AN ABSTRACT OF THE DISSERTATION OF

Michele L. Pruyn for the degree of Doctor of Philosophy in Wood Science and in Forest Science on June 17, 2002.
Title: Patterns of stem respiration within tree, with age, and among species in Pacific Northwest Trees.

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An increment core-based, laboratory method was used to measure tissue-level respiration under controlled temperature (termed respiratory potential) of eleven tree species from three age classes. Respiratory potential was calculated on a basis of core dry-mass, volume, carbon, or nitrogen content and live bole volume. Methods tests suggested that core carbon dioxide production was indicative of parenchyma cell respiration, and that wounding and extracting artifacts were minimal. Core respiratory potential (mass-based) decreased from inner bark to sapwood/heartwood boundary with inner bark 2 – 11 times higher than outer sapwood, which was in turn 1.3 – 2 times higher than inner sapwood. Heartwood respiratory potential was 2 – 10% that of outer sapwood, and was likely a product of microbe respiration or diffusion of stored carbon dioxide. For stems measured at multiple heights, sapwood rings of the same calendar year had 50% higher respiratory potentials at treetops than at bases. Increased respiratory potential near

apical (treetops) and cambial (inner bark) meristems suggested that parenchyma respiration was driven by proximity to substrate supply and/or involvement in cell formation and expansion.

Comparisons were also made among species. At breast height, species with narrow sapwood thickness (e.g. *Pseudotsuga menziesii*) had 50% higher core respiratory potentials (volume-based) than species with wide sapwood thickness (e.g. *Pinus ponderosa*). This pattern was not shown for inner bark, or sapwood in the crown. Whole-tree respiratory potential was higher in species of lower relative live bole volume (inner bark plus sapwood), except young *Pinus ponderosa*, where high relative live bole volume corresponded to high respiratory potential.

Additionally, possible physiological sources for the observed variations in respiratory potential were examined. Inner bark of *Pinus ponderosa* had higher total nitrogen and nonstructural carbohydrate contents, corresponding to higher respiratory potentials as compared to *Pseudotsuga menziesii*. In contrast, sapwood nitrogen was similar between the two species, and lower total nonstructural carbohydrates in *Pinus ponderosa* sapwood corresponded to higher respiratory potentials as compared to *Pseudotsuga menziesii*. Percent ray parenchyma volume of *Pseudotsuga menziesii* averaged 16% higher than *Pinus ponderosa*. Generally, nonstructural carbohydrate content was a better indicator of sapwood respiratory potential than either tissue nitrogen content or parenchyma anatomy.

Patterns of Stem Respiration within Tree, with Age, and among Species in Pacific Northwest Trees

by
Michele L. Pruyn

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Dedicated to my high school biology teacher, Ms. Sharon Mattson, and my college friend, Annie Pringle, who each taught me in her own way that my career path could be synonymous with my dreams.

PATTERNS OF STEM RESPIRATION WITHIN TREE, WITH AGE, AND AMONG SPECIES IN PACIFIC NORTHWEST TREES

1. INTRODUCTION

1.1. APPLICATION AND JUSTIFICATION

Understanding forest ecosystems is crucial to ensuring their existence in the future. Human reliance on forest products is unquestionable. To support this claim, one only need consider the value of wood and wood-associated products in society. There is an increasing demand to assure sustainability of production systems and forested ecosystems as the preservation of natural resources becomes evermore prevalent and necessary (Kaufmann and Linder 1996). Determining how stem respiration varies within and among trees, and whether there is a relationship with sapwood quantity and/or composition could enable the prediction or control of sapwood/heartwood amounts in trees. Better knowledge of sapwood function and its determinants will help forest managers understand how forestry practices impact sapwood/heartwood quantities and promote the development of silvicultural regimes that enhance favorable wood properties. This type of work has potential applications related to the processing, utilization, and economic value of sapwood versus heartwood, which differ greatly from one another in their properties, such as color, permeability, surface chemistry, decay resistance, and moisture content.

With rising atmospheric carbon dioxide and the corresponding predicted increases in ambient temperature, the subject of carbon cycling in forest ecosystems

has gained much attention. The potential for trees to absorb large amounts of carbon dioxide and subsequently store carbon for long periods has brought many researchers together on the subject of forest ecosystem response to global climate change. Because stem respiration is an important component of carbon budgets in forests, it is important to understand how sapwood amounts in trees are determined. Knowledge of respiration gradients within trees may provide modelers with in-stem respiration indices that are specific for species, age-class and season, which is potentially useful for modeling whole-tree and ecosystem carbon budgets.

1.2. BACKGROUND INFORMATION

Among tree species, sapwood can vary drastically in terms of its volume and cell content. These variations can affect the overall physiology of the tree. Sapwood is the active, water-conducting tissue in the stem and branches, which connects the leaves to the roots. Its quantity and composition determines (in part) the amount of photosynthetic products that will be stored versus metabolized, the efficiency with which water is conducted throughout the tree, as well as the stem's overall mechanical stability and structure.

The sapwood of stems and branches is composed of secondary xylem. Most of the cells in the xylem are highly specialized for transporting water. Vessels (in hardwoods) and tracheids (in hardwoods and softwoods) are more or less cylindrically shaped with hollow centers and thickened cell walls, which is an ideal structure for water conduction. To serve this function, tracheids and vessels are

metabolically inactive (dead) and hollow. Fifty to ninety-five percent of the xylem is composed of such dead cells (Panshin and DeZeeuw 1980). It is therefore not surprising that the living and respiring tissue of the xylem has not received much attention in the literature (Landsberg 1986).

Sapwood parenchyma comprise very little of the xylem, seven to forty-eight percent in hardwoods and three to eleven percent in softwoods (Panshin and DeZeeuw 1980), yet these are the living cells responsible for the majority of metabolic activity that occurs in woody tissues (except the phloem and cambium). Parenchyma cells occur throughout plants of all species and have various sizes, shapes, and metabolic and functional roles, having only the common attribute of a thin primary wall and a lack of a secondary wall (Mauseth 1988). Sapwood parenchyma cells (Figure 1.1) provide stems and branches with storage capabilities and various physiological functions, including respiration, radial transport of reserve materials and gases (aeration), water storage, regeneration after wounding via tyloses and callus, and compartmentalization of damage from wounding (Lev-Yadun and Aloni 1995).

The role of sapwood parenchyma cells in whole-tree physiology has not been completely revealed. Photosynthesis results in the production of carbon compounds, which are subsequently loaded into the phloem (tissue between the cambium and bark) for transport to the branches, stem and roots. From the phloem, these carbon compounds are directed throughout the tree and processed and metabolized to meet various physiological and environmental demands.

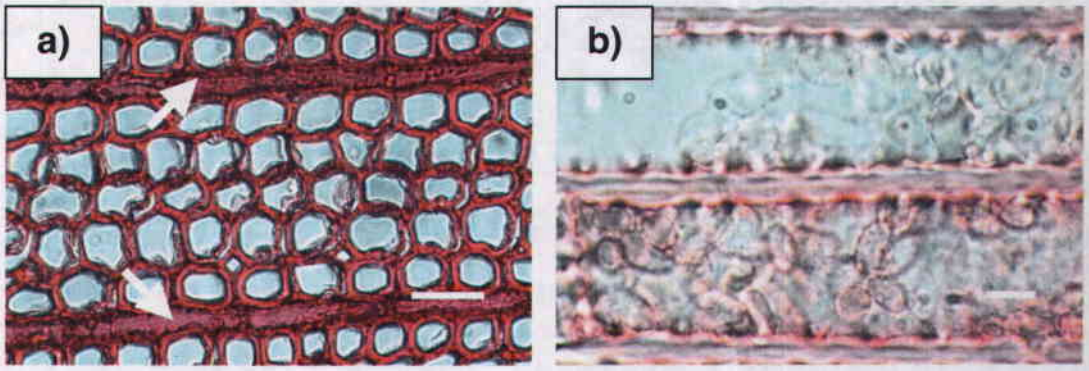

Figure 1.1. *Pseudotsuga menziesii* sapwood ray parenchyma cells. (a) Transverse section using 40 × objective (bar = 40 µm), cells indicated by arrows and (b) radial section using 100 × (bar = 10 µm), showing starch granules within cells.

Respiration breaks down photosynthetic compounds, subsequently releasing carbon dioxide and the energy (ATP) required to drive various plant metabolic processes. The way in which photosynthetic materials are directed within the plant is called carbon allocation. For example, carbon allocated to the cambium, may result in the production of new sapwood, whereas carbon allocated to the sapwood is either stored in parenchyma cells or metabolized in one of many processes. Broad questions remain unanswered regarding the regulation of carbon allocation in trees. It is thought that certain processes are more demanding of carbon reserves than are others. The central query of this dissertation was to quantify and describe the spatial and temporal patterns as well as the magnitude of the metabolic demand of sapwood parenchyma.

1.3. CONTEXT

The principle objective of this dissertation research was to understand the contribution of stem respiration to whole-tree and forest ecosystem physiology. This work was part of two broader studies, each designed by Dr. Barbara Gartner and Dr. Mark Harmon. Dr. Gartner's work has focused on the trade-offs among physiological processes that determine sapwood volume and composition. For example, the relationship between leaf area/sapwood area and ray cell anatomy and vitality was examined with regard to sapwood's role in providing storage space for photosynthetic products (Gartner et al. 2000). Also, the hypothesis was tested of whether trees operate according to a 'design criteria' to enhance sapwood hydraulic conductivity, and yet maintain mechanical resilience (Domec et al. 2002). The focus of this dissertation was to model the variation in sapwood parenchyma metabolism (respiration), anatomical characteristics, and chemical composition and determine how such gradient relate to sapwood amounts. The combination of the various studies of Dr. Gartner's research team will provide a complete picture of how materials are allocated to physiological processes and structure of sapwood.

The second broad area of interest, Dr. Harmon's research, involves monitoring carbon cycling in live and dead matter in forest ecosystems with the ultimate objective of modeling carbon budgets. Woody stem respiration contributes significantly to the carbon cycle of forested ecosystems because stems store carbon for long periods of time compared with foliage and fine roots (Lavigne and Ryan 1997). Thus, understanding the variation of stem respiration within and

among woody tree species will help reveal mechanisms of forest carbon storage and release. Respiration indices for several Pacific Northwest tree species were determined in this dissertation work and will be incorporated into a computer-based ecological model of ecosystem carbon budgets (Harmon et al. 1996).

1.4. RESEARCH OBJECTIVES

The first objective of the current study was to describe the patterns of parenchyma cell metabolic activity (respiration) within stems of mature Douglas-fir (*Pseudotsuga menziesii*, Mirb.) and ponderosa pine (*Pinus Ponderosa*, Laws.) each at separate sites in Oregon. Additionally, the findings from the older Douglas-fir and ponderosa pine were compared to measurements made on younger trees (< 10 – 50 years) of the same species at their respective sites to pursue the question of whether young trees follow trends similar to the older trees. This work required considerable methods development. Assays were devised to estimate respiration rates within stems of older and younger trees by radial and vertical position, both *in situ* and in the laboratory. The increment core-based laboratory measurements of respiration were termed respiratory potential because they were not *in situ* respiration of intact stems. Additionally, several tests were conducted to determine the technique's reliability and examine possible artifacts. This objective is presented in chapters one and two.

After describing within-stem variation of metabolic activity of Douglas-fir and ponderosa pine, the second study objective was to apply the method to other

tree species, make comparisons among species, and pursue questions on the whole-tree level. For example, the hypothesis was tested of whether the respiratory demand from woody tissues is constant among mature tree species. Thus, trees with lesser relative volumes of sapwood have higher per unit volume respiration rates than trees with greater relative volumes of sapwood. The inverse relationship between relative volume and respiration rate per volume suggests that there is a compensation for trees that maintain large volumes of sapwood. To scale within-stem, tissue-specific respiratory potential to the whole-tree level, equations were developed to predict sapwood thickness and sapwood volume from stem diameter at breast height. Data for the equations were obtained from the Forest Science Databank, which surveyed a wide range of coniferous species of the Pacific Northwest. The results of this objective are presented in chapter three.

The third and final study objective was to examine the physiological sources for the observed trends (i.e., within stems and between species, age classes, and seasons). Monthly measurements of respiratory potential and stem temperature were collected from standing trees of Douglas-fir and ponderosa pine at their respective sites to normalize laboratory measurements to field temperatures and to compare the temperature independent and dependant seasonal fluxes between the two species. Additionally, wood anatomy and chemistry were measured for both species to investigate whether parenchyma cell amount or chemical composition were correlated with respiratory potential. This objective was outlined and discussed in chapters four and five.

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2. RESPIRATORY POTENTIAL IN SAPWOOD OF OLD VERSUS YOUNG PONDEROSA PINE TREES IN THE PACIFIC NORTHWEST

Running Title: Stem respiration in young and old ponderosa pine

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2.1. SUMMARY

Our primary objective was to present and test a new technique for *in vitro* estimation of respiration of cores taken from old trees to consider respiratory trends within sapwood. Our secondary objective was to quantify effects of tree age and stem position on respiratory potential (rate of CO_2 production of woody tissue under standardized laboratory conditions). We extracted cores from 1 – 4 vertical positions in boles of 200+, 50+, and 15+ year-old *Pinus ponderosa* (Laws.). Cores were divided into five segments, corresponding to radial depths of: inner bark, outer, middle, inner sapwood, and heartwood. Data suggested that core segment CO_2 production was an indicator of its respiratory activity, and that potential artifacts from wounding and extracting were minimal. On a dry mass basis, respiratory potential of inner bark was 3 – 15 times greater than sapwood at all heights for all ages ($P < 0.0001$). Within sapwood at all heights in all ages of trees, outer sapwood had 30 – 60% higher respiratory potential than did middle or inner sapwood ($P < 0.005$). Heartwood had only 2 – 10% the respiratory potential of outer sapwood. For all ages of trees, sapwood rings produced in the same calendar year released over 50% more CO_2 at treetops than at bases ($P < 0.0001$). When scaled to the whole-tree level on a sapwood volume basis, sapwood of younger trees had higher respiratory potential than that of older trees. In contrast, the trend was reversed when using the outer-bark surface area of stems as a basis for comparing respiratory potential. The differences observed in respiratory potential calculated on a core dry mass, sapwood volume, or outer-bark surface area basis

demonstrate that the resulting trends within and among trees are determined by the way in which the data are expressed. These results must be interpreted with care since they are based on core segments rather than *in vivo* measurements, but the relative differences they indicate are probably valid even if the absolute differences are not.

Keywords: stem respiration, sapwood, phloem, cambium, tree age, tree size, ponderosa pine

2.2. INTRODUCTION

Stem and branch respiration are significant components of the carbon balance in trees (Kinerson 1975, Ryan 1990, Sprugel and Benecke 1991). Despite recent advances, many crucial details are still lacking in understanding stem respiration. On the scale of individual trees, it is not yet known where the majority of stem respiration occurs. Radial trends in respiration from bark to the heartwood/sapwood boundary have been determined from tissues extracted just above ground level in stems of *Pinus radiata* D. Don (Shain and Mackay 1973), *Fraxinus nigra* L. and *Acer rubra* L. (Goodwin and Goddard 1940), and in *Picea abies* L. and other species (Møller and Müller 1938). However, gradients of activity from bark to pith at other vertical positions, or from apical meristem to roots have not been determined (Sprugel and Benecke 1991). Also, it is not known if these gradients persist throughout a tree's life. Such patterns of sapwood activity are potentially critical to modeling whole stem respiration and its contribution to

forest ecosystem carbon budgets. Stockfors (2000) addressed this issue by measuring temperature variation among various heights and radial depths in *Picea abies* (L) Karst. Assuming a constant temperature-respiration relationship, he observed within-stem temperature variations, which resulted in scaling errors between 2 and 72% for a single sunny day and 2 and 58% for a whole year.

Furthermore, there is evidence of temperature-independent variation in CO_2 efflux from stems (Martin et al. 1994). Negisi (1982) demonstrated that stem CO_2 evolution rates in several tree species are often 50% lower than temperature-predicted respiration rates. Martin et al. (1994) provides three main theories for this discrepancy. First, increased transpiration rates on hot, sunny afternoons may lead to water deficit and stress, thus decreasing respiration (Lavigne 1987, Kakubari 1988). A second possibility is that much of respiration-evolved CO_2 is dissolved and carried away by the xylem transpirational stream (Neigisi 1972, Ryan 1990). This hypothesis is supported by the argument that oxygen is supplied to the xylem from dissolved oxygen in the flowing sap and not from radial diffusion of oxygen from the atmosphere (Hook et al. 1972, Eklund 1990). Finally, diurnal changes in rates of carbohydrate export from photosynthesizing tissue could lead to variation in substrate concentration, possibly affecting respiration rates (Azcón-Bieto et al. 1983).

The present study was designed to examine variations of sapwood respiration within stems independent of the effects of temperature and transpiration. We measured rates of CO_2 production under controlled laboratory conditions of

cores taken from stems of 15+, 50+, and 200+ year-old *Pinus ponderosa* L. trees. We refer to the reported values as respiratory potential rather than respiration rate because the conditions of our measurements on these excised samples are probably different from those within the tree. The purpose of this study was to identify bark-to-pith and treetop-to-base trends in respiratory potential. We examined potential artifacts of measuring respiration of cores extracted from tree stems, and the implications of scaling such measurements to the whole-tree level. We also considered the likely physiological mechanisms responsible for variations in respiration within trees and across tree ages.

2.3. MATERIALS AND METHODS

2.3.1. Species and site characteristics

We collected samples from 15+, 50+, and 200+ year-old trees of ponderosa pine, *Pinus ponderosa*, L. (ponderosa pine) located just east of the Cascade Range in central Oregon, near Gilchrist (N43° 28' W121° 41') at elevation 1355 m. Unless indicated otherwise, most samples were collected in early March of 1999 (200+) and 2000 (15+, 50+) from trees prior to bud break and wood production, which occurs in early to mid-June in this region. All cores were collected either prior to (March) or after (October or February) the growing season to ensure that measured respiration rates represented only maintenance respiration (McCree 1970, Thornley 1970). We therefore avoided growth respiration because it is more likely

to depend on hormonal or other stimuli, and carbohydrate supply from outside the immediate xylem stores.

2.3.2. Tree felling and sampling

All trees sampled were free of broken tops, stem deformities, or disease. Six trees each were chosen from three age classes: 200+, 50+, and 15+ year-old trees, and the diameter at 1m recorded (Table 2.1). After felling the 200+ year-old trees, we sawed 20-cm-tall stem disks from stems at node 220, and just above (to avoid branch whorls within the crown) nodes 65, 50, and 15 (years from the treetop). Tree height measurements were taken from tree base to each node, base of the live crown (first stem position above ground level with three live branches), and to treetops (Table 2.1).

Disks for respiration measurements were transported to the laboratory wrapped in extra-strength black garbage bags with moist paper toweling inside to reduce desiccation. Stem disks were stored at 4°C. Within one week after harvesting, three 12mm diameter increment cores were extracted from each node, wrapped in plastic bags and returned to cold storage. For 50+ and 15+ year-old trees, cores were extracted directly from the felled stems in the field. Three cores each were sampled from nodes 50 and 15 in the 50+ year-olds, and from node 15 in the 15+ year-olds. Cores were wrapped in plastic bags and stored on ice until returned to the laboratory, where they were stored at 4°C.

TABLE 2.1. Mean tree age, height, diameter, total leaf area, and growth rates of ponderosa pine trees. For age, height, diameter, and total leaf area means ± standard error (n = 6). For growth rates, LSMEANS and 95% confidence intervals from a 1-way ANOVA. For each row, different letters indicate significant differences among means (FPLSD, P – values < 0.05).

	Tree Age Class		
Parameter	**200+ yrs.**	**50+ yrs.**	**15+ yrs.**
Age (years)	223 ± 27	72 ± 5	31 ± 3
Range	123 – 314	47 – 79	17 – 43
Height (m)			
Base to node 15	31.0 ± 0.3	8.6 ± 0.9	1.1 ± 0.3
Base to node 50	25.5 ± 1.2	1.6 ± 0.2	--
Base to node 65	21.4 ± 1.2	--	--
Base of the live crown	13.3 ± 1.0	(< 0.5 m above node 50)	(< 0.1 m from ground)
Base to node 220	0.43 ± 0.04	--	--
Total	33.3 ± 0.4	12.4 ± 0.9	2.92 ± 0.01
Range	32 – 34	9 – 15	2.7 – 3.1
Diameter at 1 m (cm)	62 ± 2	27 ± 2	10 ± 0.5
Range	53 – 67	21 – 35	9 – 12
Total leaf area (m^2)	540 ± 35	71 ± 16	11 ± 2
Growth rates over last 15 years			
Height ($cm \cdot year^{-1}$)			
node 15 to treetop	15 ± 3^{ab}	21 ± 3^{a}	11 ± 3^{b}
Diameter 1 m from ground ($cm \cdot year^{-1}$)	0.21 ± 0.02^{a}	0.15 ± 0.02^{a}	0.20 ± 0.02^{a}
Stem wood biomass ($kg \cdot year^{-1}$)	$18\ (16, 20)^{a}$	$1.6\ (1.0, 2.4)^{b}$	$0.21\ (0.04, 0.54)^{c}$
Stem bark biomass ($kg \cdot year^{-1}$)	$3.2\ (2.8, 3.7)^{a}$	$0.5\ (0.3, 0.7)^{b}$	$0.11\ (0.04, 0.21)^{c}$
Stem wood + bark biomass / leaf area ($kg \cdot m^{-2} \cdot year^{-1}$)	0.042 ± 0.007^{a}	0.038 ± 0.007^{a}	0.031 ± 0.007^{a}

A second, small disk (< 5 cm tall) was taken from each vertical position of all trees and returned to the laboratory. After kiln drying, radii measurements and ring counts from pith to the distal edge of each tissue (outer and inner bark, sapwood, and heartwood) were recorded from the small disks taken at each stem height, for all age classes. Also, average annual ring width for the last 15 years was measured from the lowest disk for each age class, for use in diameter and stem biomass growth calculations.

2.3.3. Stem and bark biomass growth and leaf area calculations

We calculated stem wood and bark biomass growth (kg / year) for each tree using equations from Gholz et al. (1979) for ponderosa pine (Table 1). Diameter (at 1 m) of 15+ year-old trees was just below the minimum recommended diameter range in the equations (15.5 – 79.5 cm). We also calculated stem wood plus bark biomass growth per unit leaf area (kg / m^2 · year, Table 1). To estimate leaf area, we divided each tree into from one to four sections (i.e. 15+ year-olds, 1 section; 50+, 2; and 200+, 4). We hand-clipped 25% (one of every four leaf bundles) of the leaves (including attached woody material) from large crown sections (node 50 – 220), 100% from small sections (node 15), and recorded the fresh mass of the clipped material (B.L. Gartner, JC Domec, M. Pruyn, and R. Spicer, unpublished data). We took sub-samples of the clipped material, recorded the fresh mass, returned them to the laboratory for oven drying, and then separated leaves from woody twigs and branches and recorded dry mass for each. In the field, we also

took 10 needle fascicles from the clipped material back to the laboratory and stored them at -20°C, until we were ready to record leaf dimensions to calculate leaf area. We then dried the leaves, and recorded dry mass. Using proportions of leaf area to dry leaf mass to fresh leaf mass, we calculated total leaf area for each tree.

2.3.4. Respiration measurements

All cores were analyzed within one week of sampling. Respiration rates of isolated tissues have been shown to remain constant, as long as storage time was < 15 days in two hardwoods (Goodwin and Goddard 1940), *Pinus radiata* (Shain and Mackay 1973), and *Pseudotsuga menziesii* Mirb. (Pruyn, unpublished data). Twenty-four hours prior to measurement, cores were cut longitudinally to divide the core into five segments: inner bark (phloem and cambium); outer, middle, and inner sapwood; and heartwood. Outer, middle, and inner sapwood were defined by dividing sapwood into three equal radial lengths. The exception was in cores extracted from older trees, where sapwood width was greater than 100 growth rings. In this case, core segments of 10 – 15 growth rings in length were taken from the outer and inner sapwood boundaries and from the center of the sapwood. Heartwood samples (< 10 – 15 growth rings in length) were taken one ring interior (towards pith) of the transition zone rings (one or two lighter colored rings at the sapwood/heartwood boundary).

The number of rings per segment was recorded, so that an age could be determined for each radial position. Transition zone rings plus one ring of

innermost sapwood were excluded from the inner sapwood sample. These segments were weighed, wrapped tightly in plastic, and stored at 25°C overnight. The lag in measurement after coring and cutting the wood allowed metabolic activity in core segments to stabilize, thus minimizing the potential effect of accelerated respiration in response to tissue damage sustained by the cores (Goodwin and Goddard 1940, Hari et al.1991, Levy et al. 1999).

Immediately prior to measurement, core segments were re-weighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, carbon dioxide concentration within the vials was measured with a Hewlett-Packard (5700A) gas chromatograph (*GC*, Hewlett Packard, Rt. 41, Avondale PA 19311, USA) immediately after closing the vials and again after an incubation period. Because CO_2 production rate could vary with length of incubation, incubation period was held constant within experiments. For any given experiment, an incubation period of 6 or 20 hours was used, with the longer incubation necessary to accommodate the processing time of large numbers of samples. The *GC* used a He carrier and a thermal conductivity detector. A standard gas mix of 1% CO_2 and 20% O_2 in a blend of N_2 was used to calibrate the *GC*. The rate of CO_2 production by core segments, referred to here as respiratory potential, was the gauge for tissue metabolism. The respiratory potential k (nmoles CO_2 per grams dry-mass per second) of core segments was calculated as:

$$k = (\Delta CO_2/100) \cdot (1/M_{OD}) \cdot (1/T) \cdot V_H \cdot 40.91 \cdot 10^9 \qquad (1)$$

where ΔCO_2 is the net percent increase of CO_2 concentration during the incubation period, T (s) is the incubation period, M_{OD} is the oven dry mass (g) of the core segment, V_H is the volume of headspace ($2.5 \cdot e^{-05}$ m^3 minus core sample volume), 40.91 is the constant for converting CO_2 molar volume (ml) to moles at 25°C laboratory temperature and one atmospheric pressure, and 10^9 is the conversion factor for moles to nanomoles. The formula was modified to represent a rate per unit volume by replacing M_{OD} with the appropriate core segment volume.

Core segments were incubated at 25°C between *GC* measurements. Immediately following the analysis, core segments were weighed a third time. The three successive wet masses verified that there was no substantial water loss between sampling and measurement (e.g. approximately 1 – 3% moisture was lost during laboratory incubations). The fresh volume of core segments was estimated as immersed weight in distilled water using Archimedes' principle (ASTM 1998). Dry masses were determined after drying at 60°C for 48 hours.

2.3.5. Testing of methods

Because of the invasive nature of our sampling method, four tests were conducted to examine potential problems. To determine whether rate of CO_2 production by core segments was constant with respect to time and $[CO_2]$ (in the vial), we extracted one core from each of four stem positions (node 15, 50, 65, and 220 from treetop) of the six 200+ year-old trees from the harvest described above. After extraction, cores were prepared as described above. Vial CO_2 concentration

was analyzed with the *GC* immediately, and at 11, 22, and 46 hours. Respiratory potential on a mass basis was calculated as described above for each incubation period (i.e. 0 – 11, 11 – 22, and 20 – 32 hours).

To determine whether core segment respiratory potential responded to increasing temperature as expected of biological processes, four cores each were extracted from breast height of five 200+ year-old trees in late February of 2000. Cores were wrapped and stored as described above. In the laboratory, cores were assigned randomly to one of four temperatures (5, 10, 15, 25°C), segmented into four radial positions (heartwood was excluded), and stored at the assigned temperature the night prior to analysis. Cores segments were weighed, placed in septum-sealed vials, and analyzed as described above for a 6-hour incubation at their assigned temperatures. The Q_{10} of respiratory potential of core segments was calculated from the formula:

$$Q_{10} = (k_2 / k_1)^{[10/(T_2 - T_1)]} \qquad (2)$$

where k_2 and k_1 were the respiratory potentials of core segments incubated at temperatures T_2 and T_1 °C, respectively (Chen et al. 2000).

