphorous fertilizers. Res. Pap. PNW-346. Portland. OR: USDA Forest Service. Pacific Northwest Research Station 14p.

Radwan, M.A., J.S. Shumway, D.S. DeBell, and J.M. Kraft 1991 Variation in response of pole-size trees and seedlings of Douglas-fir and western hemlock to nitrogen and phosphorus fertilizers. Can. J. For. Res. 21(10):1431-1438.

Raich, J.W., and K.J. Nadelhoffer 1989 Belowground carbon allocation in forest ecosystems: Global trends. Ecology 70(5): 1346-1354.

Reekie, E.G., and R.E. Redman 1987 Growth and maintenance respiration of perennial root systems in a dry grassland dominated by *Agropyron dasystachyum* (Hook.) Scribn. New Phytologist 105(4):595-603.

Running, S.W. and J.C. Coughlan 1988 A general model of forest ecosystem processes for regional applications. I. Hydrologic balance, canopy gas exchange and preliminary production processes. Ecol. Model 42:125-154.

Russell, E.J., and A. Appleyard 1915 The atmosphere of the soil, its composition and cause of variation. J. Agric Sci.7:1-44.

Russell, R.S. 1977 Plant root systems: Their function and interaction with the soil. McGraw-Hill Book Company (UK) Limited.

Ruth, R.H. and A.S. Harris 1979 Management of western hemlock-sitka spruce forest for timber production. General Technical Report PNW-88.

Ryan, M.G. 1988 The importance of maintenance respiration by living cells in the sapwood of subalpine conifers. Ph. D. Thesis. Oregon State University. Corvallis, Oregon.

Ryan, M.G. 1991a Effects of climate change of plant respiration. Ecological Applications 1(2):157-167.

Ryan, M.G. 1991b A simple method for estimating gross carbon budgets for vegetation in forest ecosystems. Tree Physiol. 9:255-266.

Rygiewicz, P.T. 1983 Effects of mycorrhizas and pH on nitrogen and potassium fluxes in Pacific Northwest coniferous roots. Ph.D. Thesis. University of Washington, Seattle, Washington.

Rygiewicz, P.T., S.L Miller, and D.M. Durall 1988 A root-mycocosm for growing ectomycorrhizal hyphae from host roots while maintaining symbiotic integrity. Plant and Soil 109:281-284.

Saglio, P.H., and A. Pradet 1980 Soluble sugars, respiration, and energy charge during aging of excised maize root tips. Plant Physiol. 66:516-519.

Sarjala, T. H. Raitio, E. -M. Turkki 1987 Nitrate metabolism in scots pine seedlings during their first growing season. Tree Physiol. 3:285-293.

Singh, J.S., S.R. Gupta 1977 Plant decomposition and soil respiration in terrestrial ecosystems. The Bot. Rev. 43(4):449-528.

Sission, W.B. 1983 Carbon balance of *Yucca elata* Engelm. during a hot and cool period *in situ*. Oecologia 57:352-360.

Sowell, J.B., and G.G Spomer 1986 Ecotypic variation in root respiration rate among elevation populations of *abies lasiocarpa* and *Picea engelmannii*. Oecologia 68:375-379.

Steinfrover, E 1981 The relationship between Cyanide-resistant and the storage of sugars in the taproots in *Daucus carota* L. J.Exp. Bot. 32(130):911-919.

Tjepkema, J.D., and L.J. Winship 1980 Energy requirement for nitrogen fixation in actinorhyzal and legume root nodules. Science 209:279-281.

Tripepi, R.R., and C.A. Mitchell 1984 Stem hypoxia and root respiration of flooded maple and birch seedlings. Physiol. Plant. 60:567-571.

Tschaplinski, T.J., and T.J. Blake 1994 Carbohydrate mobilization following shoot defoliation and decapitation in hybrid poplar. Tree Physiol. 14:141-151.

Van der Werf, A., T. Hirose, and H. Lambers 1990 Variation in root respiration; causes and consequences for growth. ED: H. Lambers *et al*. Causes and consequences of variation in growth rate and productivity of higher plants. pp227-240. SPB Academic Publishing bv, The Hague, The Netherlands.

Van der werf, A., M.Van Nuenen, A.J. Visser, and H. Lambers 1993 Effects of N-supply on the rates of photosynthesis and shoot and root respiration of inherently fast- and slow-growing monocotyledonous species. Physiol. Plant. 89:563-569.

Van Veen, J.A., R. Merckx, and S.C.Van der Geijin 1989 Plant- and soil related controls of the flow of carbon from roots through the soil microbial biomass. Plant and Soil 115:179-188.

Vartanian, N., and M. Chauveau 1986 *In Vitro* study of root respiration recovery in mustard and rape following a drought stress period. Plant and Soil 92(2):255-264.

Veen, B.W. 1981 Relation between root respiration and root activity. IN: Structure and function of plant root. ED: R. Brouwer et al.. Martinus Nijhoff/Dr W. Junk Publisher.

Vessey, J.K. and D.B. Layzell 1986 Regulation of assimilate partitioning in soybean. Plant Physiol. 83:341-348.

Vogt, K.A. 1991 Carbon budgets of temperate ecosystems. Tree Physiol. 9:69-86.

Vogt, K.A., and D. J. Vogt 1989 Methodological considerations in measuring biomass, production, respiration and nutrient absorption for tree roots in natural ecosystems. IN: Applications of continuous and steady-state methods to root biology. ED: J.G. Torrey and L.J. Winship. Kluwer Academic Publisher. Printed in Netherlands.

Wiant, H.V., Jr. 1967 Contribution of root to forest "soil respiration". IN: Advancing frontiers of plant sciences. ED: L. Chandra 18:163-168.

Wijk. C.K., and H.B.A. Prins 1993 The kinetics of NH_4^+ and NO_3^- Plant and Soil 151:91-96.

Wollny, E. 1897 Die zersetung der organischen stoffe und die Humusbidungen mit Rucksicht auf die Bodenkutur. C. Winter, Heidelberg.

Yan, Feng, S. Schubert, and K. Mengel 1992 Effect of low root medium pH on net proton release, root respiration, and root growth of corn (*Zea mays* L.) and Broad Bean (*Vicia faba* L.). Plant Physiol. 99:415-421.

Zacheo, G., and S. Molinari 1987 Relationship between root respiration and seedling age in tomato cultivars infected by *meloidogyne incognita*. Ann. Appl. Biol. 111:589-595.

Zaerr, J.B., and C.J. Brown 1978 Physiology of western hemlock roots. IN: Western hemlock management. ED: W.A. Atkinson and R.J.Zasoski. College of Forest Resources, University of Washington, Seattle, Washington.