To test the possible effect of tissue surface-area-to-volume ratio on CO_2 production from small (5 mm) and large (8 or 12 mm) diameter cores, one 5 mm core was extracted from each of the 200+ and 15+ year-olds from the above harvest, one 8 mm from each of the 200+ year-olds, and one 12 mm from each of

the 15 + year-old trees. Cores were prepared and analyzed as described above. Only inner bark, outer, and inner sapwood tissues were sampled to capture the extremes of activity, with the exception of 15+ year-old trees, where only inner bark and outer sapwood were sampled to save time. The incubation period was six hours.

To determine whether the observed CO_2 production was due to diffusion of residual, stored CO_2 within cores, respiratory potential in cores treated with chloropicrin (a fumigant that kills live cells, often used as an insecticide) were compared to controls in six 200+ and six 50+ year-old trees in early October of 2000. Two cores were extracted from breast height of each tree. Cores were prepared as described above and placed on a tray in a desiccator jar (without desiccant). Chloropicrin (one ml) was pipetted into a vial at the desiccator bottom. Vacuum grease was used to seal the lid onto the desiccator. Untreated (control) core segments were stored similarly with the exception of the vial of chloropicrin. Core segments were stored 18 hours in a fume hood at an average temperature of 23°C, and then placed in sterile vials under a sterile, laminar-flow hood, septum-sealed and analyzed as described above. Immediately following the analysis, core segments were submerged in a 1% aqueous triphenyl tetrazolium chloride (TTC) solution and incubated overnight at 25°C. The colorless TTC is reduced to a deep-red compound by dehydrogenases in the cytoplasm of living cells (Feist et al. 1971, Ryan, 1990). Following incubation, core segments were cut in half lengthwise to examine whether the dye penetrated the core centers. We predicted that

chloropicrin-treated core segments would release no CO_2 and not stain red, whereas controls would release CO_2 and stain red. The CO_2 release by chloropicrin-treated core segments that did not stain red would indicate that stored CO_2 contributed to the CO_2 release from live segments.

2.3.6. Scaling-up core-based measurements

To compare respiration among various stem nodes, we scaled core segment volumes to stem cylinder volumes at each radial position (i.e. inner bark, and outer, middle, and inner sapwood) from each vertical position (node 220 – 15). Stem cylinder height was 12 mm (core radius), and cylinder radius was the distance from the pith to outer edge of each radial position. The volume of each radial position was calculated by subtracting consecutive cylinders (e.g., inner bark – sapwood = inner bark). Each radial position's volume was then multiplied by the respective respiratory potential on a core segment volume basis to calculate a volumetric rate of CO_2 production for each radial position at each node. We calculated weighted respiratory potentials for each node by summing the volumetric rates of each radial position and dividing by each node's total outer-bark surface area, live wood volume (inner bark + sapwood), or stem wood volume (bark + sapwood + heartwood).

To understand how our core-based measurements of respiratory potential compared across the three age classes in this study, and to intact stem-level rates from the literature, we scaled our measurements to the whole-tree level. To enable

uniform comparison across age classes, we divided tree stems into two parts: tree base to node 15 and node 15 to treetop. For node 15 to treetop, we used node 15 core respiratory potentials for each radial position. For the tree base to node 15, we averaged core respiratory potential from nodes 220 – 50 for each radial position. We assigned node 15 respiratory potential to stem segments from node 15 to treetop rather than from another vertical position (e.g. node 49 to treetop in 50+ or 200+ year-old trees) so that we could scale-up consistently for each age class.

Wood volume from the tree base to node 15 was calculated using the formula for a frustum of a right circular cone, and from node 15 to the treetop, with the formula for a right circular cone. Volumes of frustums or cones were calculated for each radial position. Consecutive frustums or cones were subtracted to obtain a volume for each radial position (e.g., inner bark – sapwood = inner bark). We calculated volumetric rates of CO_2 production for each radial position in each stem segment and weighted respiratory potentials for each whole-stem segment for all age classes using the same calculations as described above for respiratory potential by node. In this manner, we calculated weighted respiratory potentials for whole-trees.

2.3.7. Statistical analysis

All data were analyzed in Statistical Analysis Systems software, release 7.0 (SAS Institute Inc. 1998). The Shapiro-Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or

natural log) was performed when necessary to meet assumptions of normality and constant variance. Means reported ± standard error, except transformed means, where confidence intervals are used.

Repeated measures analysis in PROC GLM was used to test the effects of incubation time on respiratory potential. Each tissue at all vertical positions was analyzed independently from the other tissues. The P – values are reported for the effect of time, vertical position, and their interaction, as well as whether the response was linear or quadratic with respect to time. Regression analysis in SAS-Assist was used to describe the relationship between temperature and respiratory potential, the procedure of which is described in the results section. To compare Q_{10} values among the four radial positions at different temperature ranges, least squares means (LSMEANS) were generated using PROC MIXED analysis, with randomized block design and strip-plot (split-block) treatments (Little and Hills 1978, Milliken and Johnson 1984). Trees were blocks and the effects of tissue radial position, temperature range, and their interaction were tested. Pair-wise comparisons (*t*-tests) among tissue radial positions and temperature ranges were conducted using Fisher's Protected Least Significant Difference (FPLSD) procedure (Fisher 1966).

Paired *t*-tests were used to compare the respiratory potential between small and large cores of the same tissue. A strip-plot analysis was also used to generate LSMEANS and make comparisons (using FPLSD) among tissues at various radial and vertical positions within trees. Because experiments were carried out during

different years for the different aged trees, each age class was analyzed separately. We tested the effects of tissue radial position, vertical position, and their interaction. Heartwood was also analyzed independently because its activity was substantially lower than that of live wood, having a large impact on constant variance and normalcy of the data.

For all calculations involving scaling core-based measurements to the whole-stem segment or whole-tree level and leaf area calculations, standard errors were not pooled at each step of the calculation. For simplicity, they were instead computed from the pool of six final parameter values (e.g. whole-tree respiratory potential per unit outer-bark surface area) within each age class. To make comparisons among tree ages (i.e. growth rates and stem-segment or whole-tree respiratory potentials), a one-way ANOVA in PROC GLM was used to generate LSMEANS, with the understanding that year-sampled may have been a confounding variable. Specific pair-wise comparisons among age class means were conducted using FPLSD.

2.4. RESULTS AND DISCUSSION

2.4.1. Effects of incubation time

The rate of CO_2 production (respiratory potential) for inner bark was constant over the incubation time periods of 11, 22, and 46 hours at all vertical positions (Figure 2.1a). A repeated measures analysis revealed no effect of

position, incubation time, or their interaction ($P > 0.1$). There was also no evidence of a linear or quadratic trend between incubation time and respiratory potential among the three time intervals tested for inner bark ($P = 0.3$ for both). In contrast, incubation time affected sapwood respiratory potential (Figure 2.1b, c, d). Incubation time and position had significant effects on the response for all sapwood tissues measured ($P < 0.0001$), as did their interaction ($P < 0.05$). Sapwood respiratory potential tended to increase with respect to time at all vertical positions, except node 15, where it was fairly constant (Figure 2.1b, c, d).

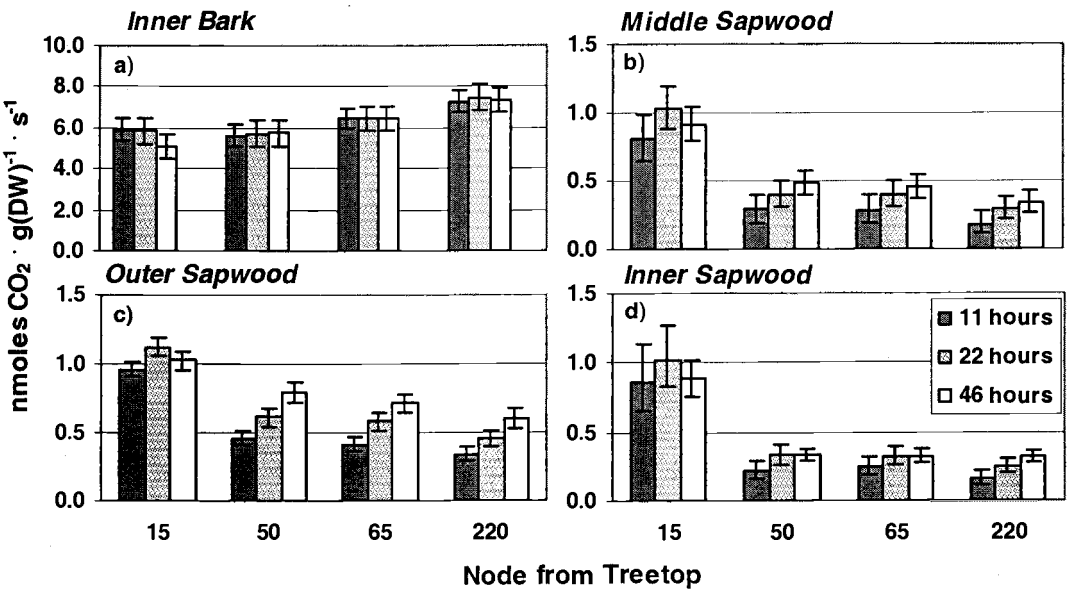

Figure 2.1. Core segment respiratory potential (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$) at 25°C with respect to incubation time for each radial position at each vertical position (note, inner bark has different y-axis scale). Different bar colors represent different incubation periods. Time intervals between gas chromatography readings are not equal. Means $\pm$ standard error (inner bark and outer sapwood), or confidence intervals (middle and inner sapwood), $n = 6$.

The null hypothesis that there was no trend with incubation time could not be rejected (all sapwood tissues, $P < 0.0002$). Significant variation in sapwood respiratory potential over time validated the necessity of maintaining constant incubation periods within experiments.

The $[CO_2]$ surrounding cells or whole organisms has been observed to have both *indirect* and *direct* effects on their respiration rates: indirect effects are due to the $[CO_2]$–history of the plant, whereas direct effects result from the $[CO_2]$ at the time of respiration measurement (Amthor 1991). The current results from pine sapwood indicated that as $[CO_2]$ within the vials gradually accumulated to levels of 1 – 5 %, an effect of increased respiration rate was initiated. Indirect effects of increased rates of apparent respiration have been observed, but primarily in crop plants, leaves (e.g., Azcón-Bieto et al. 1983), and tree roots (e.g., McDowell et al. 1999). This effect was hypothesized to result from high tissue-levels of non-structural carbohydrates and increased involvement of the alternative (cyanide-resistant) pathway of respiration (Amthor 1991, Lambers 1998). Because the gaseous environment of sapwood is probably CO_2-enriched (Eklund 1990, Hari et al. 1991, Levy et al. 1999) and its parenchyma cells store non-structural carbohydrates (Lev-Yadun and Aloni 1995), such indirect effects of $[CO_2]$ reported for storage organs and roots may explain the findings presented here. Clearly, further investigation is needed to better understand responses of stem wood respiration to changes in intercellular $[CO_2]$.

2.4.2. Temperature effects

In regressing respiratory potential on temperature, respiratory potential was log-transformed to correct the problem of non-constant variance in the residual plots. Because the response variable was still not linear and non-constant variance persisted, we modeled the response by adding a quadratic (x^2) term to the equation. (We selected x^2 over $x^{0.5}$ because extra sum of squares F-tests revealed that the x^2 was significant for all the radial positions (Table 2), whereas $x^{0.5}$ was significant for sapwood but not for inner bark). The y-intercept ($\beta_o = 0.28$) from the log of inner bark respiratory potential on temperature was not significant to the fitted quadratic equation ($P = 0.06$), indicating that the intercept was probably near zero (Table 2.2). However, because we did measure respiratory potential at 0°C, we did not drop β_o from the equation.

TABLE 2.2. Equation parameter estimates for the response of *ln* (respiratory potential) to temperature for each radial position. The F – statistics and P – values (Extra Sum of Squares F – test) are given for x^2 parameters (β_2). Estimates ± standard error, and significance to equation: * $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Radial Position	***Parameter Estimates*** ($\ln y = \beta_o + \beta_1 \cdot x + \beta_2 \cdot x^2$) β_o	β_1	β_2	β_2 F_{stat}
Inner Bark	0.28 ± 0.14	0.12 ± 0.02***	-0.0015 ± 0.0007*	$F_{1,16} > 4.74^{*}$
Sapwood				
Outer	-2.95 ± 0.20***	0.20 ± 0.03***	-0.0045 ± 0.0010***	$F_{1,17} > 20.69^{***}$
Middle	-3.51 ± 0.24***	0.20 ± 0.03***	-0.0045 ± 0.0012**	$F_{1,17} > 13.60^{**}$
Inner	-3.55 ± 0.25***	0.19 ± 0.04***	-0.0041 ± 0.0012**	$F_{1,17} > 11.23^{**}$

Inner bark and sapwood both responded to increasing temperature by increasing their respiratory potentials (Figure 2.2a-d, Table 2.2). This response has often been attributed to increased rates of respiration's enzymatic reactions at higher temperatures (Ryan et al. 1994). The curvature in respiratory response at 25°C suggested the approach of an optimal temperature range for enzymatic activity, where enzyme or substrate availability, and not temperature, was the limiting factor of respiration rate. To account for correlation among the four radial positions when comparing core respiratory response to temperature, we calculated Q_{10}*'s* for each of three temperature ranges at each radial position and used strip-plot treatments to model the covariance among the positions.

Temperature range, and the interaction of temperature range and tissue radial position, each had significant effects on Q_{10} ($P < 0.0001$). The significant interaction term was largely because inner bark Q_{10} dropped from approximately 2.6 to 1.5 between the ranges of 10-15°C and 15-25°C, whereas the sapwood Q_{10} dropped from approximately 6.0 to 1.4 between the ranges of 5-10°C and 10-15°C (Figure 2.2). Decreasing Q_{10} with increasing temperature is in accordance with Larcher (1983), who suggested that plant Q_{10}*s* approach 2.0 at 5 – 25°C, increase to $\geq$ 3.0 below 5°C, and drop to $\leq$ 1.5 above 25-30°C. This temperature dependence of Q_{10} is due to a shift in activation energy of respiration enzymes (Lyons 1973). This shift occurred at a higher temperature range for inner bark than sapwood in the current study. Effect of tissue radial position alone was insignificant ($P = 0.8$)

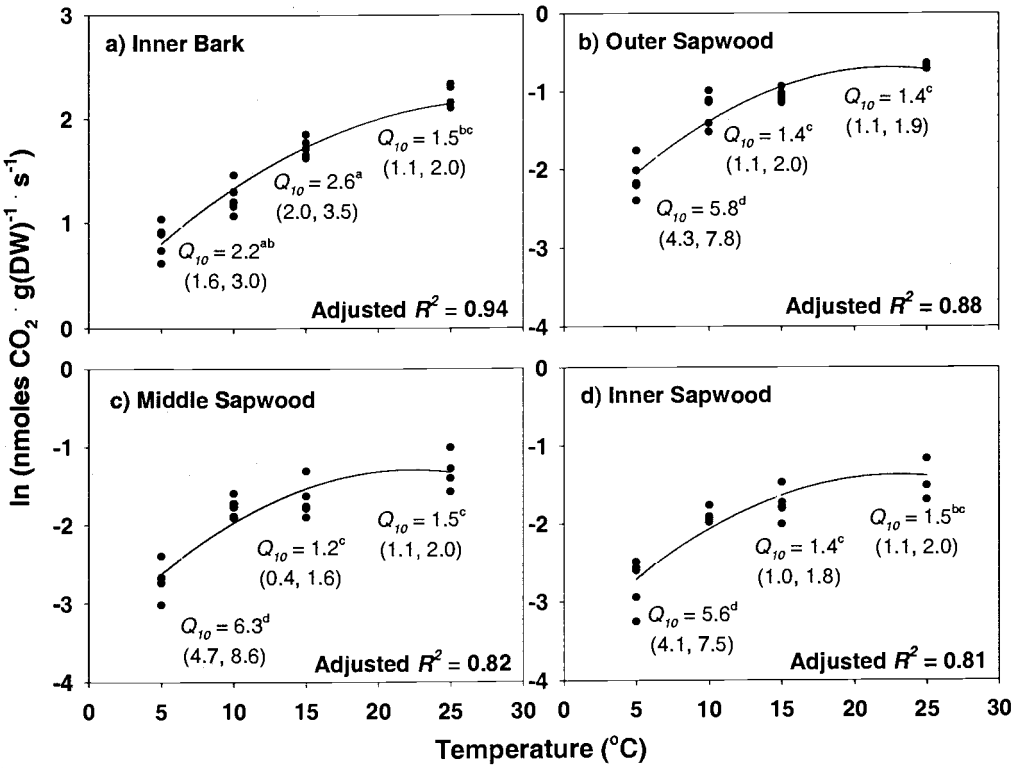

FIGURE 2.2. Relationship between temperature (°C) and natural log of stem respiratory potential (nmoles CO_2 · g(DW)$^{-1}$ · s^{-1}) of core segments extracted from breast height of 200+ year-old trees. Responses are shown for a) inner bark b) outer sapwood c) middle sapwood and d) inner sapwood (note, difference in y-axis scale for inner bark). A quadratic equation was fit to the data, the parameters of which are described in Table 2. Adjusted R-squares are given for each plot. Mean Q_{10} (Equation 2) and confidence intervals (n = 5) are given for each temperature range (i.e. 5-10, 10-15, and 15-25°C). Different letters represent significant differences among all means (strip plot model in PROC MIXED, P – values < 0.05).

because at any given temperature range, sapwood Q_{10} was fairly uniform among the three radial positions (Figure 2.2b, c, d). This uniformity of sapwood Q_{10} (and inner bark at 15 – 25°C, Figure 2.2a) was notable since cambial age ranged from 1 – 100+ years from bark to sapwood/heartwood boundary. Thus, respiratory enzymes in the live stem wood of these pines responded to temperature similarly, regardless of tissue age.

2.4.3. Effects of core size

Respiratory potential did not differ significantly between large and small core segments in three of the five comparisons ($P > 0.1$) for both 200+ and 15+ year-old trees; it was significantly higher in larger than smaller inner sapwood segments of 200+ year-olds ($P = 0.03$) and significantly lower in larger than smaller inner bark segments of 15+ year-olds ($P = 0.05$, Table 2.3). Large core segments had 40 – 70% the surface-to-volume ratios of small segments, so if wounding increased respiration, contrary to results shown here (except inner bark of 15+ year-old trees), one would have expected higher respiratory potential in the small, not large segments.

TABLE 2.3. Effect of core diameter on core segment respiratory potential at 25°C, extracted from breast height of 200+ and 15+ year-old trees. Means ± standard error ($n = 6$). For each tree age class and radial position, P – values from paired t – tests between small and large cores are as follows: * $P < 0.05$.

	Respiratory Potential by radial position (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$)		
Tree Age, Core Size	Inner bark	Outer Sapwood	Inner Sapwood
Old (200+) Trees			
8mm	7.4 ± 0.7	0.40 ± 0.03	0.21 ± 0.02*
5mm	5.1 ± 1.0	0.36 ± 0.03	0.12 ± 0.02*
Young (15+) Trees			
12 mm	6.8 ± 0.4	0.60 ± 0.06	--
5 mm	8.0 ± 0.7	0.59 ± 0.04	--

2.4.4. Effects of chloropicrin treatment

Chloropicrin-treated core segments (inner bark, outer and inner sapwood) released < 1% the CO_2 of controls in both 200+ and 50+ year-old trees, indicating that there was little stored CO_2 within segments (Figure 2.3). Additionally, chloropicrin-treated core segments formed no red color when treated with triphenyl tetrazolium chloride (TTC) solution, whereas untreated segments were nearly 100% stained with a deep red color. The red color did not penetrate to core centers. The results of positive CO_2 release and TTC staining in untreated core segments, and little CO_2 release and no staining in chloropicrin-treated segments, provided strong evidence that CO_2 production was linked to metabolic activity and not diffusion of CO_2 stores. Further, because TTC staining was fairly uniform throughout core segments (except for the very center), there was no evidence that coring caused a wound response (from heat of the core borer) by killing cells on segment surfaces.

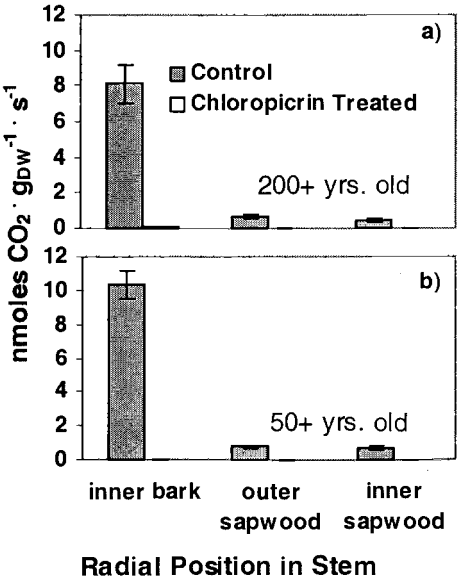

FIGURE 2.3. Effect of chloropicrin treatment on respiratory potential (nmoles CO_2 · $g(DW)^{-1}$ · s^{-1}) at 25°C of core segments extracted from breast height from 200+ and 50+ year-old stems. Means ± standard error, $n = 6$.

2.4.5. Growth rates in young versus old trees

Stem height growth over the last 15 years in 200+ year-old trees was not significantly different from either 50+, or 15+ year-olds ($P = 0.1$), yet was significantly higher in 50+ versus 15+ year-olds (Table 2.1, $P = 0.01$). Stem diameter growth (at 1 m height) over the last 15 years was not significantly different among the three age classes (Table 2.1, $P = 0.1$). Stem wood and bark biomass growth was significantly higher in 200+ than in either 50+ or 15+ year-old trees, and was also higher in 50+ than in 15+ year-olds (Table 2.1). However, biomass growth per unit leaf area was not significantly different among age classes, indicating that growth efficiency did not vary with tree age (Table 2.1). These

Black Butte, OR (Ryan et al. 2000), where biomass growth per unit leaf area in young trees (10 – 80 years-old) was 2 – 10 times greater than that of old trees (390 years-old). Also, growth rates of young pines from the former study were 2 – 6 times those of the young pines in the current study, whereas rates of the former old pines were equal to, or 1.4 – 2 times greater than the old pines discussed here. Thus, the current study's young pines may have been suppressed, and the three age classes may not represent a true chrono-sequence for pines.

2.4.6. Radial trends in core segment respiratory potential

In all tree age classes and at all vertical positions on stems, rate of CO_2 production (nmoles $CO_2 \cdot g\ (DW)^{-1} \cdot s^{-1}$) was highest in the inner bark. Adjacent sapwood respiratory potential was much lower and declined from outer bark toward the sapwood/heartwood boundary. Almost no CO_2 was released from the heartwood (Figure 2.4). Rate of CO_2 production in sapwood was substantial and likely the product of parenchyma cell respiration. Gradients of decreasing activity from bark to sapwood/heartwood boundary were reported for O_2 uptake (Goodwin and Goddard 1940, Shain and Mackay 1973), as well as for CO_2 production (Møller and Müller 1938) of isolated stem tissue (*in vitro*) from various tree species. However, the current study's values for CO_2 production (Table 2.4) were two to six times greater than those for O_2 uptake in the earlier studies.

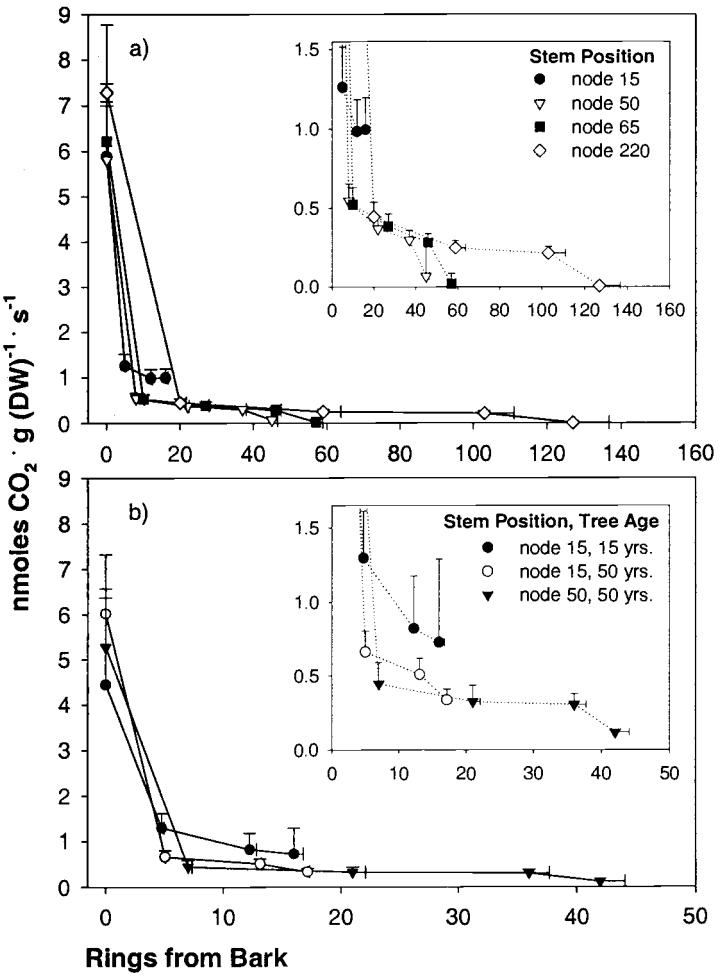

Figure 2.4. Relationship between tissue age and respiratory potential (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$) of core segments extracted from 1-4 vertical positions in a) 200+, b) 50+, and 15+ year-old stems. For each stem position, the highest point represents inner bark activity, followed by outer, middle, inner sapwood, and heartwood. Cores from node 15 had no heartwood. Smaller graphs within larger show closer views of sapwood and heartwood trends. For clarity, only (+) confidence intervals appear in graphs. Means and confidence intervals (n = 6) given in Table 2.4.

Possible explanations for this discrepancy were differences in sample dimensions (earlier: 0.01 – 0.5 cm radial thickness versus current: 2 – 5 cm) and measurement techniques (earlier: volumetric respirometer versus current: *GC*).

Effects of tissue radial position on respiratory potential were significant ($P < 0.0001$) for all three ages of trees. Respiratory potential of outer sapwood for all ages and at all positions, was significantly higher than that of inner sapwood, and in many cases the middle sapwood rate was also significantly higher than inner sapwood rate (Table 2.4). Heightened respiratory activity of outer sapwood rings was potentially related to their role in supporting growth and secondary cell wall formation occurring in the cambial zone (Goodwin and Goddard 1940), along with other physiological activities associated with xylem maintenance (Lev-Yadun and Aloni 1995). The decline in activity of middle and inner sapwood rings may be explained by age-related decline and/or dormancy of metabolic activity in sapwood parenchyma cells. These theories are supported by findings that ray cell nuclear morphology changed from outer to inner sapwood in various conifer species, thus indicating decreased ray vigor (Frey-Wyssling and Bossard 1959, Yang 1993, Gartner et al. 2000). Also, heartwood formation or wound repair has been associated with enzymatic or chemical changes in rays of middle or inner sapwood rings, suggesting that these rings may be genetically programmed to remain dormant until reactivation by signals from the cambial and / or apical meristem (Shain and Mackay 1973, Bamber 1976).

TABLE 2.4. Core segment respiratory potential at 25°C by radial position, tree age, and vertical position. LSMEANS (strip-plot model in PROC MIXED) and 95% confidence intervals, except 50+ year-old heartwood, where ± standard error was used, ($n = 6$). Different letters indicate significant differences among means (FPLSD, P – values < 0.05). Each age class was analyzed separately. Within each tree age class, heartwood was analyzed independently from inner bark and sapwood.

		Respiratory Potential (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$)				
Age	Node	Inner Bark	Outer Sapwood	Middle Sapwood	Inner Sapwood	Heartwood
200+	15	5.89[a]	1.26[b]	0.98[d]	1.00[d]	--
		4.89, 7.09	1.05, 1.52	0.81, 1.21	0.83, 1.20	--
	50	5.81[a]	0.54[c]	0.37[e]	0.30[f]	0.07[a]
		4.82, 7.00	0.45, 0.65	0.31, 0.45	0.25, 0.36	0.02, 0.28
	65	6.21[a]	0.52[c]	0.38[e]	0.28[f]	0.02[a]
		5.16, 7.48	0.43, 0.63	0.31, 0.46	0.23, 0.34	0.01, 0.08
	220	7.29[a]	0.45[ce]	0.25[fg]	0.21[fg]	0.01[ab]
		6.05, 8.78	0.37, 0.54	0.21, 0.30	0.17, 0.25	-0.006, 0.03
50+	15	6.02[a]	0.66[b]	0.51[c]	0.34[d]	--
		4.95, 7.32	0.54, 0.8	0.42, 0.62	0.28, 0.41	--
	50	5.27[a]	0.45[c]	0.33[d]	0.31[d]	0.12
		4.33, 6.41	0.37, 0.55	0.27, 0.40	0.26, 0.38	± 0.04
15+	15	4.44[a]	1.30[b]	0.82[bc]	0.73[c]	--
		3.09, 6.37	0.91, 1.86	0.57, 1.18	0.51, 1.05	--

2.4.7. Vertical trends in core segment and stem node respiratory potential

The effects of stem vertical position on core segment respiratory potential per unit dry mass was significant in both 200+ and 50+ year-old trees ($P < 0.0001$ and = 0.002, respectively). Respiratory potential in 200+ year-old trees was uniform throughout the main portion of the stem, not increasing significantly until node 15 from the treetop (Table 2.4). The interaction of tissue radial position and vertical position was highly significant in both 200+ and 50+ year-old trees ($P <$ 0.0001 and = 0.005, respectively), indicating that rate of decline in activity from bark to the sapwood/heartwood boundary was not the same at all stem vertical positions. The result of respiratory potential being fairly uniform throughout the 200+ year-old stems (node 220 – node 50) agreed with Stockfors (2000), who modeled whole-tree respiration in *Picea abies*, and Ryan et al. (1996), who measured CO_2 efflux per unit area at three vertical positions in *Pinus radiata* D. Don. Both concluded that respiration at breast height generally provided an acceptable estimate of whole-tree respiration.

When core segment respiratory potential was scaled-up to an entire node of respiring woody tissue, effect of vertical position on respiratory potential on an outer-bark surface area basis was significant ($P < 0.0001$) in 200+ year-old trees, declining from tree base to top, yet not significant ($P = 0.5$) in 50+ year-olds (Table 2.5). In contrast, node respiratory potential on a volume basis (live and stem wood) was significantly higher ($P < 0.0003$) near treetops (node 15) of 200+ and 50+ year-olds, and significantly lower ($P < 0.04$) near bases (node 220) in 200+

year-olds, than either node 50 or 65 (Table 2.5). Thus, mass- and volume-based respiratory potentials both increased significantly toward treetops.

The increased respiratory potential near ponderosa pine treetops in the current study was notable, and may be explained by the close proximity of node 15 to substrate supply, where physiological activities, such as growth, substrate metabolism and transport are high. Alternatively, node 15 may have had a higher percentage of ray parenchyma in its sapwood than the other nodes. Gartner et al. (2000) found that both ray frequency and volume were higher in the first 10 growth rings proximal to the pith of 34 year-old *Pseudotsuga menziesii* Mirb. A third possibility is that within-tree differences in metabolism are related to changes in carbohydrate synthesis that are triggered by the onset of maturation, such as the tissue in treetops, which formed at a different stage of the tree's life than at the base (Haffner et al. 1991).

2.4.8. Old versus young comparison – stem segment, and whole-tree level trends

When respiratory potential was scaled to whole-stem segments, the rate of CO_2 production per unit outer-bark surface area near treetops (node 15 to treetop) was not significantly different across age classes (Table 2.5, $P = 0.5$). However, the rate of production per unit volume (live and stem wood) was significantly lower near treetops of 50+ year-old trees than in 200+ or 15+ year-olds (Table 2.5, $P < 0.02$). Respiratory potential per outer-bark surface area was significantly higher in 200+ than in 50+ year-old bases (tree base to node 15, Table 2.5, $P < 0.0001$). In

TABLE 2.5. Outer bark surface-area, live wood volume (inner bark + sapwood), and stem wood volume (bark + sapwood + heartwood) by stem segment for each age class in ponderosa pine stems. Respiratory potential per unit outer bark surface area, and live and stem wood volume are also given by node and by stem segment. For stem geometry, means ± standard error, $n = 6$. For respiratory potential LSMEANS ± standard error, or 95% confidence intervals in parentheses ($n = 6$) are from a strip-plot model in PROCMIXED (by node), or from a 1-way ANOVA model (by segment). Different uppercase letters in each column (node), or different lowercase letters in each row (segment) indicate significant differences among means (Fisher's protected least significant difference procedure, $P < 0.05$). Values for total respiratory potential represent weighted averages from node 15 to treetop and tree base to node 15.

TABLE 2.5.

Parameter, stem segment	*Tree Age Class* 200+ yrs.	50+ yrs.	15+ yrs.
Stem geometry			
Outer bark surface area (m^2)			
Node 15 to treetop	0.3 ± 0.06	0.6 ± 0.08	0.35 ± 0.04
Node 50 to node 15	2.7 ± 0.6	5.0 ± 0.7	--
Node 65 to node 50	3.5 ± 0.2	--	--
Tree base to node 65	31.6 ± 2.0	--	--
Live wood volume (m^3)			
Node 15 to treetop	0.003 ± 0.001	0.008 ± 0.002	0.004 ± 0.001
Node 50 to node 15	0.10 ± 0.03	0.14 ± 0.03	--
Node 65 to node 50	0.20 ± 0.02	--	--
Tree base to node 65	3.1 ± 0.2	--	--
Stem wood volume (m^3)			
Node 15 to treetop	0.003 ± 0.001	0.010 ± 0.002	0.005 ± 0.001
Node 50 to node 15	0.12 ± 0.04	0.20 ± 0.04	--
Node 65 to node 50	0.23 ± 0.03	--	--
Tree base to node 65	3.9 ± 0.5	--	--

TABLE 2.5. (Continued).

Parameter, stem segment	Tree Age Class 200+ yrs.	50+ yrs.	15+ yrs.
Respiratory potential			
Per outer bark surface area (μmoles $CO2 \cdot m^{-2} \cdot s^{-1}$) by node			
Node 15	10.0 ± 1.6 A	9.1 ± 0.8 A	9.4 ± 1.3[a]
Node 50	14.9 ± 1.5 B	9.3 ± 0.8 A	--
Node 65	17.2 ± 1.5 B	--	--
Node 220	25.0 ± 1.5 C	--	--
Per outer bark surface area (μmoles $CO2 \cdot m^{-2} \cdot s^{-1}$) by segment			
Node 15 to treetop	6.3 ± 1.0[a]	6.1 ± 0.9[a]	7.5 ± 0.9[a]
Tree base to node 15	23.4 ± 1.1[a]	7.9 ± 1.1[b]	--
Total	23.3 ± 1.1[a]	7.7 ± 1.1[b]	7.5 ± 1.1[b]
Per live wood volume (μmoles $CO2 \cdot m^{-3} \cdot s^{-1}$) by node			
Node 15	682 (583, 798) A	447 ± 22 A	707 ± 49
Node 50	308 (267, 355) B	272 ± 22 B	--
Node 65	257 (222, 296) B	--	--
Node 220	202 (175, 234) C	--	--
Per live wood volume (μmoles $CO2 \cdot m^{-3} \cdot s^{-1}$) by segment			
Node 15 to treetop	694 ± 54[a]	447 ± 49[b]	707 ± 49[a]
Tree base to node 15	229 ± 16[a]	283 ± 16[b]	--
Total	230 ± 36[a]	292 ± 36[a]	707 ± 36[b]
Per stem wood volume (μmoles $CO2 \cdot m^{-3} \cdot s^{-1}$) by node			
Node 15	554 (489, 623) A	387 ± 20 A	592 ± 43[a]
Node 50	271 (229, 314) B	189 ± 20 B	--
Node 65	216 (178, 254) B	--	--
Node 220	164 (129, 195) C	--	--
Per stem wood volume (μmoles $CO2 \cdot m^{-3} \cdot s^{-1}$) by segment			
Node 15 to treetop	558 ± 47[a]	387 ± 43[b]	591 ± 43[a]
Tree base to node 15	185 ± 12[a]	204 ± 12[a]	--
Total	185 ± 33[a]	213 ± 33[a]	591 ± 33[b]

contrast, respiratory potential per live wood volume was significantly lower in 200+ than in 50+ year-old tree bases ($P = 0.04$), and there was no significant difference in respiratory potential per unit stem wood volume between 200+ and 50+ year-old bases (Table 2.5, $P = 0.3$). Whole-tree rate of CO_2 production per unit outer-bark surface area was significantly higher in 200+ than in either 50+ or 15+ year-old trees ($P < 0.0001$), whereas production rate per unit volume (live and stem wood) was significantly lower in 200+ and in 50+ than in 15+ year-olds (Table 2.5). Whole-tree level respiratory potential per unit volume of the 50+ and 200+ year-old pines was 14 – 20 times the *in situ* respiration rates of mature *Pinus ponderosa* (e.g., Ryan et al. 1995, Carey et al. 1997), confirming that the current measurements were not typical of stem respiration under natural conditions.

The discrepancy between trends in respiratory potential on a surface area versus a volume basis demonstrated that estimates of whole-tree level respiration are strongly determined by the method used in scaling-up. Respiratory potential per unit surface area was higher in large, old stem segments than in small, young ones because the surface area to volume ratio was smaller in 200+ than in 15+ year-old trees, 10:1 m and 30:1 m, respectively. Whole-tree level respiration was also influenced by how node or segment level rates were averaged to calculate whole-tree level rates. For example, using only node 15 respiratory potential per sapwood volume to represent whole-tree level respiration instead of the weighted average of all nodes overestimated whole-tree respiration by 200%, whereas using only node 220 respiratory potential underestimated whole-tree respiration by 12% (Table 2.5).

Further research is needed to ascertain whether similar scaling complexities exist within other species and incorporate diurnal or seasonal effects. Tissue nitrogen content or parenchyma cell volume should also be explored as a possible basis for respiratory potential.

2.5. ACKNOWLEDGEMENTS

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3. WITHIN-STEM VARIATION OF RESPIRATION IN *PSEUDOTSUGA MENZIESII* (DOUGLAS-FIR) TREES

Running Title: Respiratory Gradients within Stems of Douglas-fir

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3.1. SUMMARY

- We investigated a technique for measuring *in vitro* respiration to learn why rates were higher than those reported *in vivo* and to elucidate trends within mature *Pseudotsuga menziesii* trees.
- We divided extracted increment cores into 3 – 4 radial depths and used a gas chromatograph to compare respiration rates radially and vertically within stems.
- Respiration of inner bark was 2 – 3 times greater than sapwood, and 50 – 70% higher in outer than inner sapwood. Inner bark and outer sapwood released > 40% more CO_2 at treetops than at bases. Trends were robust for CO_2 production on a core dry-mass, volume, or total carbon basis. In contrast, CO_2 production on a nitrogen basis showed almost no significant variation.
- This *in vitro* technique provided an effective index for relative differences in respiration within tree stems. Discrepancies between *in vitro* and *in vivo* measurements may be related to the gaseous environment in stems. The estimated within-stem gradients in respiration were possibly determined by enzyme quantity and availability and could be useful in scaling to whole-trees.

Keywords: stem respiration, stem aeration, inner bark, sapwood, Pseudotsuga menziesii (Douglas-fir)

3.2. INTRODUCTION

In most studies, estimates of whole tree and stand level respiration rates are obtained from scaling up small sample measurements acquired using infra red gas analysis (IRGA) chambers at one or more position(s) on tree stems (e.g., Kinerson 1975, Edwards & Hanson 1996, Ryan et al. 1995). Stem surface area beneath the chamber has often been used as an index of the amount of living tissue to associate with the measured respiration rate (e.g., Linder & Troeng 1981, Matyssek & Schulze 1988). This rate can then be extrapolated to the entire surface area of the stem and from there to the ecosystem level. However, sapwood volume has proved to be a better index for scaling maintenance respiration to whole trees or ecosystems (Sprugel & Benecke 1991, Ryan & Waring 1992), probably because sapwood volume is proportional to the amount of living parenchyma cells therein (Ryan 1990, Larson 1994, Stockfors & Linder 1998).

Scaling respiration to the whole-tree level by sapwood volume from measurements at only one location assumes uniform respiration rates among all the stem parenchyma cells. This assumption is unlikely to be valid because sapwood is not uniform in age (number of years since the cells developed) or maturity (cambial age at which cells were produced). Tissue age and maturity depend on radial and vertical position in the stem, both of which may impact respiration. Radial trends in sapwood respiration have been recorded from inner bark to the heartwood/sapwood boundary for tissues extracted just above ground level in stems of *Pinus radiata* (Shain & Mackay 1973), *Fraxinus nigra* and *Acer rubra*

(Goodwin & Goddard 1940), and in *Picea abies* and other species (Møller & Müller 1938). Radial and vertical trends in sapwood respiration were recorded for *Pinus ponderosa* (Pruyn et al. 2002).

The impact of such within-stem variations on whole-tree level respiration rates was addressed in *P. abies* (Stockfors 2000). Assuming a constant temperature-respiration relationship, stem temperature was measured at multiple heights and radial depths to predict respiration. Within-stem temperature variations were observed that resulted in scaling errors between 2 and 72% for a single sunny day and 2 and 58% for a whole year. Within-stem variation in respiration was also accounted for by averaging respiration rates (measured via IRGA) from various heights on stems (Lavigne 1987, Ryan et al. 1996), or from different compass directions (Edwards & Hanson 1996). In a third approach, the distribution of live cell volume in sapwood samples was estimated by using vital staining techniques and image analysis tools, which provided a percentage of living tissue for scaling respiration rates to the whole stem-level (Ryan 1990, Stockfors & Linder 1998).

Although these three methods are improvements for representing variation of respiration within sapwood, multiple sources for inaccuracy remain. First, the use of temperature to predict respiration rates may be problematic because of temperature-independent variation in CO_2 efflux from stems (Martin et al. 1994). Second, the use of IRGA systems may sometimes underestimate respiration because all sapwood CO_2 production may not reach the stem surface. Some respired CO_2 could be dissolved in the transpiration stream (Neigisi 1975, Sprugel

1990, Levy et al. 1999), re-fixed by bark photosynthesis in some species (Nilsen 1995, Cernusak & Marshall 2000), or stored within sapwood parenchyma cells (Lev-Yadun & Aloni 1995). Third, vital staining techniques only indicate whether cells are alive or dead, they do not directly convey the degree of respiratory activity among the living cells.

In a previous study, we measured rates of CO_2 production under controlled laboratory conditions of cores extracted from mature *Pinus ponderosa* (ponderosa pine) stems. A primary objective was to quantify within-stem variation of respiration, and then scale to the whole-tree level (Pruyn et al. 2002). We learned that respiration in sapwood of those pine trees was not homogenous and that scaling to whole-trees resulted in rates substantially higher than those reported *in situ* from the literature. In the current study, we first examined the basis for higher *in vitro* than *in vivo* respiration rates of excised cores of *Pseudotsuga menziesii* (Douglas-fir) by exploring potential artifacts of the method. Second, we determined whether bark-to-pith and treetop-to-base trends in respiratory potential also existed within Douglas-fir stems. Third, we compared core respiratory potential on four different indices: dry mass, volume, moles carbon, and moles nitrogen. Finally, we discussed the likely physiological mechanisms responsible for variations in respiration within trees, across sites, and between seasons.

3.3. MATERIALS AND METHODS

3.3.1. Species and site characteristics

We collected samples from mature (60 – 112 years-old) Douglas-fir trees, *Pseudotsuga menziesii* (Mirb.), from a site just east of the Coast Range in southern Oregon, near Riddle (N42° 57' W123° 22', elevation 215 m) and in McDonald-Dunn Research Forest in the Williamette Valley, near Corvallis, Oregon (N44° 38' W123° 17', elevation 305 m). Unless indicated otherwise, samples were collected either prior to the growing season for this region, in early March of 1998 and 1999, or afterwards in October of 1999, 2000, and 2001. The rationale behind selecting these sampling dates was to capture maintenance respiration, and thus avoid the complications of growth respiration in estimating core respiration.

3.3.2. Tree sampling

All trees sampled were free of broken tops, stem deformities, or visible disease. Twenty to thirty trees were selected randomly from each site to be used for one or more of the following experiments. Stem diameter 1 m from the ground ranged from 40 – 57 cm (Corvallis) or 55-70 cm (Riddle). Tree age at breast height was 64 – 109 years (Corvallis, from cores) and 110 – 112 years (Riddle, from cross-sectional disks).

3.3.3. Respiration measurements

Respiratory potential was estimated from 12 mm increment cores extracted from either standing stems (1m from ground), or felled trees (1 m from ground and nodes 35 and 15 from treetop). When extracting multiple cores from a specific stem height, we took them evenly from about the stem's circumference. All cores were analyzed within one week of sampling. Twenty-four hours prior to measurement, cores were cut into four segments: inner bark (phloem and cambium) and outer, middle, and inner sapwood. Sapwood was defined as the woody tissue extending from the first growth ring interior to the inner bark to the last growth ring interior to the transition zone (one or two lighter colored rings at the sapwood/heartwood boundary). Outer, middle, and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. For this study, respiration of heartwood was not measured because preliminary data revealed almost no CO_2 evolution from heartwood samples (< 0.01 nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$, Pruyn, unpublished data). Number of rings per segment was recorded, so that a mean age could be determined for each segment. These segments were weighed, wrapped tightly in plastic, then stored at 25°C overnight to allow metabolic activity in core segments to stabilize (Goodwin & Goddard 1940, Hari et al. 1991, Levy et al. 1999).

Immediately prior to measurement, core segments were re-weighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, carbon dioxide concentration within vials was measured

with a Hewlett-Packard (5700A) gas chromatograph (GC, Hewlett Packard, Rt. 41, Avondale PA 19311, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25°C (unless indicated otherwise) between GC measurements. Incubation period was held constant at either 6 or 20 hours, with the longer incubation necessary to accommodate the processing time of large numbers of samples. Details of GC analysis and calculation of respiratory potential (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) are in Pruyn et al. (2002). From this point onward, we refer to the reported values as respiratory potential, rather than respiration rate because the conditions of our measurements on these excised samples are probably different from those within the tree.

Immediately following the GC analysis, core segments were weighed a third time. The three successive wet masses verified that water loss was low (between 1-3%) between sampling and the end of the measurement period. Fresh volume of core segments was estimated as displaced water by submerged samples (D2395, ASTM 2001). Dry masses were determined after oven drying at 60°C for 48 hours. The OSU Central Analytical laboratory determined total core carbon and nitrogen content using a CNS-2000 Micro Analyzer (LECO, 3000 Lakeview Ave., St. Joseph, MN 49085-2396, USA).

3.3.4. Potential artifacts of technique

To address the question of why *in vitro* respiration rates from the current technique differ from those reported *in vivo*, we conducted six methods tests to examine potential artifacts from extraction, handling, and storage prior to analysis.

3.3.4.1. Effects of time in cold-storage at 4°C

To determine whether rate of core segment CO_2 production was affected by storage time prior to respiration measurement, we extracted four 12 mm diameter cores at breast height from six trees from the Corvallis site in late August of 2000. For each tree, each core was assigned to one of four treatments: one, two, four, and nine days of storage at 4°C prior to GC measurement. The night prior to GC analysis, cores were segmented according to radial position as described above, with the exception that only inner bark, outer, and inner sapwood tissues were sampled to capture the extremes of activity. Core segments were then analyzed for CO_2 production over a 6-hour incubation period. We tested published results that a storage time of < 15 days would have no effect on core segment respiratory potential (Goodwin & Goddard 1940, Shain & MacKay 1973). The presence of a trend between core segment CO_2 production rate and storage time would indicate that the response was not stable and thus not likely indicative of respiration. Decreased CO_2 production rate with increased storage time may suggest a decreasing wound response, decreasing diffusion of stored CO_2 from within the core as it equilibrates to atmospheric concentrations, or increasing parenchyma cell

death; whereas increased CO_2 production with storage time suggests the increased contribution of microbial respiration.

3.3.4.2. Microbial presence and respiration rate

To determine the extent to which microbial respiration contributed to the observed CO_2 production of core segments, we compared the respiratory potential of samples two days and one month after coring. One core each was extracted from three of the six trees in the storage time experiment, segmented according to radial position, and measured for core respiratory potential as described above. Core segments were then placed separately into sterile petri dishes containing a malt extract agar (1.5% malt extract, 1% agar), sealed with Para-film "M" ® laboratory film, (American Can Company, Greenwich, CT 06830) and then stored at 20°C. After two and four weeks, the type of microbe (fungal or bacterial) visible to the naked eye on each core was recorded. We predicted that microbial growth would be visible on core surfaces. After the fourth week, we again measured core respiratory potential. To verify that CO_2 produced by these core segments was exclusively from microbes and not sapwood parenchyma, we exposed the cores to vital stain. Immediately following the GC analysis, we submerged them in a 1% aqueous triphenyl tetrazoilium chloride solution (TTC), which is reduced to a deep-red color in the presence of living cells (Feist et al. 1971, Ryan 1990). Red-stained tissues were considered alive and non-stained tissues dead.

3.3.4.3 Using differential scanning calorimetry to measure core respiratory potential

To verify that the CO_2 released by core segments was a product of respiration, we compared our GC measurements to core segment metabolic heat rate, i.e. the rate at which heat is produced by respiration (Criddle et al. 1991), using a differential scanning calorimeter (DSC, Hart Scientific Model 7707, Calorimetry Sciences Corp., 7900 E. Utah Valley Dr., American Fork, UT 84003, USA) in the isothermal mode (25°C). Because the DSC ampules were only 1 cm^3, we reduced the size of each sample to 0.5 cm diameter x 0.8 cm length. Two replicates for each radial position (inner bark, outer sapwood, and inner sapwood) from twelve trees from the Corvallis site were used for the experiment because of high variability of preliminary results (Pruyn, unpublished data). Extracted cores were stored at 4°C, until the night before analysis, when they were segmented as described above and stored at 25°C. The DSC analysis was implemented according to Criddle et al. (1991) and Anekonda et al. (1994), with the exception that each phase of the reaction required 75 instead of 45 minutes because respiration in stem tissues is considerably less than in leaves. We analyzed one tree per day, and each replicate set (inner bark, outer sapwood, and inner sapwood) separately, which enabled us to keep cores intact until the night prior to analysis, thereby reducing risk of desiccation.

3.3.4.4. Effects of temperature on respiration rate

To determine if response of core segment respiratory potential to temperature was consistent with literature values from intact Douglas-fir trees, four 12 mm diameter cores were extracted from breast height from each of five trees at each site (Corvallis and Riddle) in early March 2000 for a total of 20 cores per site. For each tree, each core was assigned to one of four temperatures (5, 10, 15, 25°C). Core response to temperature was measured, and the Q_{10}s (coefficient for changes in respiration with respect to temperature) for the 5 – 15°C and 15 – 25°C temperature ranges were calculated as described in Pruyn et al. (2002).

3.3.4.5. Seasonal variation of within-stem CO_2 and O_2 concentration

We examined the seasonal pattern of within-stem O_2 and CO_2 concentrations *in vivo* to gain insight as to how different these conditions were from the environment of cores *in vitro*. To determine the CO_2 and O_2 concentration within stems, four holes of different depths were drilled into twelve trees at breast height (1m from ground) in early March of 2000 from the Corvallis site. This experiment was modeled after Eklund (1990). Depths were as follows: outer bark (1 – 2 cm), inner bark (1.5 – 3 cm), middle sapwood (3 – 7 cm), and sapwood/heartwood (5 – 13cm). Depths were assigned to alternating directional faces by tree to distribute the depths evenly among the four aspects (N, S, E, W). There were a few exceptions to this design due to overshooting the drilling depths, which introduced a confounding variable of direction on depth. To determine the

four drilling depths for each tree, we extracted one 5 mm increment core from each of the NE and SE aspects. Before drilling into each tree face, we extracted a 5 mm core to the assigned depth. This sample core was compared to the neighboring diagonal core, to verify that the depth was correct. For example, this comparison would indicate if the bark thickness had changed, or whether we had overshot the sapwood/heartwood boundary. We marked the confirmed depth on a 12 mm, auger drill-bit and then drilled into the 5 mm increment-core hole. Stainless steel tubes were inserted all the way into the holes so that only 1 cm^2 of wood (tangential face) was exposed to the air in the tube and the other end of the tube emerged from the tree. The area around the tube at the point of insertion was sealed with silicone to avoid leakage, and a gas-tight rubber stopper was inserted into the end of the tube.

For trees in which resin initially threatened to block some of these stainless steel tubes, we installed drainage apparatuses, which consisted of a *Y*-connecting glass tube inserted into the septum on the tree. Each exposed end of the *Y*-tube was connected to either a 2 ml glass vial for gas collection, or to a 10 ml vial via a septum for resin collection (pointed ground-ward to facilitate flow of resin). The gas collection vial was sealed with a silicone septum and open-top screw cap and connected to the *Y*-tube via a double-ended needle that penetrated the seal of each tube. All septa connecting *Y*-tubes to steel tubes or glass vials were tightened with plastic cinch-straps. Additionally, all exposed septa surfaces were sealed with Parafilm "M" ® and covered with duct tape to impede environmental degradation of the rubber and thus minimize gas leakage. All septum-sealed, steel tubes

without drainage apparatuses were connected directly to gas-collection vials via double-ended needles. The connections to the gas-collection vials were initiated on April 1st and maintained for 3 weeks. The sealed glass vials were then removed and the inside gas composition determined using the GC. Immediately after removing the sealed glass tube, a replacement was connected to the steel tube for the next 3-week period. Vials were collected during the morning (800 – 1000 hours), once every three weeks from April through October. Drainage apparatuses were replaced as resin-collection vials filled-up, as were any rubber septa that showed excessive degradation. To compare the measured fluxes of stem O_2 and CO_2 to seasonal climate trends, daily mean maximum and minimum temperature and precipitation for the site were obtained from the Oregon State University (OSU) Integrated Pest Management Weather Data web site (http://www.orst.edu/Dept/IPPC/wea/).

3.3.4.6 Responses in core respiratory potential to variations in ambient CO_2/O_2 concentrations

To understand the effect of CO_2/O_2 concentration on core segment respiratory potential, we extracted four 12 mm diameter cores from five trees at the Corvallis site in October 1999. Each core was assigned to one of four treatments: ambient in the laboratory (control, 0.04% CO_2 / 21% O_2), O_H (2% CO_2 / 5% O_2), O_L (10% CO_2 / 2% O_2), and nitrogen-flushed (0% CO_2 / 0% CO_2). Cores were cut into the three radial segments and stored at 25°C overnight. The morning of the

experiment, segments were placed in test tubes and septum-sealed. Test tubes were flushed with nitrogen using a two-way syringe system. Control segments were not flushed. For the O_H and O_L treatments, a volume of gas equal to that which would be added was removed, then the appropriate volume of each gas was added to each test tube. Appropriate volumes were calculated by an application of the Ideal Gas Law ($concentration_1 \cdot volume_1 = concentration_2 \cdot volume_2$). Gas concentrations ($CO_2$ and O_2) in the test tubes were analyzed using the GC initially, as well as at 5, 10, and 32 hours.

3.3.5 Application of the Method – Stem radial and vertical trends in core respiratory potential

We selected and felled three trees from the Riddle site. After felling, we sawed 20 cm tall stem disks from stems at the 100th (mean $\pm$ SE, 0.3 $\pm$ 0.02 m from ground), and just above (to avoid branch whorls within the crown) the 35th (35 $\pm$ 1 m from ground) and 15th (39 $\pm$ 1 m from ground) nodes (years) from the treetop. Total stem height averaged at 44 $\pm$ 2 m and base of the live crown (first stem position above ground level with three live branches) at 28 $\pm$ 2 m. A second, short disk (< 5 cm tall) was taken from each vertical position for calculations of inner bark, sapwood, and heartwood thickness and for determining the age of the tissues. The tall disks for respiration measurements were wrapped in 4 mm-thick black plastic bags with moist paper toweling inside to reduce desiccation before experimentation. Tall disks were stored at 4°C. The short disks were kiln dried,

and then radial distances and ring counts from pith to the distal edge of each tissue (outer and inner bark, sapwood, and heartwood) were recorded. Within one week after harvesting, five 8 mm diameter increment cores were extracted from each height position (tall disk), wrapped separately in plastic bags and immediately returned to cold storage. The evening before GC analysis, cores were cut into four radial positions (inner bark, outer sapwood, middle sapwood, and inner sapwood), re-wrapped in plastic, and stored overnight at 15°C. The following morning the samples were placed in vials, septum-sealed, and analyzed for CO_2 production as described above. The incubation period was 22 hours at 15°C.

3.3.6. Statistical Analysis

All data were analyzed in Statistical Analysis Systems software, release 8.0 (SAS Institute Inc. 1998). The Shapiro-Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or natural log) was performed when necessary to meet assumptions of normality and constant variance. Least squares means (LSMEANS), generated from the various SAS procedures described below, are reported ± pooled SE, or confidence intervals for transformed variables. Within a specific table or figure, if confidence intervals were required for one variable, they were presented for all.

A one-way ANOVA in PROC GLM was used to make comparisons among cold-storage time treatments. Specific pair-wise comparisons among treatments were conducted for each radial position separately using Fisher's Protected Least

Significant Difference (FPLSD) procedure (Fisher 1966). Paired t – tests were used to compare respiratory potential of control cores before and after 30 days on agar medium. Comparisons among radial positions for the DSC analysis of core respiratory potential were made using PROC MIXED, with randomized block design and strip-plot (split-block) treatments (Little & Hills 1978, Milliken & Johnson 1984). Trees were blocks and the effect of radial position was tested. Pair-wise comparisons among tissue radial positions were conducted using FPLSD procedure. Comparisons among Q_{10} values at different temperature ranges were made using a strip-plot analysis in PROC MIXED. Trees were blocks, and the effects of tissue radial position, temperature range, site, and all possible interactions were tested. Pair-wise comparisons among tissue radial positions and temperature ranges were conducted using FPLSD.

Repeated measures analysis in PROC MIXED was used to test the effects of sampling date, radial position, and their interaction on respiratory potential (Little et al. 1996). We initially included aspect (N, S, E, W) and the interaction of aspect and sampling date in the model, but dropped these effects when they proved to be non-significant. Trees were treated as blocks, and the tree by treatment interaction was also blocked using the SUBJECT option in the repeated statement with a TYPE=UN covariance structure. A repeated measures analysis in PROC MIXED was also used to test the effects of incubation time, atmospheric (CO_2/O_2) treatment, and their interaction on core respiratory potential. This repeated

measures model was identical to the previous model, except that tree was included as a random effect and each radial position was analyzed separately.

A strip-plot analysis was used to make comparisons (using FPLSD) among tissues at various radial and vertical positions within trees. We tested the effects of tissue radial position, vertical position, and their interaction. This analysis was also used to test the effects of radial position, season (Corvallis site) or vertical position (Riddle site), and their interaction on respiratory potential on four different indices (i.e. core dry mass, volume, carbon, or nitrogen).

3.4. RESULTS

3.4.1. Effects of cold-storage time and microbial respiration

There were no significant differences in respiratory potential (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) of inner sapwood from Douglas-fir trees on the Corvallis site, stored for 1, 2, 4, or 9 days at 4°C prior to GC analysis ($P \geq 0.05$, Table 3.1). Outer sapwood respiratory potential was also fairly constant, regardless of storage length, with the exception of tissue stored for 4 days, which respired significantly higher than tissues stored for 1, 2, or 9 days ($P < 0.04$, Table 3.1). In contrast, inner bark showed a trend of increasing activity with storage time that was significant ($P = 0.05$) after 9 days of storage, when respiratory potential was 45% higher than after the first day. After 30 days on agar medium, microbial growth (fungal and/or bacterial) was visible on all core segments (except one inner bark sample). We

concluded that microbial respiration probably did not contribute significantly to the respiratory potential of freshly sampled cores because respiratory potential of older cores with visible microbe growth was either > 50% less than (inner bark and outer sapwood) or equal to (inner sapwood) that of fresh cores with no microbes visible (Table 3.1). Further, we verified that CO_2 produced by cores with visible microbe growth was exclusively from microbes because the TTC only stained red where microbes were visible.

3.4.2. Differential scanning calorimetry (DSC)

Respiratory potential measured using DSC was significantly higher in inner bark than in sapwood, but not higher in outer than inner sapwood (Table 3.1). Inner bark and sapwood respiratory potentials measured with the DSC were comparable to GC-measured respiratory potentials from core segments in the storage time experiment (Table 3.1). However, DSC respiratory potentials for inner sapwood were generally higher than when measured with the GC.

TABLE 3.1. Respiratory potential at 25°C of core segments from three radial positions, testing three different variables or methods: storage period, microbial presence after one-month incubation, and differential scanning calorimetry. All cores extracted from breast height of (n) trees at the Corvallis site. Least Squares Mean (LSMEAN, ANOVA or Strip-plot analysis in PROC MIXED) or Mean (t – test) ± 95% confidence intervals. For each test, different lowercase letters indicate significant differences within columns and different uppercase letters indicate significant differences within rows (LSMEANS by FPLSD, Means by t – tests, $P < 0.05$).

	Respiratory Potential (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) by Radial Position		
Test	Inner bark	Outer Sapwood	Inner Sapwood
Storage Period (days at 4ºC) ($n = 6$)	LSMEAN	LSMEAN	LSMEAN
1	3.3 (2.6, 4.0)[a]	0.63 (0.53, 0.74)[a]	0.27 (0.22, 0.32)[a]
2	4.1 (3.4, 4.9)[a]	0.60 (0.49, 0.70)[a]	0.27 (0.21, 0.33)[a]
4	3.9 (3.1, 4.6)[a]	0.79 (0.69, 0.90)[b]	0.32 (0.27, 0.37)[a]
9	4.8 (4.1, 5.5)[b]	0.61 (0.50, 0.71)[a]	0.30 (0.24, 0.35)[a]
Microbial Presence ($n = 3$)	Mean	Mean	Mean
(days after coring, microbe status)			
2 days, no growth visible	5.1 (5.0, 5.2)[a]	0.63 (0.57, 0.69)[a]	0.27 (0.20, 0.34)[a]
30 days, microbe growth visible	0.69 (0.62, 0.76)[b]	0.30 (0.20, 0.39)[b]	0.19 (0.18, 0.20)[a]
Differential Scanning Calorimeter ($n = 12$)	LSMEAN	LSMEAN	LSMEAN
	3.9 (3.4, 4.5)[A]	0.7 (0.5, 1.0)[B]	0.6 (0.4, 0.8)[B]

3.4.3. Temperature effects

For all radial positions in trees from both sites, the Q_{10} for the 5-15°C temperature range, averaging between 4.5 and 7.6, was significantly higher than for the 15-25°C range, averaging 2.0 (Table 3.2). The effect of temperature on Q_{10} was thus significant ($P < 0.0001$). The large variability of the Q_{10} average at the lower temperature range resulted from outer and inner sapwood samples from the Riddle site. Thus, these two positions were exceptions to the trend of no significant variation among radial positions for each temperature range and the effect of radial position on Q_{10} was not significant ($P > 0.2$). However, the interaction of temperature by radial position and the interaction of temperature by site were significant ($P = 0.01$), likely a result of high outer and inner sapwood $Q10$s at the Riddle site.

TABLE 3.2. Mean Q_{10} for three radial positions in stems at both the Corvallis and Riddle sites. All cores extracted from breast height of ($n = 5$) trees at each site. Least Squares Mean (Strip-plot analysis in PROC MIXED) ± 95% confidence intervals are given for each temperature range (i.e. 5-15 and 15-25°C). Different letters represent significant differences among all means (FPLSD, $P < 0.05$).

	Radial Position		
	Inner Bark	***Outer Sapwood***	***Inner Sapwood***
Q_{10} (Temperature Range ºC)			
Corvallis			
Q_{10} (5-15)	4.5 (3.3, 5.7)[a]	4.8 (3.6, 6.0)[a]	5.3 (4.1, 6.5)[a]
Q_{10} (15-25)	2.2 (1.0, 3.4)[b]	1.9 (0.7, 3.1)[b]	2.0 (0.9, 3.2)[b]
Riddle			
Q_{10} (5-15)	4.5 (3.3, 5.7)[a]	7.6 (6.4, 8.8)[c]	7.6 (6.4, 8.8)[c]
Q_{10} (15-25)	2.4 (1.2, 3.6)[b]	1.8 (0.6, 2.9)[b]	1.9 (0.8, 3.1)[b]

3.4.4. Seasonal variation of within stem CO_2 and O_2 concentration

The effects of aspect (N, S, E, and W) and the interaction of aspect and sampling date were not significant to the repeated measures model for response variables, O_2 and CO_2 concentration ($P > 0.1$). Thus, we found no evidence in these data of a confounding variable of aspect on gas concentration by radial position and dropped these effects from the model. However, we could not completely rule out the possibility of confounding because of the experimental design, which had an unequal number of radial positions assigned to each compass direction. Within stem O_2 and CO_2 varied significantly by sampling date ($P < 0.0001$) and radial position ($P < 0.0001$). The interaction of sampling date by radial position was significant for stem O_2 ($P = 0.003$), suggesting that the relationship between O_2 and sampling date varied among the four radial positions. This result was in contrast to the non-significant interaction of sampling date by radial position for stem CO_2 ($P = 0.2$).

The trend in O_2 concentration by sampling date and radial position were essentially inversely related to the corresponding trend in CO_2: when O_2 was low, CO_2 was high. Lowest within stem O_2 (mean = 6.4 %) and highest CO_2 (mean = 3.0%) occurred at the sapwood/heartwood boundary during the growing season on Julian day 146 (Figure 3.1a – d). This hypoxic environment within stems corresponded with the onset of the growing season, higher temperatures, and less precipitation (Figure 3.1e). As the growing season ended in late September – early October (~ Julian day 280), within stem O_2 gradually returned to the atmospheric

FIGURE 3.1. Seasonal flux of within-stem gas concentrations of trees at the Corvallis site. Concentration of carbon dioxide (% CO_2) and oxygen (% O_2) in the gas phase in equilibrium with a) outer bark, b) inner bark, c) middle sapwood, and d) heartwood. e) Daily mean maximum and minimum temperature and precipitation. Least Squares Means $\pm$ 95% confidence intervals (n = 12 trees) in PROC MIXED.

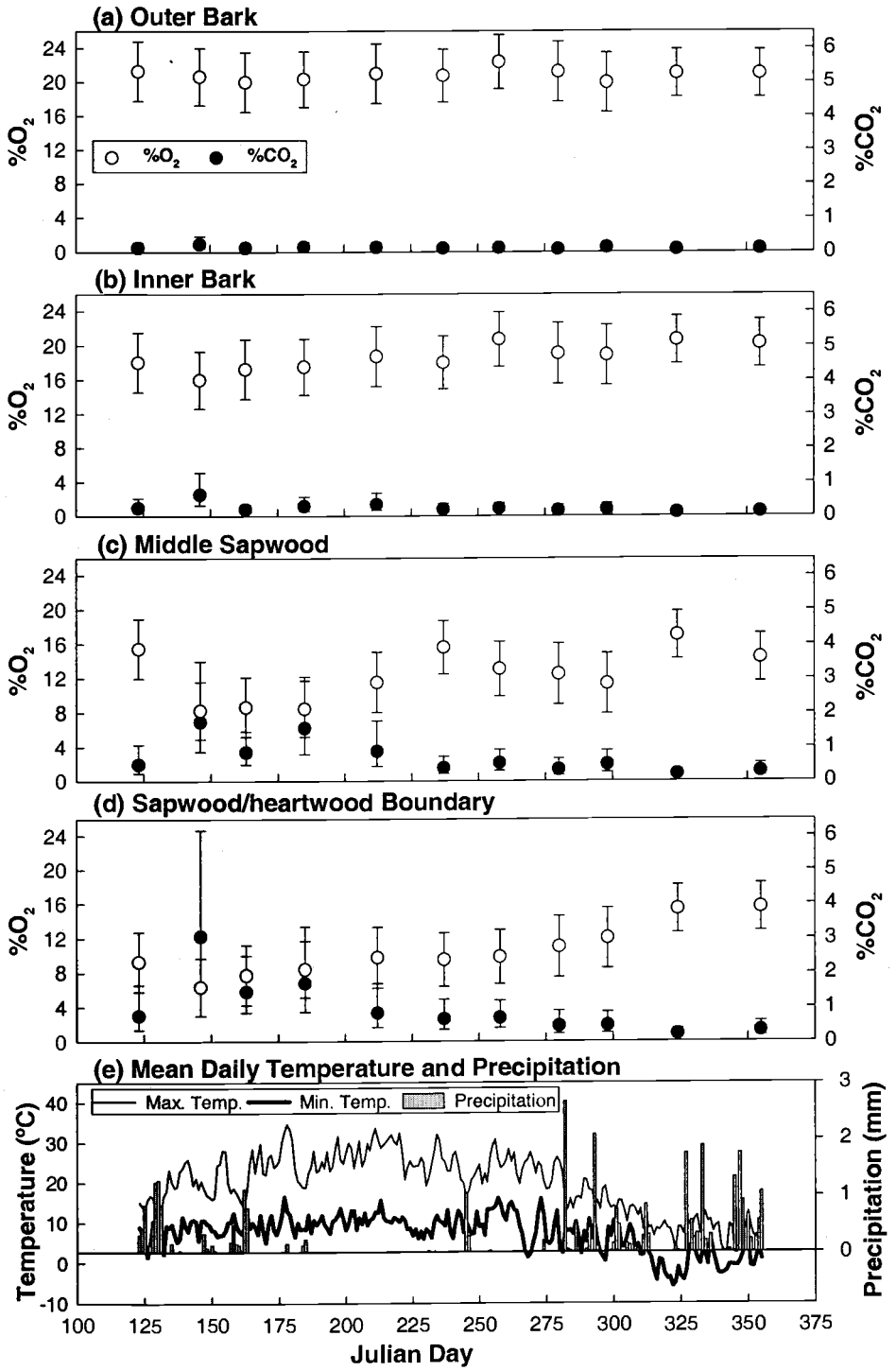
(a) Outer Bark
(b) Inner Bark
(c) Middle Sapwood
(d) Sapwood/heartwood Boundary
(e) Mean Daily Temperature and Precipitation
%O2
%CO2
Max. Temp.
Min. Temp.
Precipitation
Temperature (°C)
Precipitation (mm)
Julian Day

FIGURE 3.1.

levels recorded on the first sampling date in early May (Julian day 123). Within stem CO_2 followed a similar pattern, yet returned to atmospheric levels more rapidly. Middle sapwood and the heartwood/sapwood boundary were nearly always more hypoxic than the inner and outer-bark, yet significant differences between these two outer, or two inner radial positions were rare.

3.4.5. Responses in core respiratory potential to variation in atmospheric CO_2 / O_2 concentration

The initial gas concentration treatments (ambient, O_H – 5% O_2, 2% CO_2; O_L – 2% O_2, 10% CO_2; and N_2 flushed) changed over the course of the experiment because of core segment respiration (oxygen uptake or carbon dioxide production) and gas diffusion (stored gas from within the cores into the headspace of the vials or visa versa, Figure 3.2). As a result, gas concentrations were nearly equal among the four treatments as the experiment reached completion, which changed the effect of treatment on core respiratory potential over time.

Respiratory potential, carbon dioxide production (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) and oxygen uptake (nmoles $O_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) rates varied significantly by incubation time ($P < 0.0005$) and treatment ($P < 0.005$) for all three radial positions (Figure 3.2). The interaction between time and treatment was significant for O_2 uptake in inner bark and outer sapwood ($P < 0.05$), but not for inner sapwood ($P = 0.07$). In contrast, the interaction for CO_2 production was significant for all three

FIGURE 3.2. Effects of four different gaseous environments on respiratory potential at 25°C, O_2 uptake (nmoles $O_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) and CO_2 production (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$), of cores extracted at breast height from trees at the Corvallis site. Positive values for O_2 rates and negative values for CO_2 rates are the result of the respective diffusion of O_2 out of, or CO_2 into the core. Each lettered pair of graphs shows gas concentration ($\%CO_2$ or $\%O_2$) within test tubes, and respiratory potential (O_2 uptake or CO_2 production) of tissues from each radial position at three incubation times. Least Squares Means $\pm$ SE (n = 5 trees) from repeated measures analysis in PROC MIXED. Above the x-axis, different letters indicate significantly different means among treatments at each incubation time (treatment effects), and below the x-axis, different letters indicate significantly different means among incubation times for each treatment (time effects) (FPLSD, $P < 0.05$). For each effect, data without letters are not significantly different from one another.

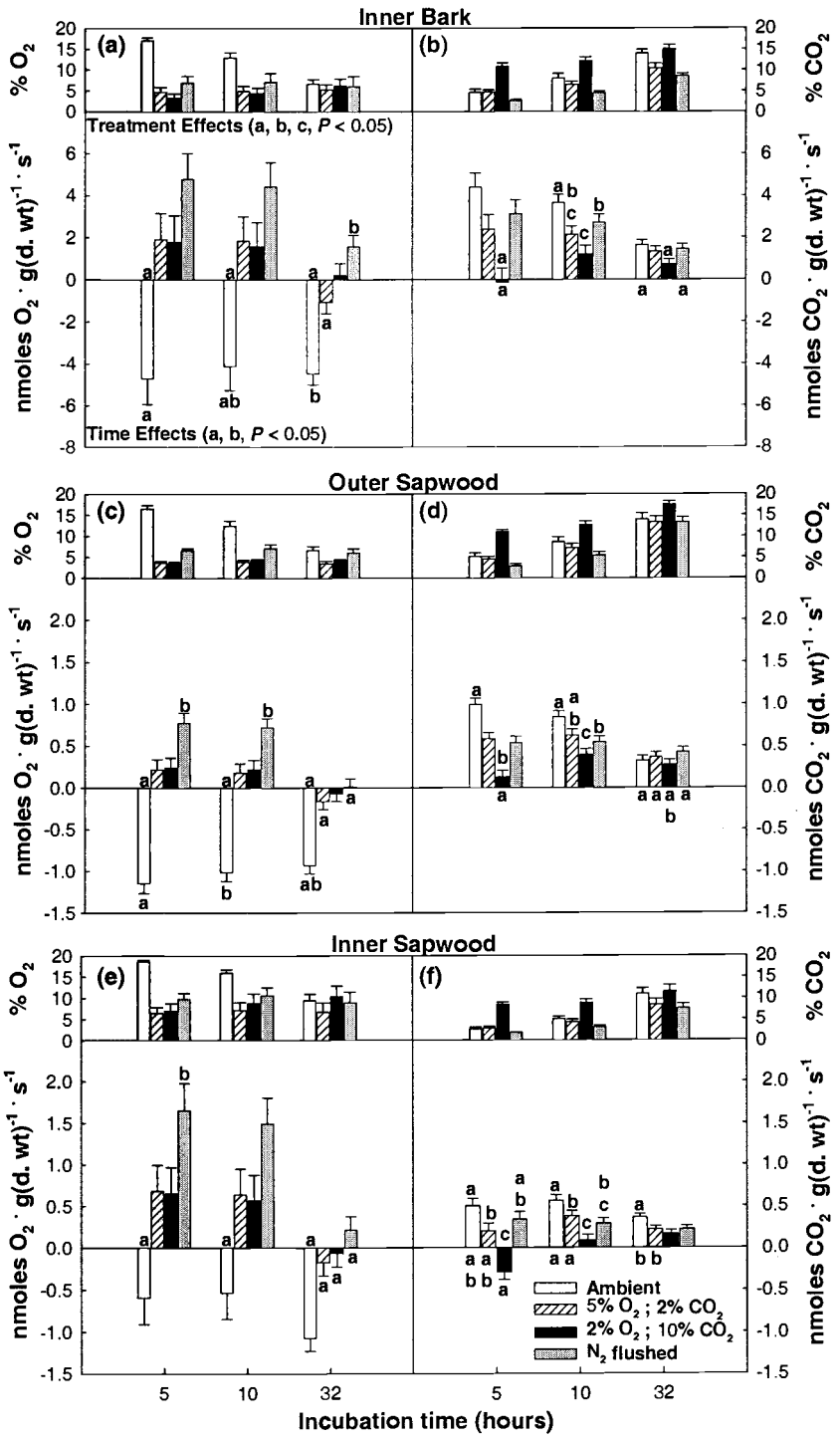
Inner Bark
(a)
(b)
% O2
% CO2
Treatment Effects (a, b, c, P < 0.05)
Time Effects (a, b, P < 0.05)
nmoles O2 · g(d. wt)-1 · s-1
nmoles CO2 · g(d. wt)-1 · s-1
Outer Sapwood
(c)
(d)
Inner Sapwood
(e)
(f)
Ambient
5% O2 ; 2% CO2
2% O2 ; 10% CO2
N2 flushed
Incubation time (hours)

FIGURE 3.2

radial positions ($P < 0.01$). This result indicated that the trend in core respiratory potential over time varied by treatment. All radial positions of core segments under ambient conditions (control) showed the highest rates of O_2 uptake and CO_2 production among the four treatments tested (Figure 3.2). As O_2 concentration in the vials decreased and CO_2 increased over time, O_2 uptake and CO_2 production of the inner bark and outer sapwood controls decreased significantly (Figure 3.2a – d). Inner sapwood O_2 uptake and CO_2 production for the controls did not show any significant trends over time (Figure 3.2e – f).

For the O_H, O_L, and N_2 flushed treatments O_2 uptake was not evident after 5 or 10 hours of incubation in samples from any of the radial positions (Figure 3.2a, c, e). The positive values for O_2 rates were likely the result of O_2 diffusion from within the core segments into vial headspace. After 32 hours, it was evident that all core segments (from the O_H, O_L, and N_2 flushed treatments) were consuming O_2 because their O_2 uptake rates had decreased or become negative (indicating O_2 uptake). Inhibition of core segment respiratory potential was evident by the trend of decreased CO_2 production in the treated core segments as compared to the controls (Figure 3.2b, d, f). The most notable inhibition of CO_2 production rates occurred in the O_L treated core segments. The most extreme cases of this trend were in the inner sapwood and to a lesser extent inner bark, where negative CO_2 rates were recorded, indicating the likely diffusion of CO_2 from the vial headspace into the core segment.

3.4.6. Stem radial and vertical trends in core respiratory potential

When considering only stem sapwood respiratory potential, the effects of stem radial position ($P = 0.0003$) and the interaction of radial by vertical position ($P = 0.04$) were significant, whereas the effect of stem vertical position was not significant ($P = 0.15$). At all three vertical positions, outer sapwood respiratory potential was > 60% higher than middle or inner, and there was no significant difference between middle and inner sapwood (Figure 3.3a). The significant interaction term indicated that the relationship between respiratory potential and radial position (ring number) varied by stem vertical positions. When both inner bark and sapwood respiratory potential were compared, effects of stem radial position, vertical position, and their interaction were all significant ($P < 0.03$, Figure 3.3b). At node 15 from the treetop, inner bark respiratory potential was > 3 times higher than all sapwood positions (Figure 3.3b). However, at node 100 inner bark respiratory potential was equal to that of all sapwood positions, except inner sapwood. Outer and middle sapwood positions at node 15 from the treetop were at least 50% higher than their corresponding radial positions at node 100 (Figure 3.3b).

3.4.7. Respiratory potential using moles carbon or moles nitrogen as a basis

At both the Corvallis and Riddle sites, core segment respiratory potential per moles carbon (μmoles $CO_2 \cdot$ moles $C^{-1} \cdot sec^{-1}$ at 25°C) followed trends similar to the mass and volume based indices (Table 3.3). The effect of radial position was

significant at both sites for all three indices ($P < 0.0001$). For the carbon, mass, and volume indices, effects of season and the interaction of radial position by season were not significant ($P > 0.2$), whereas effects of vertical position and the interaction of radial by vertical position were ($P < 0.01$). For these indices respiratory potential at treetops was at least 1.5 times greater than at the bases. The within-stem trends for respiratory potential per moles nitrogen (μmoles $CO_2 \cdot$ moles $N^{-1} \cdot sec^{-1}$ at 25°C) were not as obvious as when indices of carbon, mass, or volume were used (Table 3.3). For example, although the effect of radial position on respiratory potential per unit nitrogen was significant (Riddle site, $P = 0.01$), the inner bark value was only 1.2 times greater than that of sapwood as compared to > 5 times greater for any of the other indices. Moreover, there were no significant differences among stem radial and vertical positions for respiratory potential per moles nitrogen at the Corvallis site.

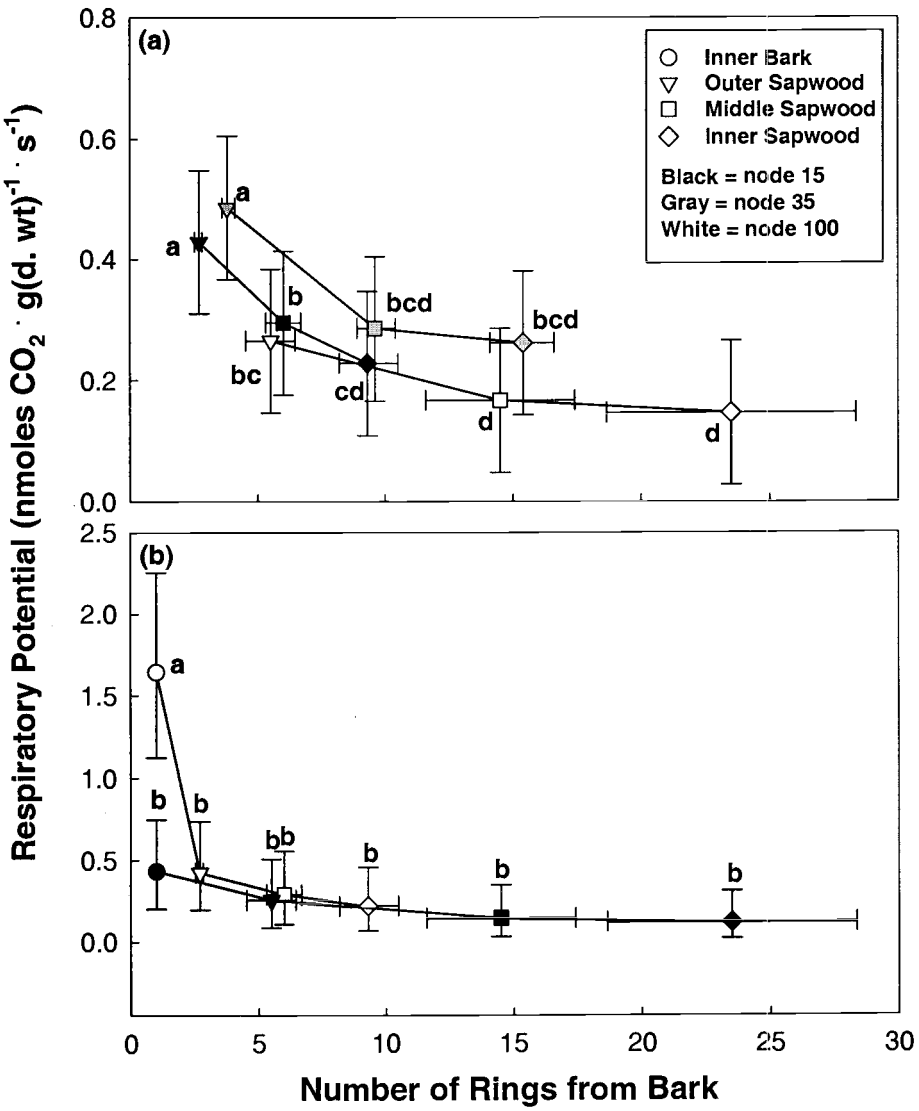

FIGURE 3.3. Relationship between tissue age and respiratory potential at 15°C (nmoles $CO_2 \cdot g(d.\ wt)^{-1} \cdot s^{-1}$) of cores extracted from 2 – 3 vertical positions within (4a) sapwood and (4b) inner bark and sapwood of trees from the Riddle site. Different shapes represent each stem radial position, and different shades represent each vertical position. Least Squares Means ± 95% confidence intervals from strip-plot analysis in PROC MIXED ($n = 3$ trees). In each panel, different letters indicate significant differences (FPLSD, $P < 0.05$). For clarity, not all significant differences were indicated in (4b), namely: node 100, inner bark versus inner sapwood; outer sapwood, node 15 versus node 100; and middle sapwood, node 15 versus node 100.

TABLE 3.3. Respiratory potential on a carbon, nitrogen, mass, or volume basis of cores extracted from 1 – 2 stem vertical positions from trees at the Corvallis (March and October) and Riddle (March) sites. Note: mass and volume indices are in units of 10^{-3} μmoles CO_2, whereas molar indices are in μmoles CO_2. For each measurement, Least Squares Means (Strip-plot analysis in PROC MIXED) $\pm$ 95% confidence intervals for Corvallis (n = 5) and Riddle (n = 3) trees. For each group of two rows, different letters indicate significant differences among means for all radial positions (FPLSD, $P < 0.05$).

Carbon and Nitrogen measurements by location, season, and stem position	***Radial Position***		
	Inner bark	***Outer sapwood***	***Inner sapwood***
Corvallis, OR – March and October (1 m from ground)			
Respiratory potential per moles Carbon at 25°C (μmoles $CO_2 \cdot$ moles $C^{-1} \cdot s^{-1}$)			
March	0.13 (0.10, 0.17)^{a}	0.024 (0.018, 0.033)^{b}	0.016 (0.012, 0.022)^{bc}
October	0.09 (0.07, 0.13)^{a}	0.024 (0.018, 0.033)^{b}	0.012 (0.009, 0.016)^{c}
Respiratory potential per moles Nitrogen at 25°C (μmoles $CO_2 \cdot$ moles $N^{-1} \cdot s^{-1}$)			
March	35 (20, 49)^{abc}	29 (15, 43)^{bc}	22 (8, 36)^{c}
October	49 (35, 63)^{a}	41 (27, 55)^{ab}	33 (19, 48)^{bc}
Respiratory potential per grams dry mass at 25°C (10^{-3} μmoles $CO_2 \cdot$ g(d. wt)$^{-1} \cdot s^{-1}$)			
March	5.5 (4.1, 7.5)^{a}	1.03 (0.77, 1.39)^{b}	0.68 (0.50, 0.92)^{cd}
October	3.9 (2.9, 5.2)^{a}	0.97 (0.72, 1.31)^{bc}	0.48 (0.35, 0.64)^{d}
Respiratory potential per core volume at 25°C (10^{-3} μmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$)			
March	2.7 (2.0, 3.6)^{a}	0.5 (0.4, 0.7)^{b}	0.33 (0.24, 0.44)^{c}
October	1.8 (1.3, 2.4)^{a}	0.5 (0.4, 0.7)^{b}	0.26 (0.19, 0.34)^{c}

TABLE 3.3. (Continued).

Carbon and Nitrogen measurements by location, season, and stem position	***Radial Position***		
	Inner bark	***Outer sapwood***	***Inner sapwood***
Riddle, OR – March			
(Node 15 and Node 100 from treetop)			
Respiratory potential per moles Carbon at 15°C (μmoles $CO_2 \cdot$ moles $C^{-1} \cdot s^{-1}$)			
Node 15	0.05 (0.04, 0.06)[a]	0.01 (0.003, 0.02)[b]	0.006 (0.0, 0.01)[b]
Node 100	0.01 (0.003, 0.02)[b]	0.006 (0.0, 0.01)[b]	0.003 (0.0, 0.01)[b]
Respiratory potential per moles Nitrogen at 15°C (μmoles $CO_2 \cdot$ moles $N^{-1} \cdot s^{-1}$)			
Node 15	6.3 (0.2, 11.7)[a]	12.8 (2.9, 21.8)[a]	9.1 (0.6, 13.8)[a]
Node 100	3.8 (0.9, 16.5)[a]	10.2 (4.4, 25.5)[a]	5.0 (2.4, 20.2)[a]
Respiratory potential per grams dry mass at 15°C (10^{-3} μmoles $CO_2 \cdot$ g(d. wt)$^{-1} \cdot s^{-1}$)			
Node 15	2.2 (2.0, 2.4)[a]	0.4 (0.2, 0.6)[b]	0.2 (0.1, 0.4)[bc]
Node 100	0.4 (0.2, 0.6)[b]	0.2 (0.1, 0.4)[bc]	0.1 (0.0, 0.3)[c]
Respiratory potential per core volume at 15°C (10^{-3} μmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$)			
Node 15	1.04 (0.94, 1.14)[a]	0.23 (0.14, 0.31)[bc]	0.13 (0.04, 0.22)[d]
Node 100	0.28 (0.20, 0.37)[b]	0.16 (0.07, 0.24)[cd]	0.08 (0.0, 0.17)[d]

3.5. DISCUSSION

The methods tests indicated that respiration rates in core segments were stable and not likely from wounding or microbial respiration (Table 3.1). Previous studies have also shown stable respiration rates in stored samples of tree stem tissue, for storage lengths < 15 days, in two hardwoods (Goodwin & Goddard 1940) and in *Pinus radiata* (Shain & Mackay 1973). In the current study, inner bark respiratory potential was less stable than the sapwood tissues and thus should be analyzed within the first few days of sampling. In a previous study, we concluded that the CO_2 production of extracted ponderosa pine cores was from the respiration of living parenchyma cells and not from degassing of stored CO_2 by demonstrating that there was no respiration from cores exposed to a toxic fumigant as compared to controls (Pruyn et al. 2002). Results from differential scanning calorimetry in the current study further supported this conclusion.

In the current study, the mean Q_{10} of 2.0 for all tissues at both sites for the 15 – 25°C range is consistent with literature values for intact Douglas-fir stems (L.A. Cernusak, N. McDowell, N. Balster, & J.D. Marshall, unpublished data). The higher sapwood Q_{10}s in the current study for the 5 – 15°C temperature range are unusual as compared to Larcher (1983), who reported that plant Q_{10}s approach 2.0 at 5 – 25°C, but have been recorded for decomposing root tissues (e.g. Chen et al. 2000). These data suggest that at lower temperatures (5 – 15°C), Douglas-fir sapwood may have been more reactive to temperature increases than would be expected from literature values. Further research is necessary to ascertain whether a

Q_{10} lower than 2.0 should be used when normalizing respiration measurements of Douglas-fir sapwood to temperatures in the 5 – 15°C range. The relative uniformity of sapwood Q_{10} within a given temperature range (excluding the 5 – 15°C range at the Riddle site) was notable because sapwood age ranged from 2 – 30 years. Thus, respiratory enzymes in live stem wood of these Douglas-fir trees responded to temperature similarly, regardless of tissue age.

The O_2 and CO_2 concentrations within Douglas-fir stems *in vivo* differed from the ambient concentrations of the standard *in vitro* set-up used in the current study, which likely impacted the respiration rates of extracted cores. Low O_2 and high CO_2 within stems was also recorded in *Pinus strobes* and *Picea abies* during the growing season (Chase 1934, Eklund 1990, respectively). Mean O_2 from the Douglas-fir trees in the current study did not reach levels as low, nor did mean CO_2 reach levels as high as in these previous studies. However, individual Douglas-fir trees did reach such levels of O_2 (2%) and CO_2 (9%), but trees were not synchronized by sampling date, resulting in the lower means reported here. The inverse relationship between O_2 and CO_2 suggests that respiration of the living cells in the sapwood regulates their concentrations (Eklund 1990). Further, the seasonal hypoxia within tree stems has been attributed to the formation of heartwood extractives in *Acacia mearnsii* (Carrodus 1971) and latewood formation in *Picea abies* (Eklund 1990).

Oxygen supply to sapwood is hypothesized to be a function of both radial influx into the trunk through intercellular gas spaces and transport of dissolved oxygen via transpiration in the xylem (Gansert et al. 2001). Considerable soil water deficiency during dry periods may reduce transpiration and prevent the entry (O_2) and exit (CO_2) of dissolved gases via the transpiration stream. The resulting CO_2 build-up is augmented by high respiration rates, characteristic of the growing season and associated with cambial divisions and photosynthate mobilization and storage (Chase 1934). Hypoxia within stems may create negative partial pressures, favoring the radial influx of oxygen through bark lenticels to intercellular spaces of the cortex and phloem. The neighboring cambium and xylem are relatively impermeable to gas because they lack a continuum of intercellular spaces (Hook et al. 1972), which explains the CO_2 increase and O_2 decrease from outer bark to the sapwood/heartwood boundary in the current study, Chase (1934), and MacDougal & Working (1933). Evidence that O_2 is also supplied to sapwood from the soil and then the transpiration stream was demonstrated in *Picea abies* stems, where oxygen levels decreased with stem height during the growing season (Eklund 2000). Further evidence of the transpiration stream as an O_2 source within stems is reported in ***Betula pedula***, where sap flow was found to be a major determinant for the diurnal flux of dissolved O_2 concentrations (Gansert et al. 2001).

When vial CO_2 concentrations were increased in the current study to mimic within-stem conditions *in vivo*, core respiratory potential was decreased as compared to controls. This result is consistent with reports of a direct, immediate,

and reversible effect of reduced respiration in isolated tissues (Amthor 1991) or intact plants (Griffin et al. 1996) under conditions of elevated CO_2. The effect of respiratory potential inhibition was not as evident from patterns of O_2 uptake in treatments of low O_2 because of O_2 diffusion from within core segments into the vial headspace. This diffusion of dissolved O_2 from within cores into the vial headspace was likely the result of a positive partial pressure inside cores, created by the low O_2 treatments. The living cells within core segments were thus able to access and use gaseous and dissolved O_2 in respiration and produce CO_2, even when vial O_2 concentration was low or near zero. This ability of parenchyma cells to respire under low gaseous O_2 availability suggests a mechanism for how cells respire deep within stem sapwood. Accumulation of intracellular CO_2 from aerobic and/or anaerobic respiration (depending on the oxygen micro-environment of each cell) may create a gradient for O_2 diffusion into cells from nearby intercellular air spaces, or the transpiration stream within conducting elements of the xylem. Other mechanisms, such as the alternative pathway (cyanide-resistant) or anaerobic respiration may enable respiration to continue despite high ambient CO_2, simultaneously maintaining a gradient for O_2 influx while providing energy for stem tissue anabolism (Hook et al. 1972, Amthor 1991).

The trends of increasing respiratory potential from pith to bark and tree base to treetop that were identified within Douglas-fir stems at the Riddle site were similar to those in ponderosa pine (Pruyn et al. 2002). Respiratory potential (normalized to 25°C) was similar in both species, with the exception of inner bark,

which was 3 – 4 time higher in ponderosa pine than Douglas-fir. Also, respiratory potential from breast height of the Riddle trees was nearly equal to values reported for O_2 uptake in stem tissue samples from *Fraxinus nigra* and *Acer rubrum* (Goodwin & Goddard 1940), but twice that of *Pinus radiata* (Shain & Mackay 1973). Possible explanations for the latter discrepancy were differences in sample dimensions (0.5 cm radial thickness versus our 2-5 cm long cores) and measurement techniques (volumetric respirometer versus our gas chromatograph) between studies. Further research is needed to ascertain whether similar within-stem trends exist in other species, and to incorporate diurnal or seasonal effects.

Potentially, the heightened respiratory activity of outer sapwood was related to its role in supporting growth and secondary wall formation in the cambial zone (Goodwin & Goddard 1940), and/or other physiological activities associated with xylem maintenance (Lev-Yadun & Aloni 1995). Enhanced activity may also be linked to a role in carbohydrate storage. Studies have shown increased amounts of soluble carbohydrates and starch in the outer sapwood and inner bark as compared to the inner sapwood and heartwood of *Pinus sylvestris* (Saranapää & Höll 1989). The reduced activity of middle and inner sapwood may be explained by age-related changes and/or dormancy of metabolic activity in sapwood parenchyma cells. These theories are supported by findings that ray cell nuclear morphology changed from outer to inner sapwood in various conifer species, thus indicating decreased ray vigor (Frey-Wyssling & Bossard 1959, Yang 1993, Gartner et al. 2000). Also, heartwood formation or wound repair has been associated with enzymatic or

chemical changes in rays of middle or inner sapwood, suggesting sapwood parenchyma cells may be genetically regulated to remain dormant until reactivation by signals from the cambial and/or apical meristem (Shain & Mackay 1973, Bamber 1976). The notably higher respiratory potential within the inner bark and outer sapwood at nodes 15 and 35 compared to the same positions at node 100 may also be explained by their location, which was within the crown, where physiological activities, such as growth, substrate supply, metabolism, and transport are high.

Our results showed rates of CO_2 production that were nearly equal for the various radial positions when computed on a nitrogen basis versus on a core carbon content, dry mass, or volume basis, which is likely because tissue nitrogen content is an index of enzyme amounts in the tissue. Values for respiratory potential on a nitrogen basis for outer and inner sapwood in the current study were 1.5 – 2 times higher than oxygen consumption on a nitrogen basis of outer and inner sapwood extracts from two hardwoods (Goodwin & Goddard 1940). Although carbon and nitrogen may be more representative of living cell material within stem tissue, it cannot be an absolute basis for measurement because some of the extracted carbon and nitrogen was likely obtained from dead cells (Goodwin & Goddard 1940). Thus, other indices in addition to tissue nitrogen content should be explored for correlations to respiratory trends, such as total non-structural carbohydrates (sugar, starch, and lipids), or percent live parenchyma cell volume.

Carbon dioxide production of extracted wood cores was generally 3 – 15 times higher than IRGA measurements of Douglas-fir stem respiration (Cernusak et al., unpublished data) and thus did not represent normal production within intact tree stems. The current results suggest that wounding, microbial respiration, and leakage of stored CO_2 are not likely explanations for this discrepancy. We suggest three possible reasons why extracted cores respired at higher rates: (1) Inner bark and sapwood Q_{10} (for temperature ranges $\leq$ 15°C) may be higher than has been indicated previously by IRGA measurements, which has implications for normalizing respiration to $\leq$ 15°C (2) within stem concentrations of high CO_2 and low O_2 may inhibit respiration *in situ*, and (3) some respired CO_2 may exit the stem via the transpiration stream and not radial efflux, so that not all respired CO_2 is captured by the IRGA systems. Gradients of core respiratory potential within and among trees could be used as an index for scaling to the whole-tree level, in conjunction with IRGA measurements to provide a reference for *in situ* respiration rates. However, the IRGA rates will have to account for respired CO_2 that has left the stem undetected via the transpiration stream. Core based estimates for respiration could also be applied to such research areas as storage physiology, metabolism, wood development, ecosystem and individual-tree modeling of carbon pools, and wood function in different life forms and growth habits.

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4. STEM RESPIRATORY POTENTIAL IN SIX SOFTWOOD AND FOUR HARDWOOD TREE SPECIES IN THE CENTRAL CASCADES OF OREGON

4.1. SUMMARY

Mature and old growth tree species of varying sapwood thickness were compared with regard to stem respiration. An increment core-based, laboratory method under controlled temperature was used to measure tissue-level respiration (termed respiratory potential) of ten different tree species. Bark, sapwood, and heartwood thickness measurements were used to predict sapwood volume from stem diameter outside bark for four of the ten species. These predictions of sapwood volume were used to scale the core-based measurements of respiratory potential to the whole-tree level. At breast height, species that maintained narrow sapwood (< 10 cm) such as *Pseudotusga menziesii*, *Taxus brevifolia*, and *Thuja plicata*, had sapwood core respiratory potentials that were 50% higher than species with wide sapwood (> 10 cm), such as *Abies amabilis*, *Pinus monticola*, and *Tsuga heterophylla*. This pattern was not shown for inner bark respiratory potential, or for sapwood respiratory potential within the crown. There was no strong relationship between core respiratory potential and species-specific literature values of the volumetric proportion of ray parenchyma cells in sapwood. However, when tissue-level rates were scaled to the whole-tree level, there was an inverse relationship between whole tree respiration rate per unit volume and fraction of live bole (inner bark plus sapwood) in the stem. The implication was that tree species

supporting lower percentages of live bole respired more per unit volume of live bole than species with larger percentages of live bole.

Keywords: stem respiration, scaling, sapwood thickness, sapwood volume, hardwoods, softwoods

4.2. INTRODUCTION

Estimating stem respiration with the objective of scaling to the ecosystem level has received much attention over the last 25 years, as researchers have tried to understand forest responses to the environment (e.g., Linder and Troeng 1981, Ryan and Waring 1992, Lavigne and Ryan 1997). Estimates of stem respiration's contribution to total autotrophic respiration of forests have been reported to range from 12% (sub-alpine *Pinus contorta*, Ryan and Waring 1992 and Australian *Pinus radiata*, Ryan et al. 1996) to 42% (temperate forests, Waring and Schlesinger 1985). Rising atmospheric CO_2 concentrations and the predicted corresponding increases in mean annual temperature will likely result in increases in stem respiration, which could ultimately affect forest carbon budgets significantly (Carey et al. 1997).

In most cases, estimates of whole-tree and stand level respiration rates are obtained from scaling up small sample measurements, acquired using infrared gas analysis (IRGA) chambers at one or two positions on tree stems (e.g., Kinerson 1975, Edwards and Hanson 1996, Ryan et al. 1995). Stem surface area beneath the

chamber often has been used as an index of the amount of living tissue to associate with the measured respiration rate (e.g., Linder and Troeng 1981, Matyssek and Schulze 1988). This rate can then be scaled to the entire surface area of the stem and to the ecosystem level. However, sapwood volume has proved to be a better index for scaling maintenance respiration to whole trees or ecosystems (Sprugel 1990, Sprugel and Benecke 1991, Ryan and Waring 1992), probably because sapwood volume is proportional to the amount of living parenchyma cells therein (Ryan 1990, Larson 1994, Stockfors and Linder 1998).

The assumption that respiration is uniform throughout the stem causes uncertainty in scaling to the whole-tree level from one or two measurements from the stem's surface. Small-scale, tissue-based, respiration measurements have indicated significant differences within stems, with tissue in close proximity to apical (treetops) and/or cambial (inner bark) meristems respiring at higher rates than other areas in the stem. On a dry mass basis, respiratory potential of *Pinus ponderosa* inner bark was 3 – 15 times greater than the neighboring outer sapwood, which was in turn 30 – 60% higher than middle or inner sapwood at different stem heights in three different age classes of trees (Pruyn et al. 2002 a). Also, sapwood rings produced in the same calendar year released over 50% more CO_2 at treetops than at bases. Similar patterns were found within stems of *Pseudotsuga menziesii* (Pruyn et al. 2002 b). The implications of these small-scale measurements to whole trees varied depending on what scaling method was used. For example, given the greater relative volume of respiring tissue at the tree base versus the treetop, tissue-

level respiration rates at the base of the tree had greater influence on whole stem respiration than tissue-level rates near the top (Pruyn et al. 2002 a).

Other uncertainties exist in scaling to the whole-tree level associated with inner bark and sapwood volume prediction of the tree from stem diameter at breast height. Species-specific values of inner bark thickness are available in the literature (Smith and Kozak 1971), as are equations for predicting sapwood thickness (Lassen and Okkonen 1969) or bole volume (Brown 1962, Chambers and Foltz 1979). In contrast, species-specific equations for predicting inner bark or sapwood volume are less abundant. Allometric relations for sapwood volume versus stem diameter were developed for *Pinus* spp., *Populus* spp. and *Picea mariana* (Gower et al. 1993, 1997) by destructively sampling stems, measuring sapwood cross-sectional area and stem lengths, and using frustum equations to calculate volume (Husch et al. 1972). However, because of the intensive nature of this work, these studies only measured 18 – 40 trees on 2 – 3 different sites, thus limiting the scope of inference for the equations.

Further, the relationship between whole-tree sapwood volume and stem respiration is not well understood. Strong, positive relationships have been developed between sapwood volume under the IRGA chamber and the corresponding bole CO_2 efflux (nmoles $\cdot$ s^{-1}) ($R^2 = 0.72$, *Abies amabilis*, Sprugel 1990; $R^2 = 0.85$, *Pinus ponderosa*, Ryan et al. 1995). Thus, trees with large sapwood volume, likely have high values of total CO_2 efflux, as compared to trees with small sapwood volume. However, trees with larger sapwood volumes do not

necessarily have higher stem respiration rates per unit volume than smaller trees. One hypothesis is that trees maintain a constant respiration load from stem tissues. Trees may attain this constant load by having low stem respiration rates (per unit volume) if maintaining a large sapwood volume, or by having high woody tissue respiration rates if maintaining a small sapwood volume. Thus, trees may implement a compensation mechanism via this inverse relationship between stem respiration rate (per unit volume) and sapwood volume. Alternatively, there may be no limit to the stem respiratory load manageable by trees, suggesting a positive relationship between the two variables. Lastly, stem respiratory loads may not be related to sapwood volume and may instead be regulated by an independent parameter, such as leaf area, tree size, or tree age.

In the current study, we asked whether small-scale differences in respiration within stems and among species of varying sapwood thickness were evident on the whole-tree level. The first objective was to quantify potential stem respiration on the tissue level in six softwood and four hardwood tree species of the Pacific Northwest. Second, a series of equations were developed to predict inner bark and sapwood volume of individual trees from stem diameter at breast height to enable scaling tissue-based respiration to the whole-tree level. Finally, the relationship between species-specific stem respiration and live bole volume (inner bark plus sapwood) was analyzed and discussed in terms of its significance to stem physiology and ecosystem function.

4.3. MATERIALS AND METHODS

4.3.1. Study areas

Respiration measurements were conducted on trees sampled from various sites in the H.J. Andrews Experimental Forest, located in the western Cascade Range, Oregon, USA (N 44° W 122°, elevation 410 – 1630 m). Sapwood thickness and tree volume measurements were obtained from the Oregon State University's Department of Forest Science Databank (FSDB). Sapwood thickness measurements were conducted in and near the H.J. Andrews Experimental Forest in study areas dominated by coniferous forest, primarily of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) (Dyrness et al. 1974). Tree volume measurements were conducted on trees sampled from reference stands of mature and old growth forests throughout the Pacific Northwest region (details in Acker et al. 1998).

The H.J. Andrews Experimental Forest is in the 6400 ha drainage basin of Lookout Creek, a tributary of the Blue River and the McKenzie River. Generally representative of the mountainous landscape of the Pacific Northwest, the Andrews Forest contains extensive examples of the region's conifer forests (including old-growth stands over 500 years old) and associated wildlife and stream ecosystems. Forest communities in the central portion of Oregon's western Cascades vary according to moisture and temperature gradients and have often been defined to occur within two distinct forest zones, which are largely a function of temperature

or elevation (Dyrness et a. 1974). For example, the *Tsuga heterophylla* zone occupies an elevational range of 300 – 1050 m, whereas the *Abies amabilis* zone ranges from 1050 – 1550 m.

4.3.2. Respiratory measurements – field methods

Respiratory potential was estimated from 12 mm increment cores extracted from breast height of standing stems (1 m from ground) of ten different mature tree species (Table 4.1). Four trees of each species were selected randomly within H.J. Andrews Experimental Forest from sites where they grew. Also, four younger trees were selected from each of two species, *Pseudotsuga menziesii* and *Tsuga heterophylla*, from the same sites as the older, same-species trees. Because the younger *Tsuga heterophylla* were growing in the understory of mature/old-growth stands, they were considered suppressed. The younger *Pseudotsuga menziesii* were not considered suppressed because they grew on the edges or in canopy gaps.

In four of the ten species (*Abies amabilis*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*), respiratory potential within stems was also measured on a 12 mm core from the base of the live crown and from the base of the top 1/5 of the tree's height (Table 4.1). Stem diameter outside bark was measured at each point of core extraction, and heights to the crown samples and treetops were measured using a Forest Pro ® hypsometer (Laser Technology Inc., 7070 Tucson Way, Englewood, Colorado 90112, USA). Tree age was determined from breast

TABLE 4.1. Ranges of age, diameter, and height measurements for the ten species of trees sampled at HJA Experimental Forest. Note: Acronyms listed were used in tables and figures to follow.

Species	Acronym	Age (years)	Diameter (cm)	Total Height (m)	Height to Base of Live Crown (m)	Height to Base of top 1/5 of Tree (m)
Abies amabilis ([Dougl.] Forbes)	ABAM	170 – 370	43 – 80	40 – 46	16 – 20	33 – 41
Acer macrophyllum (Pursh.)	ACMA	15 – 47	26 – 49	--	--	--
Alnus rubra (Bong.)	ALRU	12 – 31	13 – 35	--	--	--
Castonopsis chrysophylla (Dougl.)	CACH	37 – 58	8 – 26	--	--	--
Pinus monticola (Dougl.)	PIMO	105 – 120	55 – 60	--	--	
Populus trichocarpa (Torr. & Gray)	POTR	25 – 40	37 – 75	--	--	--
Pseudotsuga menziesii (old) ([Mirb.] Franco)	PSMEo	300 – 600	115 – 157	40 – 53	20 – 32	35 – 42
Pseudotsuga menziesii (young)	PSMEy	13 – 23	18 – 28	19 – 26	--	--
Taxus brevifola (Nutt.)	TABR	51 – 81	14 – 41	--	--	--
Thuja plicata (Donn. ex. D. Donn)	THPL	100 – 500	68 – 142	21 – 36	8 – 14	17 – 26
Tsuga heterophylla (old) ([Raf.] Sarg.)	TSHEo	107 – 220	53 – 100	38 – 45	17 – 26	28 – 40
Tsuga heterophylla (young suppressed)	TSHEy	26 – 100	11 – 29	20 – 23	--	--

height cores. All sampled trees were free of broken tops, stem deformities, or obvious signs of disease. Unless indicated otherwise, samples were collected in the dormant season: in early March of 1998 and 1999, and in late September of 2000 and 2001. We selected these sampling dates to capture maintenance respiration, and thus avoid the complications of growth respiration in our estimation of core respiratory potential (McCree 1970, Thornley 1970).

4.3.3. Sapwood thickness – field measurements

Sapwood thickness was used to calculate sapwood volume in four of the ten tree species (*Abies amabilis, Pseudotsuga menziesii, Thuja plicata, and Tsgua hetrophylla*). Approximately ten trees per stand were measured for a representative sample of the diameter classes present. For *Abies amabilis* (sample size, n = 24 trees) sapwood thickness data were taken from Harmon (1992). For *Pseudotsuga menziesii* (n = 600), *Thuja plicata* (n =50), and *Tsuga heterophylla* (n = 200) data were taken from FSDB: TV0404, TV0504, and TV0524. For each tree, sapwood thickness was estimated from two increment cores taken at breast height (Means et al. 1999).

4.3.4. Sapwood volume – field measurements

Sapwood volume was calculated for the same four species as for sapwood thickness. To enable these calculations, the outside dimensions of individual standing trees were determined using an optical dendrometer (Barr and Stroud

FP15). It was used to separate each tree into 5 to 6 logs and provided each log's bottom and top outside bark stem diameters, as well as its length. Bark thickness was determined at breast height, and by assuming the bark to wood ratio was constant along the bole, the diameter of wood (without bark) was calculated. Approximately thirty trees per stand were measured for a representative sample of the diameter classes present. These stem diameter data for *Abies amabilis* (n = 140 trees), *Pseudotsuga menziesii* (n = 220), *Thuja plicata* (n = 50), and *Tsuga heterophylla* (n = 350) were taken from FSDB TV0096.

4.3.5. Respiratory measurements – laboratory methods

All extracted cores were analyzed within one week of sampling, using a previously developed protocol (Pruyn et al. 2002 a, b). Twenty-four hours prior to measurement, cores were divided into three segments: inner bark (phloem and cambium) and outer and inner sapwood. Any green tissue (photosynthetic) that was visible just underneath the bark surface on the inner bark samples from was carefully removed with a razor blade. Sapwood was defined as the woody tissue extending from the first growth ring proximal to the inner bark to the last growth ring interior of the transition zone (one or two lighter colored rings at the sapwood / heartwood boundary). Outer and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. For this study, respiratory potential of heartwood was not measured because preliminary data revealed almost no CO_2 evolution from heartwood samples (< 0.01 nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$,

Pruyn, data not shown). Number of rings per segment was recorded, so that a mean age could be determined for each segment. These segments were weighed, wrapped tightly in plastic, and then stored at 25°C overnight to allow metabolic activity in core segments to stabilize (Goodwin & Goddard 1940, Hari et al. 1990, Levy et al. 1999).

Immediately prior to measurement, core segments were re-weighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, carbon dioxide concentration within vials was measured with a Hewlett-Packard (5700A) gas chromatograph (*GC*, Hewlett Packard, Rt. 41, Avondale PA 19311, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25°C for six hours (unless indicated otherwise) between *GC* measurements. Details of *GC* analysis and calculation of respiratory potential (nmoles $CO_2 \cdot m^{-3} \cdot s^{-1}$) are in Pruyn et al. (2002 a) with the exception that in the current study core volume was used as a basis for respiration rather than dry mass. Immediately following the *GC* analysis, core segments were weighed a third time. Changes in the three successive wet masses verified that water loss was low (between 1 – 3%) between sampling and measurement. Fresh volume of core segments was estimated as the water displaced when samples were submerged (D2395, ASTM 2001). From this point onward, the reported values are referred to as respiratory potential, rather than respiration rate, because measurement conditions of these excised samples were probably different from those within the tree.

4.3.6 Volumetric proportion of sapwood parenchyma in the stem

Core respiratory potential (averaged over March 1999, September 2000, and 2001) of the inner bark, outer sapwood, and inner sapwood for each species were plotted against the corresponding species-specific volumetric proportion of sapwood parenchyma (percent parenchyma volume) in the stem. Values of percent parenchyma volume were obtained for each species from those listed in Panshin and DeZeeuw (1980), with the exception of *Alnus rubra*, which was taken from Gartner et al. (1997) and *Abies amabilis*, taken from Myer (1922). Because there were no species-specific literature values for *Populus trichocarpa* and *Taxus brevifolia,* values from the closest related species were used, i.e. *Populus deltoides* and *Taxus distichium*, respectively (Panshin and DeZeeuw 1980). Because there were no literature values for *Castanopsis chrysophylla* or any related species within the genus, *Castanea dentata* was used because it was the species that keyed out the closest to *Castanopsis chrysophylla* according to the dichotomous key for minute anatomy (Panshin and DeZeeuw 1980). Values of percent parenchyma volume included axial and radial parenchyma cells for hardwoods, whereas only radial parenchyma were included for softwoods because axial parenchyma are not abundant (e.g. ≤ 0.2 %, Panshin and DeZeeuw 1980).

4.3.7. Estimating sapwood and inner bark volumes on the whole-tree level

The purpose of the following series of equations was to enable the scaling of core-based respiration to the whole-tree level. Thus, these equations were only

performed on the four species for which we had respiratory measurements at multiple heights (i.e. *Abies amabilis*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*).

4.3.7.1. Sapwood thickness

The means of sapwood thickness (S_T) were grouped into classes of stem diameter outside bark at breast height (D_B) by every 2.54 cm (1 in), and for $D_B >$ 76.2 cm (30 in) by every 12.7 cm (5 in), following Lassen and Okkonen (1969). The D_B and S_T were then averaged for each D_B class and the following two equations were applied to the data:

$$S_T = \beta_0 + (\beta_1 \cdot D_B^{-1}) \quad (1)$$

$$S_T = \beta_0 \cdot (1 - e^{-\beta 1 \cdot D_B}) \quad (2)$$

After determining that Equation (2) was better suited for the data, it was applied to the sapwood volume database to calculate sapwood thickness along stems of three of the four species (*Pseudotusga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*).

The parameters for Equation (2) were estimated for *Abies amabilis* because there was no available data for S_T versus D_B for this species. Mean sapwood thickness for *Abies amabilis* was estimated from logs that were approximately 50 cm in diameter (Harmon 1992). This estimate was assumed to be the maximum

sapwood thickness (β_0) for this species because the relationship between S_T and D_B for the three other species from the current study suggested that maximum S_T was reached at a D_B of < 50 cm. For the rate of increase of sapwood thickness (β_1) of *Abies amabilis*, the same value was used as for *Tsuga heterophylla*.

4.3.7.2. Sapwood volume

To calculate the sapwood volume in the trees, S_T was estimated at various heights along stems. Two assumptions were used to estimate S_T along stem heights of the four species: (1) S_T was constant along stems and equal to S_T at D_B, and when $S_T \geq$ stem diameter, heartwood thickness equaled zero; (2) S_T tapered along stems and was a function of stem diameter at the point of estimation. For each assumption of S_T along stem heights, S_T was subtracted from the wood radius (without bark) from both ends of each log (measured via an optical dendrometer) to obtain heartwood radius. Using heartwood radius, total wood radius, and log length (measured via an optical dendrometer) in the formula for the frustum of a right circular cone, heartwood volume and total wood volume for a series of logs along each stem were then calculated. At the top of trees, heartwood radius was often zero, and a cone formula was used to calculate heartwood volume for the top log. However, because there were no data for the true height of the heartwood within the top log, it was assumed that the height for the heartwood cone was equal to top log's length. Heartwood volume was subtracted from wood volume to calculate sapwood volume for each log, and sapwood volume of each log along

stem lengths was summed to calculate sapwood volume for entire trees. Tree sapwood volumes calculated from each model of S_T along stem heights were compared. Sapwood volume (S_V) was regressed on stem diameter outside bark at breast height (D_B) using the following equation:

$$lnS_V = \beta_0 + \beta_1 \cdot lnD_B + \beta_2 \cdot (lnD_B)^2 \qquad (3)$$

4.3.7.3. Cumulative relative sapwood volume and relative height

To give a specific sapwood volume to core respiratory potential at different heights along tree stems, relative cumulative sapwood volume was plotted against relative stem height for each of the four tree species. From the sapwood volume database, a height from each stem was randomly selected and then cumulative sapwood volume was calculated as the sum of each log's sapwood volume from the tree base up to the selected height. Relative cumulative sapwood volume (S_{VC}, sapwood volume below a given height / total sapwood volume) was then regressed on relative stem height (H_C , given height / total tree height) using the following equation:

$$S_{VC} = \beta_0 + \beta_1 \cdot H_C + \beta_2 \cdot H_C^2 \qquad (4)$$

4.3.7.4. Inner bark thickness and volume

Equation (2) was also used to determine inner bark thickness for trees of varying stem diameter outside bark at breast height (D_B) for these four species. However, because there were no data to create the parameters for this curve, the average inner bark thickness for each species was taken from Smith and Kozak (1971) for β_0 (i.e. *Abies amabilis* 0.33 cm, *Pseudotsgua menziesii* 0.60 cm, *Thuja plicata* 0.33 cm, *Tsuga heterophylla* 0.35). The species-specific coefficient generated from the sapwood thickness curves were used for the rate of increase of inner bark thickness (β_1).

Then, using the same tree data and methods used for estimating sapwood volume (Equation 3), inner bark volume was calculated. However, in this case, inner bark thickness was added to wood radius (inside bark). Next, an inner bark volume, including the inner wood volume, was calculated for each log along the stem's height. Subtracting out wood volume, an estimate for inner bark volume was obtained. Relative cumulative inner bark volume was predicted from relative stem height using the same methods as for sapwood (Equation 4).

4.3.8. Scaling core-based respiratory potential to the whole-tree level

Using Equation (3), total inner bark volume and sapwood volume were calculated for each tree from which we took respiratory measurements. Then, using Equation (4), relative cumulative sapwood volume was calculated for each height position (see Method A, Table 4.2). To see whether the method used to

segment the trees vertically affected the estimates of whole tree respiratory potential, trees were divided vertically in three different ways (Methods 1 – 3) in addition to the tree stem segment proportions described for Method A (Table 4.2).

TABLE 4.2. Stem tree segments used for scaling core-based respiratory potential to the whole-tree level.

	Segmentation of Stems: Proportions			
	Species Specific	Uniform for All Species		
Tree Segment	*Method A*	*Method 1*	*Method 2*	*Method 3*
Top	Near Treetop to Treetop *(Below Crown)*	1/3	1/2	1/4
Middle	Base Live Crown To near Treetop *(Mid Crown)*	1/3	1/4	1/4
Bottom	Tree Base to Base Live Crown *(Top Crown)*	1/3	1/4	1/2

To determine the proportion of outer sapwood to inner sapwood, the total wood and heartwood volumes were calculated for each height position using the same methods as for total sapwood from above. Inner sapwood volume (including heartwood) was calculated using a radius of sapwood thickness divided by two plus heartwood radius. Inner sapwood and outer sapwood volumes were each calculated by subtracting out the adjacent, interior wood volume (e.g. heartwood-inclusive, inner sapwood volume minus heartwood volume = inner sapwood volume). The ratio of outer sapwood to inner sapwood volume was then calculated for each

vertical position of each species. These ratios were used to estimate outer sapwood and inner sapwood volumes for each height position of each tree (Table 4.3).

TABLE 4.3. Ratios of outer sapwood volume to inner sapwood volume for each stem segment along tree heights of four tree species. Means ± SE (n = 7 trees, from September of both years).

	Ratio of Outer to Inner Sapwood Volume		
Species	***Breast Height***	***Base of the Live Crown***	***Near Treetop***
ABAM	1.11 ± 0.004	1.17 ± 0.006	1.24 ± 0.02
PSME	1.03 ± 0.002	1.08 ± 0.005	1.11 ± 0.01
THPL	1.05 ± 0.001	1.04 ± 0.003	1.07 ± 0.01
TSHE	1.16 ± 0.004	1.23 ± 0.005	1.40 ± 0

To calculate weighted respiratory potential for the entire tree, the core-based measurements were first multiplied by the corresponding volume of tissue (inner bark, outer sapwood, or inner sapwood) for each height position on stems. The amount of potential CO_2 produced by each tissue radial position was then summed from each stem segment along the tree's height. That sum was divided by total live bole volume (inner bark plus sapwood) of the tree.

The weighted respiratory potential per unit volume for each species were plotted against the fraction of the tree that was live bole (inner bark plus sapwood divided by total bole). Data from the sapwood volume database were used to calculate the species-specific fraction of live bole, which was then regressed on stem diameter outside bark at breast height. An equation of the same form as Equation (3) was fit to the data for each of the four species, and then used to

estimate the fraction of live bole for the trees from which we took the respiratory measurements. Weighted respiratory potential per unit volume was also calculated for *Pseudotusga menziesii* (young) and *Tsuga heterophylla* (young, suppressed) using the breast height measurements of core respiratory potentials to scale to the whole-tree level. Respiratory measurements of these young trees were scaled to the whole-tree level using the equations of their older, species equivalents. The weighted respiratory potential per unit volume for mature *Pinus ponderosa* (220-years-old) was obtained from Pruyn et al. (2002 a). The weighted respiratory potential per unit volume for each species was then plotted against the maximum fraction of live bole for large diameter trees. For the young *Pseudotsuga menziesii* and *Tsuga heterophylla*, fraction of live bole was estimated from trees of 20 – 25 cm, stem diameter outside bark at breast height.

4.3.9. Statistical analysis

All data were analyzed in Statistical Analysis Systems software, release 7.0 (SAS Institute Inc. 1998). The Shapiro-Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or natural log) was performed when necessary to meet assumptions of normality and constant variance. Means were reported $\pm$ standard error (SE), except transformed means, where confidence intervals are used. Within a specific table or figure, if confidence intervals were required for one variable, they were presented for all.

To compare core respiratory potential among the three tissue radial positions of the ten tree species from the current study, least squares means (LSMEANS) were generated using PROC MIXED analysis, with randomized block design and strip-plot (split-block) treatments (Little and Hills 1978, Milliken and Johnson 1984). Trees were blocks and the effects of tissue radial position, species, and their interaction were tested for each sampling date separately. Pair-wise comparisons (*t*-tests) among tissue radial positions and species were conducted using Fisher's Protected Least Significant Difference (FPLSD) procedure (Fisher 1966).

Linear regression analysis from SigmaPlot software, release 6.0 (SPSS, Inc. 1986 – 2000), was used to describe the relationship between core respiratory potential and volumetric proportion of sapwood parenchyma cells. Non-linear regression analysis in Sigma Plot was used to describe the relationship between sapwood thickness and stem diameter outside bark at breast height. Linear Regression analysis in SAS-Assist was used to describe the relationships between (1) sapwood volume and stem diameter outside bark at breast height, (2) relative cumulative sapwood volume and relative stem height, and (3) fraction of the tree that is live wood and stem diameter outside bark at breast height.

A one-way ANOVA in PROC GLM was used to generate LSMEANS for total live wood volume (inner bark plus sapwood) and for the whole-tree CO_2 production of each species. Specific pair-wise comparisons among species means were conducted using FPLSD.

4.4. RESULTS

4.4.1. Comparison of core respiratory potential among species

At breast height the trends in core respiratory potential (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$) among tissue radial positions and among species were repeatable with respect to year and date sampled, with consistent, significant differences among species for all dates tested ($P < 0.0001$). On average for all species, inner bark respiratory potential was 2 – 11 times higher than that of outer sapwood, and outer sapwood respiratory potential was 1.3 – 2 times higher than inner sapwood (Table 4.4). There were no significant differences between the core respiration for old versus young (suppressed) trees of either *Pseudotsuga menziesii* or *Tsuga heterophylla.*

The effects of tissue radial position and the interaction between species and tissue were significant for year 2 of March and both years of September ($P < 0.03$). *Alnus rubra* and *Acer macrophyllum* had the highest inner bark respiratory potential of the ten species sampled (30 – 60% higher than the other species, Table 4.4). Inner bark respiratory potentials were also notably higher in *Pinus monticola*, *Populus trichocarpa* (September, year 2) and *Castanopsis chrysophylla* (September, year 2) than that of the other species. This pattern among species was not repeated in outer sapwood, where the respiratory potentials of *Thuja plicata* and *Taxus brevifola* were 20 – 80% higher than the other species. Older *Pseudotsuga menziesii* and the hardwoods, *Acer macrophyllum*, *Alnus rubra*, and *Castanopsis*

TABLE 4.4. Breast Height Respiratory Potential (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$ at 25°C; except March, year 1 at 15°C) of cores for the tree species sampled from HJA Experimental Forest. LSMEANS and 95% confidence intervals from Strip plot analysis in PROC MIXED (n = 4 trees; except March, year 1 n = 3). Significant differences among means from Fisher's Protected Least Squares Differences ($P < 0.05$). For all comparisons, each season (March or September) and each year (1 or 2) were analyzed separately. Different lowercase letters represent differences among tissue radial positions within each row. Different uppercase letters represent differences among species within each column.

		March	
Species	Inner bark	Outer Sapwood	Inner Sapwood
ABAM			
year 1	--	--	--
year 2	--	--	--
ACMA			
year 1	--	0.15 (0.10, 0.19)^{BC}	--
year 2	^{a}2.5 (1.9, 3.3)^{AB}	^{b}0.50 (0.38, 0.66)^{AC}	^{c}0.29 (0.22, 0.38)^{AB}
ALRU			
year 1	--	0.10 (0.05, 0.14)^{AB}	--
year 2	^{a}2.9 (2.2, 3.9)^{A}	^{b}0.49 (0.37, 0.65)^{AC}	^{b}0.41 (0.31, 0.54)^{AF}
CASH			
year 1	--	0.15 (0.11, 0.20)^{BC}	--
year 2	^{a}1.2 (0.9, 1.6)^{C}	^{b}0.52 (0.40, 0.69)^{AC}	^{c}0.27 (0.20, 0.36)^{B}
PIMO			
year 1	--	--	--
year 2	^{a}2.6 (2.0, 3.4)^{A}	^{b}0.24 (0.18, 0.31)^{B}	^{c}0.12 (0.09, 0.16)^{D}
POTR			
year 1	--	0.08 (0.04. 0.13)^{A}	--
year 2	--	--	--
PSMEo			
year 1	--	0.12 (0.07, 0.16)^{AB}	--
year 2	^{a}1.8 (1.3, 2.3)^{BC}	^{b}0.36 (0.28, 0.48)^{A}	^{c}0.18 (0.14, 0.24)^{E}
PSMEy			
year 1	--	0.12 (0.08, 0.17)^{AB}	--
year 2	^{a}1.8 (1.4, 2.4)^{BC}	^{b}0.24 (0.18, 0.32)^{B}	^{c}0.13 (0.10, 0.17)^{DE}
TABR			
year 1	--	0.21 (0.17, 0.26)^{CD}	--
year 2	^{a}1.8 (1.4, 2.4)^{BC}	^{b}0.46 (0.35, 0.61)^{AC}	--
THPL			
year 1	--	0.24 (0.19, 0.28)^{D}	--
year 2	^{a}1.6 (1.2, 2.1)^{C}	^{b}0.67 (0.51, 0.88)^{C}	^{b}0.47 (0.36, 0.62)^{F}
TSHEo			
year 1	--	0.07 (0.06, 0.11)^{A}	--
year 2	^{a}1.4 (1.1, 1.9)^{C}	^{b}0.24 (0.18, 0.31)^{B}	^{c}0.14 (0.10, 0.18)^{DE}
TSHEy			
year 1	--	--	--
year 2	^{a}1.3 (1.0, 1.5)^{C}	^{b}0.19 (0.14, 0.25)^{B}	^{c}0.12 (0.09, 0.15)^{D}

TABLE 4.4. (Continued).

Species	September Inner bark	Outer Sapwood	Inner Sapwood
ABAM			
year 1	^{a}1.1 (0.6, 2.1)^{AB}	^{b}0.25 (0.13, 0.46)^{ADE}	^{c}0.06 (0.03, 0.12)^{A}
year 2	^{a}1.5 (1.0, 2.4)^{A}	^{b}0.24 (0.15, 0.37)^{ABE}	^{b}0.14 (0.08, 0.23)^{A}
ACMA			
year 1	^{a}2.6 (1.4, 1.8)^{B}	^{b}0.30 (0.16, 0.56)^{ACE}	^{c}0.04 (0.02, 0.07)^{A}
year 2	^{a}3.0 (1.9, 4.7)^{B}	^{b}0.31 (0.20, 0.48)^{ABD}	^{c}0.10 (0.06, 0.15)^{AC}
ALRU			
year 1	^{a}2.4 (1.3, 4.5)^{B}	^{b}0.28 (0.15, 0.52)^{ACDE}	^{b}0.19 (0.10, 0.36)^{B}
year 2	^{a}3.1 (2.0, 4.9)^{B}	^{b}0.37 (0.24, 0.58)^{AC}	^{c}0.15 (0.10, 0.23)^{AB}
CASH			
year 1	^{a}1.4 (0.8, 2.6)^{AB}	^{b}0.39 (0.21, 0.72)^{AC}	^{b}0.22 (0.12, 0.40)^{B}
year 2	^{a}2.2 (1.4, 3.4)^{AB}	^{b}0.38 (0.24, 0.59)^{AC}	^{b}0.23 (0.14, 0.35)^{BD}
PIMO			
year 1	--	--	--
year 2	^{a}1.9 (1.2, 3.0)^{AB}	^{b}0.19 (0.12, 0.30)^{BE}	^{c}0.08 (0.05, 0.13)^{CE}
POTR			
year 1	^{a}0.3 (0.2, 0.5)^{C}	^{b}0.10 (0.06, 0.19)^{B}	^{b}0.10 (0.06, 0.19)^{A}
year 2	^{a}2.1 (1.3, 3.3)^{AB}	^{b}0.20 (0.13, 0.31)^{BE}	^{c}0.10 (0.06, 015)^{AC}
PSMEo			
year 1	^{a}1.9 (0.9, 3.9)^{AB}	^{b}0.41 (0.22, 0.75)^{AC}	^{b}0.21 (0.11, 0.38)^{B}
year 2	^{a}1.1 (0.7, 1.7)^{C}	^{b}0.36 (0.23, 0.56)^{A}	^{c}0.17 (0.11, 0.26)^{AB}
PSMEy			
year 1	--	--	--
year 2	^{a}1.3 (0.8, 2.0)^{AC}	^{b}0.22 (0.14, 0.34)^{ABE}	^{c}0.06 (0.04, 0.09)^{CE}
TABR			
year 1	^{a}1.4 (0.8, 2.6)^{AB}	^{a}0.66 (0.36, 1.2)^{C}	^{b}0.22 (0.12, 0.40)^{B}
year 2	^{a}1.4 (0.9, 2.1)^{AC}	^{b}0.56 (0.36, 0.87)^{C}	^{c}0.29 (0.19, 0.45)^{D}
THPL			
year 1	^{a}1.6 (0.9, 2.9)^{AB}	^{b}0.48 (0.26, 0.88)^{AC}	^{b}0.33 (0.18, 0.62)^{B}
year 2	^{a}1.5 (1.0, 2.4)^{AC}	^{b}0.52 (0.33, 0.81)^{CD}	^{b}0.29 (0.19, 0.45)^{D}
TSHEo			
year 1	^{a}0.8 (0.4, 1.5)^{A}	^{b}0.12 (0.07, 0.23)^{BD}	^{b}0.07 (0.04, 0.13)^{A}
year 2	^{a}1.1 (0.7, 1.7)^{C}	^{b}0.15 (0.09, 0.23)^{E}	^{c}0.05 (0.03, 0.08)^{E}
TSHEy			
year 1	^{a}1.2 (0.6, 2.1)^{AB}	^{b}0.13 (0.07, 0.24)^{BE}	^{b}0.10 (0.06, 0.19)^{A}
year 2	^{a}0.57 (0.4, 0.9)^{D}	^{b}0.14 (0.09, 0.21)^{E}	^{c}0.05 (0.03, 0.08)^{E}

chrysophylla also had notably higher outer sapwood respiratory potentials than in other species (Table 4.4).

There were no consistent, significant trends in respiratory potential among the three tree heights in any of the four species *Abies amabilis*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterphylla*. The effect of vertical position and the interaction of species, vertical position, and tissue were significant in year 1 ($P = 0.002$), but not year 2 ($P = 0.3$). However, the effect of the interaction between species and vertical position was significant for both years ($P < 0.02$). The most evident trend (although not significant for all four species) was of increasing respiratory potential from tree base to treetop in the outer sapwood (Table 4.5). At each vertical position, *Thuja plicata* outer sapwood respiratory potential was 20 – 50% higher than the other species with the exception of near treetop, year 2 (Table 4.5).

TABLE 4.5. September measurements of core respiratory potential (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$ at 25°C) at breast height, base of the live crown, and near treetop of four species from HJA Experimental Forest. LSMEANS and 95% confidence intervals from Strip plot analysis in PROC MIXED (n = 3 trees in year 1 and 4 trees in year 2). Significant differences among means from Fisher's Protected Least Squares Differences ($P < 0.05$). For all comparisons each year (1 or 2) was analyzed separately. Different lowercase letters represent differences among tissue radial positions within each row. Different uppercase letters represent differences among species and vertical positions within each column.

Species	Inner Bark	Outer Sapwood	Inner Sapwood
		Breast Height	
ABAM			
year 1	$^{a}1.5\ (1.1, 1.8)^{AB}$	$^{b}0.4\ (0.2, 0.7)^{AB}$	$^{b}0.2\ (0.1, 0.3)^{AC}$
year 2	$^{a}1.5\ (0.9, 2.5)^{AB}$	$^{b}0.2\ (0.1, 0.4)^{A}$	$^{b}0.1\ (0.1, 0.2)^{AE}$
PSME			
year 1	$^{a}1.9\ (1.4, 2.4)^{B}$	$^{b}0.4\ (0.3, 0.7)^{AB}$	$^{b}0.2\ (0.1, 0.4)^{AB}$
year 2	$^{a}1.1\ (0.7, 1.9)^{AB}$	$^{b}0.4\ (0.2, 0.6)^{AB}$	$^{c}0.2\ (0.1, 0.3)^{AB}$
THPL			
year 1	$^{a}1.7\ (1.3, 2.1)^{AB}$	$^{b}0.8\ (0.5, 1.1)^{BE}$	$^{b}0.4\ (0.2, 0.7)^{BD}$
year 2	$^{a}1.5\ (0.9, 2.5)^{AB}$	$^{b}0.5\ (0.3, 0.9)^{BC}$	$^{b}0.3\ (0.2, 0.5)^{BD}$
TSHE			
year 1	$^{a}1.2\ (0.9, 1.6)^{AB}$	$^{b}0.2\ (0.1, 0.4)^{A}$	$^{b}0.1\ (0.04, 0.3)^{A}$
year 2	$^{a}1.1\ (0.6, 1.8)^{AB}$	$^{b}0.15\ (0.1, 0.2)^{A}$	$^{c}0.05\ (0.03, 0.09)^{C}$
		Base of the Live Crown	
ABAM			
year 1	$^{a}1.5\ (1.1, 1.9)^{AB}$	$^{b}0.4\ (0.3, 0.7)^{AB}$	$^{c}0.1\ (0.05, 0.3)^{A}$
year 2	$^{a}1.9\ (1.1, 3.1)^{AB}$	$^{b}0.3\ (0.2, 0.5)^{AB}$	$^{b}0.2\ (0.1, 0.3)^{AB}$
PSME			
year 1	$^{a}1.8\ (1.4, 2.4)^{B}$	$^{b}0.6\ (0.4, 0.9)^{B}$	$^{b}0.3\ (0.2, 0.6)^{BC}$
year 2	$^{a}1.0\ (0.6, 1.6)^{AB}$	$^{b}0.4\ (0.3, 0.7)^{B}$	$^{b}0.3\ (0.2, 0.5)^{BD}$
THPL			
year 1	$^{a}1.9\ (0.8, 1.5)^{B}$	$^{b}1.1\ (0.8, 1.5)^{C}$	$^{c}0.6\ (0.4, 0.9)^{D}$
year 2	$^{a}1.3\ (0.7, 2.4)^{AB}$	$^{a}0.7\ (0.4, 1.3)^{BC}$	$^{b}0.2\ (0.1, 0.4)^{AB}$
TSHE			
year 1	$^{a}0.6\ (0.4, 1.0)^{C}$	$^{b}0.2\ (0.1, 0.4)^{A}$	$^{b}0.1\ (0.04, 0.3)^{A}$
year 2	$^{a}0.9\ (0.5, 1.5)^{B}$	$^{b}0.2\ (0.1, 0.3)^{A}$	$^{c}0.1\ (0.05, 0.13)^{AE}$
		Near Treetop	
ABAM			
year 1	$^{a}1.8\ (1.4, 2.3)^{B}$	$^{b}0.6\ (0.4, 0.8)^{B}$	$^{c}0.2\ (0.1, 0.4)^{AB}$
year 2	$^{a}1.7\ (1.0, 2.8)^{AB}$	$^{b}0.5\ (0.3, 0.8)^{BC}$	$^{c}0.1\ (0.08, 0.2)^{AE}$
PSME			
year 1	$^{a}1.0\ (0.7, 1.4)^{A}$	$^{a}0.9\ (0.7, 1.3)^{CE}$	$^{b}0.4\ (0.2, 0.7)^{BD}$
year 2	$^{a}1.4\ (0.8, 2.3)^{AB}$	$^{ab}1.0\ (0.6, 1.7)^{C}$	$^{b}0.5\ (0.3, 0.9)^{D}$
THPL			
year 1	$^{a}2.5\ (2.1, 3.1)^{D}$	$^{a}2.1\ (1.6, 2.5)^{D}$	$^{b}0.5\ (0.3, 0.7)^{BD}$
year 2	$^{a}1.4\ (0.8, 2.3)^{AB}$	$^{b}0.3\ (0.2, 0.6)^{AB}$	$^{b}0.3\ (0.2, 0.4)^{BE}$
TSHE			
year 1	$^{a}1.2\ (0.9, 1.5)^{A}$	$^{b}0.4\ (0.2, 0.6)^{A}$	$^{b}0.2\ (0.1, 0.3)^{AC}$
year 2	$^{a}1.0\ (0.6, 1.7)^{AB}$	$^{b}0.4\ (0.2, 0.6)^{AB}$	$^{c}0.2\ (0.1, 0.3)^{AB}$

The relationship between core respiratory potential and proportion of parenchyma cells in the sapwood (literature values) was slightly negative for all three tissue radial positions (inner bark, outer and inner sapwood) in the softwoods, and either slightly positive (inner bark) or negligible (sapwood) in the hardwoods (Figure 4.1).

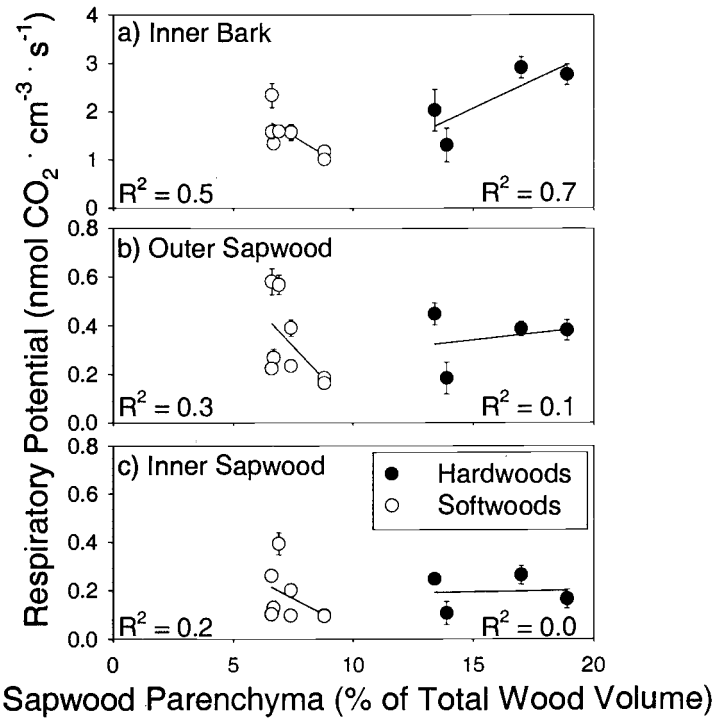

FIGURE 4.1. The relationship between core respiratory potential and proportion of parenchyma cells (literature values). Respiratory means from three sampling dates (March 1999, September 2001, 2002) ± SE.

4.4.2. Estimating sapwood and inner bark volumes on the whole-tree level

4.4.2.1. Sapwood and Inner Bark Thickness

Many of the Equation (1) parameters were not significant and did not describe the relationship between sapwood thickness (S_T) and diameter outside bark at breast height (D_B) for *Pseudotusga menziesii*, *Thuja plicata*, and *Tsuga heterophylla* ($P > 0.05$, data not shown). In contrast, for Equation (2) the parameters were all significant (Table 4.5) and the adjusted R^2 values were higher (i.e. *Pseudotsuga menziesii* Equation (1) 0.72, Equation (2) 0.76; *Thuja plicata* (1) 0.26, (2) 0.34; *Tsuga heterophylla* (1) 0.53, (2) 0.62). There were no statistical analyses for *Abies amabilis* or for the inner bark thickness of all four species because its equation parameters (Table 4.6) were derived and assigned as described in the methods section. Sapwood thickness increased with stem diameter outside bark at breast height until a certain stem diameter was reached, after which point sapwood thickness remained constant (Figure 4.2).

4.4.2.2. Sapwood and Inner Bark Volume

When sapwood volume (S_V) was regressed on stem diameter outside bark at breast height (D_B) for each of the four species, a funnel-shaped spread in the residual plot suggested the need for transformation. After applying a series of different transformations, we determined that the natural log of both axes was the best solution. However, the trend of non-constant variance in the residual plot

TABLE 4.6. Equation parameters of four tree species for predicting thickness, volume, and relative cumulative volume of sapwood and inner bark. Parameter estimates ± SE, significant at $P < 0.05$. Abbreviations: sapwood thickness (S_T), inner bark thickness (B_T), sapwood volume (S_V), inner bark volume (B_v), stem diameter outside bark at breast height (D_B), relative cumulative sapwood volume (${}^fS_v$), relative cumulative inner bark volume (${}^fB_v$), and relative height (${}^fH$).

	Sapwood Thickness versus Breast Height Diameter $S_T = \beta_0 \cdot (1 - e^{\beta 1 \cdot D_B})$		
	Adjusted R^2	β_0	β_1
Sapwood			
ABAM	--	5.9 ± 0	-0.05 ± 0.01
PSME	0.76	4.8 ± 0.1	-0.07 ± 0.01
THPL	0.34	2.2 ± 0.2	-0.05 ± 0.05
TSHE	0.62	7.8 ± 0.5	-0.05 ± 0.01
	Inner Bark Thickness versus Breast Height Diameter $B_T = \beta_0 \cdot (1 - e^{\beta 1 \cdot D_B})$		
	Adjusted R^2	β_0	β_1
Inner Bark			
ABAM	--	0.33 ± 0	-0.05 ± 0.01
PSME	--	0.60 ± 0	-0.07 ± 0.01
THPL	--	0.33 ± 0	-0.05 ± 0.05
TSHE	--	0.35 ± 0	-0.05 ± 0.01

	Sapwood Volume versus Breast Height Diameter $LnS_V = \beta_0 + (\beta_1 \cdot lnD_B) + (\beta_2 \cdot lnD_B^2)$			
	Adjusted R^2	β_0	β_1	β_2
Sapwood				
ABAM	0.97	-15.2 ± 0.7	6.0 ± 0.4	-0.52 ± 0.05
PSME	0.95	-9.5 ± 0.7	3.1 ± 0.3	-0.17 ± 0.04
THPL	0.97	-13.7 ± 0.8	4.8 ± 0.4	-0.38 ± 0.06
TSHE	0.96	-15.2 ± 0.6	6.0 ± 0.3	-0.51 ± 0.04
	Inner Bark Volume versus Breast Height Diameter $LnB_V = \beta_0 + (\beta_1 \cdot lnD_B) + (\beta_2 \cdot lnD_B^2)$			
	Adjusted R^2	β_0	β_1	β_2
Inner Bark				
ABAM	0.97	-17.2 ± 0.7	5.7 ± 0.4	-0.50 ± 0.05
PSME	0.95	-11.4 ± 0.7	2.8 ± 0.3	-0.15 ± 0.04
THPL	0.98	-16.4 ± 0.8	4.9 ± 0.4	-0.40 ± 0.06
TSHE	0.96	-17.7 ± 0.6	5.5 ± 0.3	-0.47 ± 0.04

	Relative Cumulative Sapwood Volume versus Relative Height $S_{VC} = \beta_0 + (\beta_1 \cdot H_C) + (\beta_2 \cdot H_C^2)$			
All Species	Adjusted R^2	β_0	β_1	β_2
Sapwood	0.99	0	2.02 ± 0.02	-1.02 ± 0.02
	Relative Cumulative Inner Bark Volume versus Relative Height $B_{VC} = \beta_0 + (\beta_1 \cdot H_C) + (\beta_2 \cdot H_C^2)$			
	Adjusted R^2	β_0	β_1	β_2
Inner Bark	0.99	0.004 ± 0.002	1.75 ± 0.01	-0.73 ± 0.02

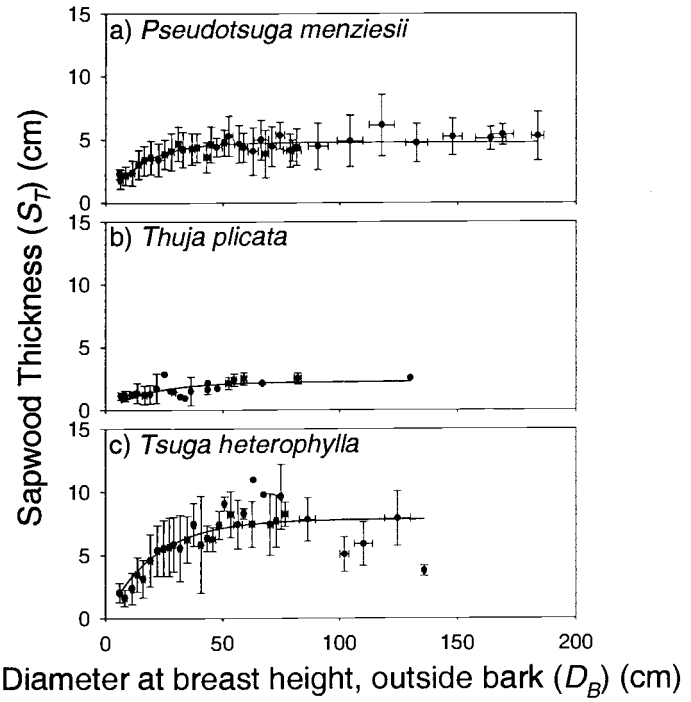

FIGURE 4.2. The relationship of sapwood thickness (cm) to stem diameter outside bark at breast height (cm) for three tree species from the Pacific Northwest. Equation parameters with standard errors and their significance listed in Table 4.5.

suggested that there was curvature in the data. A squared term (lnD_B^2) was thus added to the equation, which was significant and restored constant variance to the residual plots (Equation 3, Table 4.6).

Sapwood volume (S_V) calculated using stem diameter from breast height (assumption 1 of S_T along stem heights) was significantly higher than S_V calculated using diameter outside bark at the point of estimate (assumption 2) ($P < 0.0001$, data not shown). However, when S_V from assumption 1 was plotted against S_V from assumption 2, the slope approximated 1.0 (range: 0.98 to 1.01, among the four species) and the y-intercept, 0 (range: -0.004 to -0.028), indicating that the bias between the two assumptions was slight and likely not biologically important. Similar results were found for inner bark volume. Thus, sapwood thickness and inner bark thickness at D_B were assumed to be constant along stem heights (assumption 1) when calculating volume estimates for the four species. There was a curvilinear relationship between D_B and sapwood volume (S_v) (Figure 4.3), evidenced by the significant squared-term in the equation.

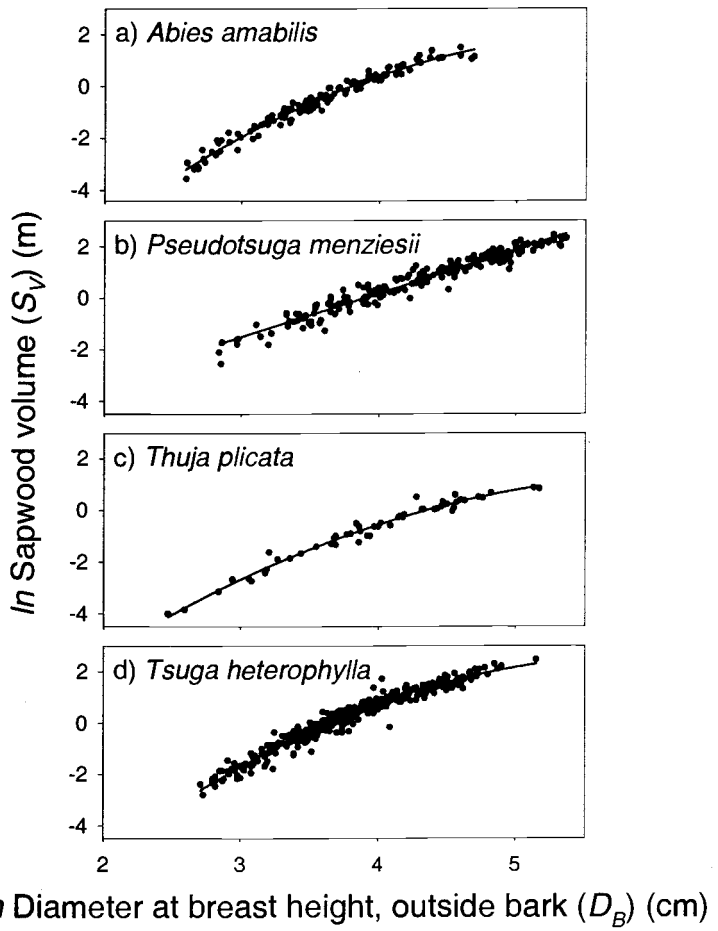

FIGURE 4.3. The relationship of sapwood volume (m^3) to diameter outside bark at breast height (cm) of four species from the Pacific Northwest. Equation parameters with standard errors and their significance listed in Table 4.5.

4.4.2.3. Cumulative relative sapwood and inner bark volume and relative height

For the regression of relative cumulative sapwood volume (S_{VC}) on relative stem height (H_C), the values at 0 and 100% relative height were defined because of the nature of the function. The relationship between S_{VC} and H_C was suspected to be similar for all four species. To test this hypothesis, species and the interaction of species by H_C was included as an effect in the model (Equation 4). The squared term (H_C^2) was also added to correct the curvature that was evident in the residual plot. Neither the effect of species, nor the interaction term, was significant ($P > 0.2$). The interaction term was dropped first, which resulted in species becoming significant ($P = 0.01$). *Pseudotsuga menziesii* was the only species with a significant parameter estimate (equal to -0.008, $P < 0.01$). Therefore, the effect of species was dropped. At this point, the y-intercept (β_0) was no longer significant ($P > 0.1$) and was dropped, and H_C and H_C^2 were the main effects (Table 4.6). The results from the regression of relative cumulative bark volume (B_{VC}) on H_C were similar to those from S_{VC} versus H_C. The exception was that *Thuja plicata* instead of *Pseudotsuga menziesii* was the only species with a significant parameter estimate (equal to 0.016, $P < 0.0001$). The effect of species was also dropped for inner bark, leaving H_C and H_C^2 as the main effects (Table 4.6).

The convex shape of the curve of relative cumulative sapwood volume versus relative stem height suggested that most of the sapwood volume was within the lower portions of the stem (Figure 4.4). As the curve tapered off at higher stem heights, less sapwood volume accumulated with each height increment. Sapwood

volume accumulated at a higher rate at stem bases because volume increases as the square with stem radius.

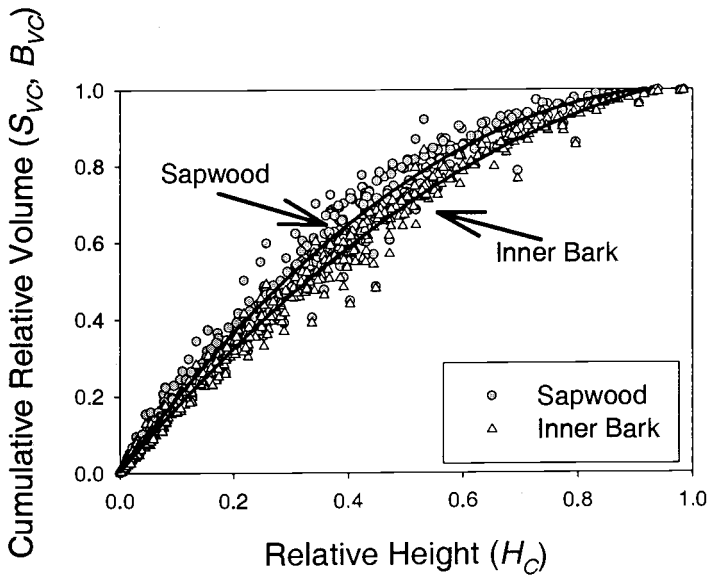

FIGURE 4.4. The relationship between relative cumulative inner bark and sapwood volume and relative height for four Pacific Northwest species, *Abies amabilis*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*. Equation parameters with standard errors and their significance listed in Table 3.5.

4.4.3. Scaling respiratory potential to the whole-tree level

Upon scaling to the whole-tree level, inner bark potential CO_2 production ($\mu moles \cdot s^{-1}$) was 15 – 50% of the whole-tree potential CO_2 production for all four species (Figure 4.5). There was a positive relationship between whole-tree potential CO_2 production and its live bole volume (Figure 4.5).

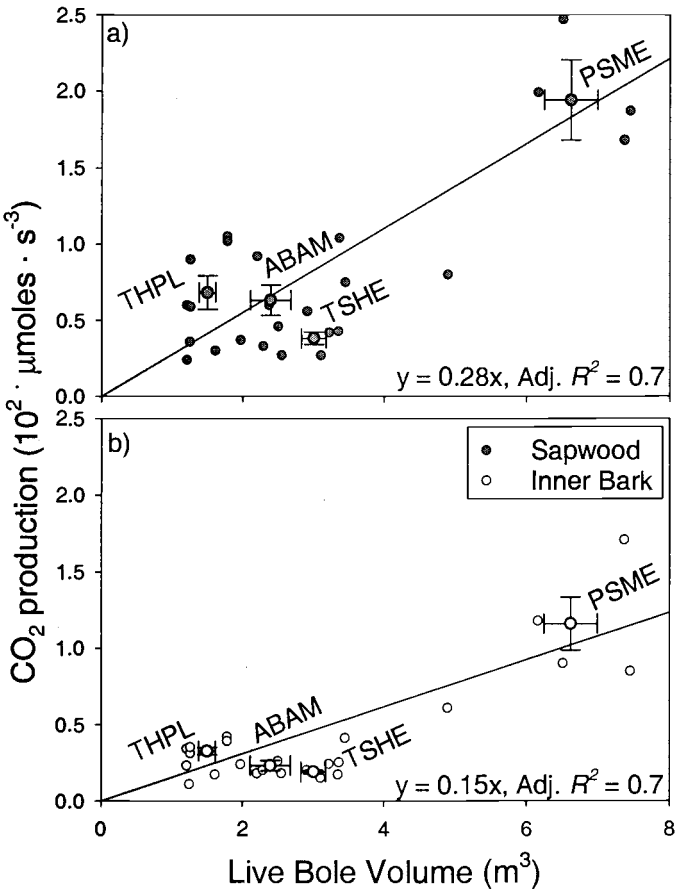

Figure 4.5. Whole-bole CO_2 production of sapwood (a) and inner bark (b) versus live bole volumes (inner bark plus sapwood) for four tree species from HJA Experimental Forest. Regression equations were fit to the scatter plots, separately for inner bark and sapwood. Means $\pm$ SE for each species show where they each were located with respect to one another and the regression line.

Tree species with larger live bole volumes (inner bark plus sapwood) did not necessarily have high fractions of live bole (inner bark plus sapwood divided by total bole). For example, *Pseudotsuga menziesii* had the highest live bole volume of the four species measured, but the fraction of live bole was lower than both *Abies amabilis* and *Tsuga heterophylla*. A log/log equation described the

relationship between fraction of live bole and stem diameter outside bark at breast height (Table 4.7).

Table 4.7. Equation parameters for the relationship between fraction of live bole and stem diameter outside bark at breast height for five tree species. Parameter estimates ± SE, significant at $P < 0.05$. Abbreviations: relative live bole volume ($^{f}L_V$) and stem diameter outside bark at breast height (D_B)

	Fraction of Live Bole versus Breast Height Diameter $Ln\ {}^{f}L_V = \beta_0 + (\beta_1 \cdot lnD_B) + (\beta_2 \cdot lnD_B^2)$			
Species	Adjusted R^2	β_0	β_1	β_2
ABAM	0.95	-1.0 ± 0.2	0.6 ± 0.1	-0.2 ± 0.01
PIPO	0.78	-2.2 ± 0.5	1.0 ± 0.3	-0.1 ± 0.03
PSME	0.97	0.3 ± 0.2	0.1 ± 0.1	-0.1 ± 0.01
THPL	0.97	-1.6 ± 0.3	0.6 ± 0.1	-0.2 ± 0.01
TSHE	0.93	-1.4 ± 0.1	0.9 ± 0.1	-0.2 ± 0.01

Trees with higher fractions of live bole (*Abies amabilis*, *Tsuga heterophylla*) had lower whole-tree respiration rates per unit volume than trees with lower fractions of live bole (Figure 4.6 a). Estimates of whole tree respiratory potential were not affected by the method used to segment the trees vertically, as the standard errors closely overlapped among the means derived from each method (described in Table 4.2). Also, young *Pseudotsuga menziesii* and young (suppressed) *Tsuga heterophylla* with fractions of live wood that were 3 – 4 times higher than older trees of the same species, had respiratory potentials that were 30% lower than the older trees (Figure 4.6 b).

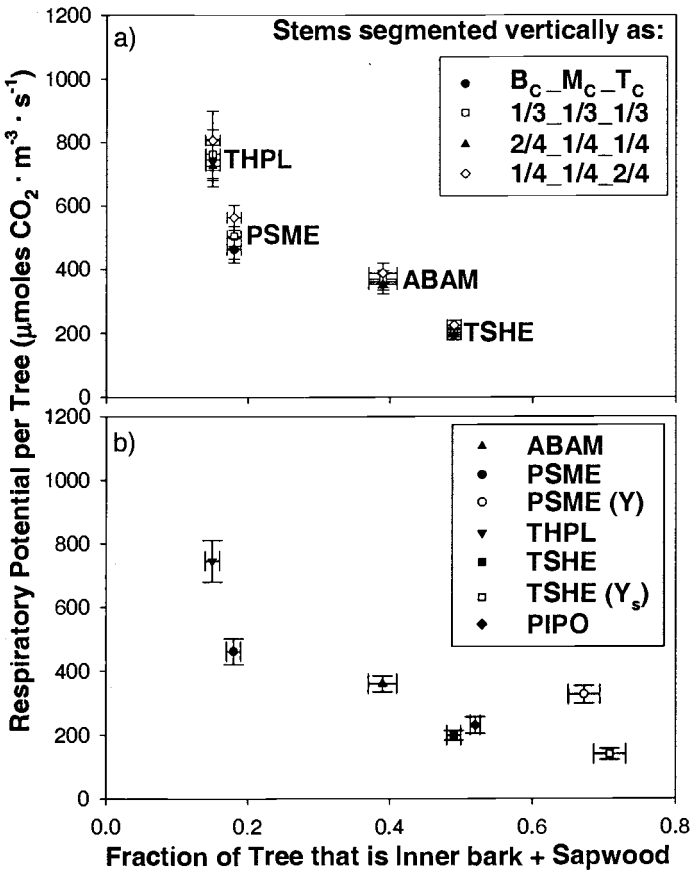

Figure 4.6. Relationship of respiratory potential on the whole-tree level to percentage of the tree that is live wood volume (inner bark plus sapwood) for five tree species from HJA Experimental Forest. Panel (a) shows species means ± SE using four different methods of scaling to the whole tree level (Table 4.2). Panel (b) shows species means ± SE using the first method of scaling from panel (a) and assuming live wood percentages of large-sized species for the older trees and of small-sized species for the younger trees. Abbreviations: B_C = Below Crown, M_C = Mid Crown, T_C = Top Crown, Y = Young, Y_S = Young

4.5. DISCUSSION

4.5.1. Core-based respiratory potential

The trends of increasing respiratory potential from pith to bark that were identified within the ten different tree species from H.J. Andrews Experimental Forest were similar to those in ponderosa pine and Douglas-fir (Pruyn et al. 2002 a, Pruyn et al. 2002 b). Potentially, the increased respiratory activity of inner bark and outer sapwood as compared to the inner sapwood positions was related to their role in supporting growth and secondary wall formation in the cambial zone (Goodwin and Goddard 1940), and/or other physiological activities associated with xylem maintenance (Lev-Yadun and Aloni 1995). Enhanced activity in the outer growth rings may also be linked to carbohydrates levels within parenchyma cells. Studies have shown increased amounts of soluble carbohydrates and starch in the outer sapwood and inner bark as compared to the inner sapwood and heartwood of *Pinus sylvestris* (Saranpää and Höll 1989). The reduced activity of middle and inner sapwood may be explained by age-related changes (Frey-Wyssling and Bossard 1959, Yang 1993, Gartner et al. 2000), dormancy of metabolic activity in sapwood parenchyma cells (Shain and Mackay 1973), and/or the onset of heartwood formation (Bamber 1976).

The basis on which respiration was calculated (e.g. core mass versus volume) was important because for two different samples of respiring tissue of the same volume, there may be differences in the constituent amount of living cells

(parenchyma), depending on species or position within the stem. For example, the volumetric proportion of the stem that is ray parenchyma ranges from 6 – 30% among hardwood species, whereas it ranges from 5 – 9% among softwoods (Panshin and DeZeeuw 1980). Therefore, expressing respiration on a different basis (e.g. tissue nitrogen content or parenchyma ray volume) could change the ranking among tissues being compared. Goodwin and Goddard (1940) found little difference in tissue level respiration rates on a mass basis between two hardwood species, *Acer rubrum* and *Fraxinus nigra*, yet detected more notable differences when respiration rates were calculated on a nitrogen basis (related to the amount of protein in living cells).

The lack of a strong relationship between inner bark or sapwood respiratory potential and the volumetric proportion of sapwood parenchyma of both softwoods and hardwoods suggested that the amount of sapwood parenchyma cells does not determine the metabolic potential of the sapwood. It is possible the respiratory potential of the tissue is instead limited by substrate type (sugars, starches, lipids) and/or concentration within the parenchyma cells. There may also be an independent mechanism that controls parenchyma cell activity, such as the gaseous environment of the tissue (Amthor 1991, Gansert 2001) or other metabolic constraints. The slightly negative relationship between softwood tissue respiratory potential and sapwood parenchyma cell amounts is interesting because it suggests that when the volumetric proportion of parenchyma is lower, the cells respire more to meet the metabolic demands of the sapwood or perhaps the whole-tree. In

contrast, the positive relationship in the hardwoods' inner bark potentially suggested that their inner bark metabolism was determined by the amount of parenchyma cells available in the sapwood, possibly for transport and storage of photosynthetic products.

The possibility also exists that the volumetric proportions of sapwood parenchyma cells from the literature were not representative of the percentage of living parenchyma in the core samples. The use of vital staining techniques revealed that live cell volume (%) declined by over 50% in the sapwood at a position 10 mm from the cambium relative to a position immediately proximal to the cambium in *Picea abies* (L.) Karst (Stockfors and Linder 1998) and *Pseudotsuga menziesii* (Gartner et al. 2000). Further, a direct, positive relationship between maintenance respiration and live-cell volume (%) was identified in *Pinus contorta* (var. *latifolia* Engelm., Ryan 1990). Thus, precise values of the proportion of living parenchyma in the sapwood of the species from this study are needed to absolutely define the relationship between core respiratory potential and parenchyma amounts.

4.5.2. Trends between stem diameter and sapwood/inner bark amounts

The stem diameter at which maximum sapwood thickness occurred differed among *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heterophylla*. The trend indicated that sapwood thickness increased rapidly with stem diameter when the stem diameters were smaller (0 – 25 cm) and increased more slowly at larger stem

diameters. This trend is similar to a previous study on six western coniferous species, including Inland and Coastal *Pseudotsuga menziesii* and *Thuja plicata*, with sapwood thickness generally increasing most rapidly during the first 15 – 20 years of growth (Lassen and Okkonen 1969). The relationship between sapwood volume and stem diameter outside bark at breast height was closely related to the trends in sapwood thickness. A log/log relationship between sapwood volume and stem diameter was also recorded for two different *Pinus* species in contrasting environments (Gower et al. 1993). However, no curvature was detected in the relationship, likely because the stem diameters ranged from 19 – 24 cm. The curvature in the log/log relationship in the current study indicated that sapwood volume increased at a slower rate with larger stem diameters. This curvature is similar to the theoretical result for a hollow, right-circular cone (sapwood minus heartwood) with sapwood reaching a maximum thickness and tapering along the stem.

4.5.3. Inter-species comparisons on the whole-tree level

The trend of lower potential whole tree respiration per unit volume in young trees versus old trees in the current study contrasted from the results of a previous study, where young *Pinus ponderosa* trees (30-years-old) respired at rates that were three times higher than older trees (220-years-old, Pruyn et al. 2002 a). However, in the previous study there was no difference in the fraction of live bole between the young and old pines (0.80 for both age classes), whereas in the current study the

fraction of live bole the younger trees was considerably higher than the older trees of the same species. Another possible explanation was that the young *Tsuga heterophylla* in the current study were suppressed and produced less photosynthetic product, resulting in lower respiration rates of the live bole. Therefore, the preliminary conclusion was that the inverse relationship between whole-tree respiratory potential and relative live bole volume was only true for species within the mature/old-growth age class and not always true for younger individuals on the same site.

The inverse relationship between whole-tree respiratory potential and fraction of live bole volume (inner bark plus sapwood divided by total stem wood) indicated that there may be a compensation for maintaining large amounts of sapwood. In this way, trees of contrasting sapwood amounts maintained a constant respiration load by having either high relative volumes of live bole with low per unit volume respiration rates, or visa versa. Trees maintaining a constant respiration load from woody tissues may be advantageous to their survival. If respiration increased indefinitely with sapwood amount, then after photosynthesis stops with the onset of the dormant season, trees may risk depleting sapwood photosynthetic reserves (via respiration) and thus limit the reserves available for wound repair, or growth during the early stages of the following growing season.

A second possible implication of this inverse relationship is that there may be constancy in the respiratory demand among species of the same size/age class. The way in which this respiratory demand is met by different species may

ultimately depend on the volume of live wood available. Thus, species with relatively large percentages of live bole volume may respire at much lower rates as compared to species with smaller percentages because there is more respiring wood available to meet the demand. Further research is necessary to ascertain whether this inverse relationship between whole-tree respiratory potential and relative live bole volume is robust among other species, age classes, and sites. It would also be interesting to determine why some young tree species (*Pinus ponderosa)* respire at much higher rates than older trees of the same species, whereas others do not follow this pattern (*Pseudotsuga menziesii*), especially in light of the fact that these two species maintain contrasting amounts of sapwood.

4.6. ACKNOWLEDGEMENTS

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5. SEASONAL RESPONSE OF STEM RESPIRATION TO TEMPERATURE FLUX IN TWO CONIFEROUS SPECIES OF THE PACIFIC NORTHWEST

5.1. SUMMARY

Stem respiration in two conifers of contrasting sapwood thickness, *Pseudotsuga menziesii* (Douglas-fir) and *Pinus ponderosa* (ponderosa pine), was estimated from the CO_2 production of increment cores measured under laboratory conditions. Using species-specific Q_{10}s calculated from respiratory response to temperature, monthly measurements of core-based respiration under laboratory conditions were extrapolated to predict seasonal trends for each species. When these trends were compared to respiration predicted strictly from sapwood temperature, a temperature-independent, seasonal fluctuation in respiration was identified. This temperature-independent flux in respiration differed between Douglas-fir and ponderosa pine. The differences between the two species were discussed in terms of their implications for the amount of sapwood maintained by trees and the resulting effects on wood quality.

5.2. INTRODUCTION

Sapwood and heartwood are characteristically very different in terms of cellular and chemical composition, density, moisture content, and decay resistance (Panshin and DeZeeuw 1980). These differences have significant implications for the processing, utilization, and economic value of sapwood versus heartwood in the forest products industry. For example, the highest valued lumber in ponderosa pine

comes from the outer shells (sapwood) of old growth logs, often used for cabinets and finish work, whereas the interior core (heartwood) of old growth and large logs have the lowest value and are often used for strength in construction (Ayer-Sachet and Fahey 1988). In general heartwood is more durable than sapwood because the extractives resist pathogen attack, but is harder to treat via impregnation with preservatives because of aspirated or encrusted bordered pits that block full penetration of the chemical into the tissue (Megraw 1986). Additionally, including heartwood in certain products can be problematic because its extractives may interfere with bleaching in the pulping process, setting of paints and varnishes, adhesion of surface films, or setting of strong glue joints (Panshin and DeZeeuw 1980, Tsoumis 1991). Better knowledge of sapwood function and the physiological mechanisms determining sapwood/heartwood quantity will help forest managers develop silvicultural regimes that favor the production of the amount and type of wood desired for the end product.

Despite research, the absolute determinants of sapwood quantity and composition remain obscure. Sapwood width as a linear measurement or in terms of the number of annual growth ring increments varies widely among species (Panshin and DeZeeuw 1980). Ponderosa pine maintains as many as 150 – 200 growth rings in its sapwood, whereas Douglas-fir maintains less than 100. Tree vigor has been identified as a control of sapwood width, where dominant trees within a species have wider sapwood (Panshin and DeZeeuw 1980). Relationships have been developed between leaf area and sapwood area via the pipe model theory

(Waring et al. 1982), implying that a certain amount of sapwood is needed to adequately supply the leaves with water, or provide sufficient space for storage of photosynthetic products. Rudman (1966) proposed that sapwood amounts in trees was a matter of metabolic efficiency, stating that when photosynthetic reserves are in excess of the tree's metabolic requirements, they are converted into heartwood extractives. Other research has suggested that biomechanical constraints may determine biomass allocation. For *Pinus sylvestris* L., which maintains a large number of sapwood rings, it was determined that most of the mechanical support required by the tree was provided by the sapwood (Mencuccini et al. 1997). They concluded that the tree possibly maintains as much sapwood as it needs to be bio-mechanically stable (in addition to providing storage space and hydraulic supply).

Given that biomass allocation to sapwood might be determined by the metabolic demands of the whole tree, the current study tested the hypothesis that differences in the respiration physiology of the sapwood in two species of contrasting sapwood thickness would potentially reveal design strategies for sapwood amounts in trees. Thus, the primary objective was to compare the seasonal trends in stem respiration of two species of contrasting sapwood thickness, *Pseudotsuga menziesii* (Douglas-fir) and *Pinus ponderosa* (ponderosa pine). Using an increment core-based, laboratory method to measure respiration of inner bark and outer, middle, and inner sapwood, the tissue-level respiratory response to temperature was modeled for both species. To enable comparison between the two species, temperature-response models were used to scale tissue-level, monthly

measurements of respiration in the laboratory to the organ-level (a volume of live stem wood) *in vivo*. To determine whether there were seasonal fluctuations in respiration that were independent of temperature, monthly trends in respiration extrapolated from laboratory measurements were compared to those extrapolated strictly from sapwood temperature. The identified, species-specific differences in seasonal respiratory trends were explored in terms of their potential implications for metabolic strategy and the resulting effects on sapwood amount.

5.3. MATERIALS AND METHODS

5.3.1. Site and species characteristics

The Douglas-fir trees, *Pseudotsuga menziesii* (Mirb.), were located on a site in McDonald-Dunn Research Forest in the Williamette Valley, near Corvallis, Oregon (N44° 38' W123° 17', elevation 305 m) and just east of the Coast Range in southern Oregon, near Riddle (N42° 57' W123° 22', elevation 215 m). The ponderosa pine trees, *Pinus ponderosa*, L., were located just east of the Cascade Range in central Oregon, near Gilchrist (N43° 28' W121° 41') at elevation 1355 m. All trees sampled were free of broken tops, stem deformities, or visible disease. Twenty to thirty trees were selected randomly from each site, sub-samples from which were used in the following experiments.

5.3.2. Sample dates, sample size, and individual tree characteristics

For the temperature response measurements, samples were collected from five trees at each of the three sites in early September 1999, March 2000, or January 2002. The rationale behind selecting these sampling dates was to capture maintenance respiration, and thus avoid the complications of growth respiration in estimating core respiration (McCree 1970, Thornley 1970). For the monthly respiration measurements, samples were collected from eight trees at each the Riddle and Gilchrist sites once a month from May 1998 through November 1999 for Douglas-fir, and from March 1999 through May 2000 for ponderosa pine. For all trees, diameter outside bark was measured at 1 m from the ground, and tree age and sapwood thickness were determined from two 5 mm increment cores extracted from breast height of each tree (Table 5.1).

TABLE 5.1. Ranges in stem diameter at 1 m from the ground, tree age and sapwood thickness for *Pseudotsuga menziesii* and *Pinus ponderosa* at the three different study sites in Oregon. Ranges: n = 10 trees (Corvallis), or n = 8 trees in (Riddle and Gilchrist)

Species (site)	Diameter at breast height (cm)	Tree Age (years)	Sapwood Thickness (cm)
Douglas-fir (Corvallis)	40 – 57	60 – 112	2 – 10
Douglas-fir (Riddle)	55 – 70	110 – 112	5 – 7
Ponderosa pine (Gilchrist)	53 – 67	250 – 400	10 – 20

5.3.3. Temperature monitoring at sites

Daily minimum and maximum temperature measurements of the sapwood for Douglas-fir (Riddle) and ponderosa pine (Gilchrist) were obtained from copper-constantin probes inserted into drilled holes (0.3 cm drill bit) to a 2.5 cm depth into the sapwood of five trees (of comparable characteristics to study trees) at each site. After inserting the probes, the hole was sealed off with silicone. A Campbell 21X data logger recorded the temperature every 15 seconds from the probes and stored an average every 5 minutes for 24 hours on each of the sampling dates. For the October and December (1999) dates at the Gilchrist site, the minimum and maximum sapwood temperatures were only from 7 am – 11 am because of malfunctioning of the data logger. Missing temperature or respiration measurements from the monthly respiration data were due to failure of the data logger, or from inability to obtain cores after increment borers broke.

5.3.4. Respiration measurements

Respiratory potential was estimated from 12 mm increment cores extracted from stems at breast height at 1m from the ground. When extracting multiple cores from an individual stem, they were taken evenly from about the stem's circumference. All cores were analyzed within one week of their extraction. Cores were stored at 4°C until twenty-four hours prior to measurement, when they were cut into four segments: inner bark (phloem and cambium) and outer, middle, and inner sapwood. Sapwood was defined as the woody tissue extending from the first

growth ring interior to the inner bark to the last growth ring interior to the transition zone (one or two lighter colored rings at the sapwood/heartwood boundary). Outer, middle, and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. The exception was in cores extracted from ponderosa pine trees, where sapwood width was greater than 100 growth rings. In this case, core segments of 10 – 15 growth rings in length were taken from the outer and inner sapwood boundaries and from the center of the sapwood. Core segments were weighed, wrapped tightly in plastic, then stored at 25°C overnight to allow metabolic activity in core segments to stabilize (Goodwin & Goddard 1940, Levy et al. 1999).

Immediately prior to measurement, core segments were re-weighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, carbon dioxide concentration within vials was measured with a Hewlett-Packard (5700A) gas chromatograph (GC, Hewlett Packard, Rt. 41, Avondale PA 19311, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25°C (unless indicated otherwise) between GC measurements. Incubation period was held constant at 6 hours. Details of GC analysis and calculation of respiratory potential (nmoles $CO_2 \cdot m^{-3} \cdot s^{-1}$) are in Pruyn et al. (2002 a) with the exception that in the current study core volume was used as a basis for respiration rather than dry mass. Immediately following the GC analysis, core segments were weighed a third time. The three successive wet masses verified that water loss was low (1-3%) between sampling

and the end of the measurement period. Fresh volume of core segments was estimated as displaced water by submerged samples (D2395, ASTM 2001). From this point onward, the reported values were referred to as respiratory potential, rather than respiration rate, because measurement conditions of these excised samples were probably different from those within the tree.

5.3.5. Effects of temperature on respiratory potential

To determine the response of core segment respiratory potential to temperature in Douglas-fir trees to a broad range of temperatures, four cores were extracted in March of 2000 and 6 cores in January of 2002 from breast height from each of five trees (different set of five trees for each date) at the Corvallis site. For each tree, each core was assigned to one of four temperatures (5, 10, 15, 25°C, March) or one of six temperatures (4, 10, 20, 28, 40, 55°C, January). Temperature response experiments were also conducted on the Douglas-fir trees at the Riddle site and ponderosa pine trees at Gilchrist to enable the prediction of their respiratory potentials at specific temperatures. Four cores were extracted from each of five trees from each site in September (1999) and March (2000). For each tree, each core was assigned to one of four temperatures (5, 10, 15, 25°C, March and 5, 15, 25, 35°C, September). For the sapwood measurements of Douglas-fir trees from both sites, only outer and inner sapwood was sampled to capture the extremes of activity. Core response to temperature was measured and modeled according to Pruyn et al. (2002 a). Also, the Q_{10}s (coefficient for changes in respiration with

respect to temperature) for the 5 – 15°C, 15 – 25°C, and 25 – 35°C temperature ranges were calculated for the Riddle and Gilchrist trees as described in Pruyn et al. (2002 a).

5.3.6. Modeling *in vivo* respiratory potential from *in vitro* measurements

Two different models were used adjusted laboratory measurements of core segment respiratory potential to field temperatures of sapwood (*in vivo*). First, *in vivo* respiratory potentials for Douglas-fir (Riddle) and ponderosa pine (Gilchrist) trees for each sampling date were estimated by regressing stem respiration rates against sapwood temperatures via the following model (Lavigne 1987):

$$R_f = \beta_0 \cdot \beta_1^{[(T_1 - T_0)/10]}, \quad (1)$$

Where R_f = respiratory potential at modeled temperature (T_1) (μmoles $CO_2 \cdot m^{-3} \cdot s^{-1}$), β_0 = stem respiratory potential at initial temperature (T_0), and $\beta_1 = Q_{10}$ for the temperature range between T_1 and T_0. For each sampling date, the difference between the laboratory temperature (25°C) and sapwood temperature was determined. If the difference was $\leq$ 10°C, the equation was simply used once to adjust the respiratory potential from the laboratory temperature to sapwood temperature. If the difference between laboratory and sapwood temperature was > 10°C, the equation was used twice: first, to adjust the laboratory respiratory

potential to 15°C, and second to adjust the respiratory potential to sapwood temperature. Sapwood temperature-adjusted, respiration rates were calculated for each tissue radial position (inner bark, outer sapwood, middle sapwood, and inner sapwood) of each tree and plotted according to sampling date along with the corresponding, unadjusted values of respiratory potential at 25°C.

The second model used the species-specific equations that were derived from laboratory measurements of the response of core segment respiratory potential to temperature for the Riddle and Gilchrist trees in September (1999) and March (2000). Respiratory potential at sapwood field temperatures was estimated for each tissue radial position on each sampling date using the following equation:

$$Ln\ R_m = \beta_0 + \beta_1\ (\text{date}) + \beta_2\ (\text{temperature}) + \beta_3\ (\text{date} \cdot \text{temperature}) + \beta_4\ (\text{temperature}^2) \qquad (2)$$

Where R_m = respiratory potential estimated from the laboratory-derived equation (2) and $\beta_0 - \beta_4$ are the parameter estimates from the regression of core segment respiratory potential on temperature. The R_m was modeled by using the monthly sapwood temperatures in two ways: (1) using September as the date reference in the equation and (2) using March as the date reference. The models for R_m for both the March and September date references were plotted according to sampling date.

5.3.7. Scaling respiratory potential: from tissue to organ

To estimate the respiratory potential of a volume of living tissue for each tree at the Riddle and Gilchrist sites, core segment volumes were scaled to stem cylinder volumes at each radial position (i.e. inner bark, and outer, middle, and inner sapwood) at breast height using the following procedure. In addition to sapwood thickness, inner bark thickness was also measured from the two cores extracted from each tree at both sites. Because outer bark thickness was not measured at this time, such measurements were taken from the data of previous studies on trees of similar characteristics at the same sites (Pruyn et al. 2002 a, Pruyn et al. 2002 b). The sum of outer bark, inner bark, and sapwood thickness was subtracted from stem diameter at breast height to determine heartwood thickness. Sapwood thickness was then divided by three, and each third added in succession to obtain the thickness from the pith to the distal edge of inner sapwood, middle sapwood, and outer sapwood, respectively. Using this thickness of each radial position as radius and the increment core's diameter (12mm) as height, stem cylinder volumes were calculated for each radial position inclusive of the inner tissues. The shell-volume of each radial position was then calculated by subtracting consecutive cylinders (e.g., inner bark – outer sapwood = inner bark). Each tissue radial position's volume was then multiplied by its respective core segment respiratory potential to calculate a volumetric rate of CO_2 production for each radial position. Weighted respiratory potentials were calculated for an entire segment of live wood at breast height by summing the volumetric rates of each

radial position and dividing by the total volume of inner bark plus sapwood. These weighted rates of respiratory potential were calculated for each tree on each sampling date in Douglas-fir (Riddle) and ponderosa pine (Gilchrist) using all four models discussed in the previous section (i.e. Equation 1: R_f at 25°C and R_f at sapwood temperature, and Equation 2: R_m from September and R_m from March).

5.3.8. Statistical analysis

All analyses were conducted using Statistical Analysis Systems software, release 8.0 (SAS Institute Inc. 1998). Equation parameter estimates for the relationship between respiratory potential and temperature, parameter estimate significance, and the adjusted R^2 were calculated using SAS Assist. Least squares means (LSMEANS), generated from SAS procedures, were reported $\pm$ pooled SE, or confidence intervals for transformed variables. The Shapiro-Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or natural log) was performed when necessary to meet assumptions of normality and constant variance. Within a specific table or figure, if confidence intervals were required for one variable, they were presented for all.

Comparisons among Q_{10} means at different temperature ranges were conducted separately for Douglas-fir and ponderosa pine, using a randomized block design with strip-plot (split-block) treatments in PROC MIXED (Little and Hills 1978, Milliken and Johnson 1984). Trees were blocks, and the effects of tissue radial position, temperature range, and their interaction were tested. Pair-wise

comparisons among tissue radial positions and temperature ranges were conducted using Fisher's Protected Least Significant Difference (FPLSD, Fisher 1966). Comparisons between live-wood respiratory potential (averaged over inner bark and sapwood) predicted from laboratory measurements (R_f, Equation 1) and those predicted from the temperature response equations (R_m, Equation 2) were not conducted statistically because the relevant repeated measures analysis required replication, which was not available for the modeled data (R_m). Comparisons for R_f among sampling dates and between species were conducted using a repeated measures analysis in PROC MIXED, testing the effects of species, sampling date, and their interaction. An unstructured covariance matrix was used in the model. To provide enough degrees of freedom in the analysis, six consecutive sampling dates were used, beginning with the July (1998) measurement for Douglas-fir and the July (1999) measurement for ponderosa pine. The January (2000) sampling date was skipped for ponderosa pine because the data logger failed to record sapwood temperature.

5.4. RESULTS

Regressing respiratory potential on temperature required a log transformation because of a horn-shaped distribution in the residual plot. After the transformation, the residual plot continued to suggest curvature in the data near the higher temperatures, and a squared term (temperature^2) was added to the equation, resulting in a normal distribution of the residuals. This trend was true in the

relationship between respiratory potential and temperature at all three sites (Tables 5.2, 5.3). For Douglas-fir at the Corvallis site, the comparison between the temperature response curves from March 2000 and January 2002 for the 5 – 30°C temperature range revealed that date, temperature, and their interaction were all significant for all three tissue radial positions (inner bark, outer sapwood, and inner sapwood, Table 5.2). The significant interaction term indicated that tissue respiratory potential responded differently to temperature on the two sampling dates. Expanding the temperature response curve of January 2002 (Corvallis) to the 5 – 55°C temperature range, revealed that the quadratic equation with the temperature^2 term did not accurately describe the data because of unequal variance in the residual plot (Table 5.2). Adding a cubic term (temperature^3) improved the problem of non-constant variance, but did not completely result in consistency, evidenced by the lack-of-fit of the curve's peak to the data for the inner bark and outer sapwood radial positions (Figure 5.1).

For Douglas-fir at the Riddle site, the relationship between respiratory potential and temperature was the same on the two sampling dates for all the radial positions, evidenced by the non-significant interaction between date and temperature (Table 5.3). The significant date term for inner bark suggested that respiratory potential differed between March and September at each temperature (Table 5.3). However, the respiratory potential at each temperature was exactly the same in March as in September because the effect of date was not significant for

TABLE 5.2. The relationship between respiratory potential (R_f) and temperature with respect to sampling date in *Pseudotsuga menziesii* (Corvallis, OR) for two different temperature ranges. Parameter estimates ± SE, significant to the equation at $P < 0.05$.

	March 2000/January 2002 Comparison (5 – 30°C) $Ln\ R_f = \beta_0 + \beta_1$ (date) $+ \beta_2 \cdot$ (temp) $+ \beta_3$ (date · temp) $+ \beta_4$ (temp^2) Date reference: January = 1, March = 0					
tissue	Adjusted R^2	β_0	β_1	β_2	β_3	β_4
Inner Bark	0.93	5.0 ± 0.2	0.49 ± 0.14	0.15 ± 0.02	-0.033 ± 0.009	-0.001 ± 0.001
Outer Sapwood	0.82	3.5 ± 0.2	0.48 ± 0.21	0.17 ± 0.03	-0.041 ± 0.013	-0.002 ± 0.001
Inner Sapwood	0.87	2.7 ± 0.3	1.60 ± 0.23	0.20 ± 0.03	-0.045 ± 0.014	-0.003 ± 0.001
	January (5 – 55°C) $Ln\ R_f = \beta_0 + \beta_1 \cdot$ (temp) $+ \beta_2$ (temp^2) $+ \beta_3$ (temp^3)					
tissue	Adjusted R^2	β_0	β_1	β_2	β_3	
Inner Bark	0.76	4.3 ± 0.6	0.40 ± 0.10	-0.015 ± 0.0 4	0.0001 ± 0.0005	
Outer Sapwood	0.77	2.8 ± 0.7	0.41 ± 0.10	-0.016 ± 0.004	0.0001 ± 0.0005	
Inner Sapwood	0.85	3.4 ± 0.5	0.33 ± 0.07	-0.011 ± 0.003	0.0001 ± 0.0003	

TABLE 5.3. The relationship between respiratory potential (R_f) and temperature with respect to sampling date in *Pseudotsuga menziesii* (PSME – Riddle, OR) and *Pinus ponderosa* (PIPO – Gilchrist, OR). Parameter estimates ± SE, significant to the equation at $P < 0.05$.

	September 1999/March 2000 Comparison (5 – 30°C) $Ln\ R_f = \beta_0 + \beta_1$ (date) $+ \beta_2 \cdot$ (temp) $+ \beta_3$ (date · temp) $+ \beta_4$ (temp^2) Date reference: September = 0, March = 1					
tissue	Adjusted R^2	β_0	β_1	β_2	β_3	β_4
PSME						
Inner Bark	0.94	4.4 ± 0.1	0.24 ± 0.08	0.18 ± 0.02	0	-0.0025 ± 0.0004
Outer Sapwood	0.93	2.1 ± 0.2	0	0.26 ± 0.02	0	-0.004 ± 0.001
Inner Sapwood	0.91	1.4 ± 0.2	0	0.28 ± 0.02	0	-0.004 ± 0.001
PIPO						
Inner Bark	0.71	5.6 ± 0.20	0	0.15 ± 0.03	0	-0.003 ± 0.001
Outer Sapwood	0.87	2.5 ± 0.2	1.0 ± 0.2	0.18 ± 0.02	-0.04 ± 0.01	-0.003 ± 0.001
Middle Sapwood	0.84	2.1 ± 0.2	0.82 ± 0.22	0.18 ± 0.03	-0.04 ± 0.01	-0.003 ± 0.001
Inner Sapwood	0.83	2.2 ± 0.2	0.70 ± 0.21	0.15 ± 0.03	-0.03 ± 0.01	-0.002 ± 0.001

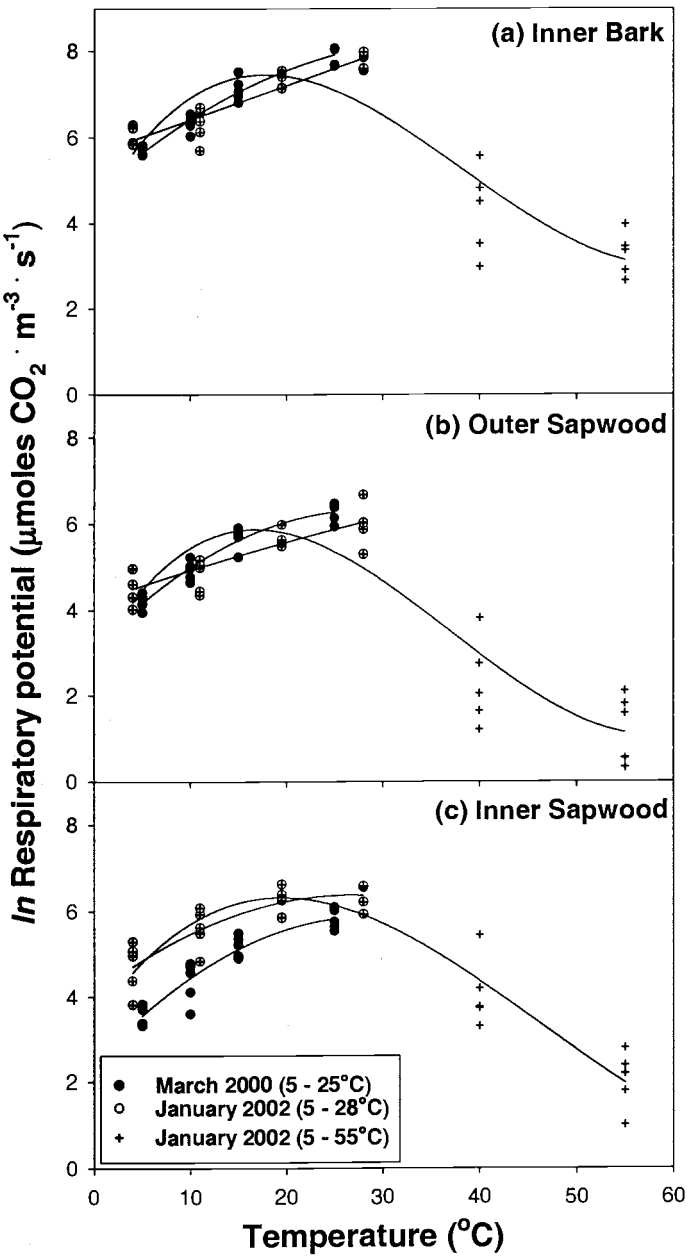

FIGURE 5.1. The relationship between the natural log of respiratory potential and temperature for *Pseudotusga menziesii* in March (2000) and February (2002) in Corvallis, OR. Equation parameters, their significance and adjusted R^2 in Table 1.

Douglas-fir outer and inner sapwood. The effect of date or the interaction between date and temperature were not significant for ponderosa pine inner bark at the Gilchrist site (Table 5.3). In contrast, the effects of temperature, date, and their interaction were all significant for ponderosa pine outer, middle and inner sapwood.

There was no effect of date on the Douglas-fir Q_{10}s ($P = 0.8$), whereas date had a significant effect on the ponderosa pine Q_{10} ($P = 0.0004$). Temperature range had a significant effect on the Q_{10} for both species ($P < 0.0001$, Table 5.4). The Q_{10} of Douglas-fir inner bark was higher than either sapwood position at the lower temperature range (5 – 15°C) and more nearly equal to sapwood Q_{10}s at the higher temperature ranges (Table 5.4), thus explaining the significant effects of tissue ($P = 0.02$) and the interaction of tissue and temperature range ($P < 0.0001$). However, there was no difference in Q_{10} among the four tissue radial positions for ponderosa pine ($P = 0.8$).

The difference between the monthly trends of live-wood respiratory potential (averaged over inner bark and sapwood) measured under laboratory conditions (R_f at 25°C) and live-wood respiratory potential modeled strictly from temperature (R_m – either model, Equation 2) revealed temperature-independent seasonal fluctuations in respiratory potential. An example of this difference was the occurrence of peaks for Douglas-fir (R_f at 25°C) in late September (1998) and October (1999) that were 50% higher than R_f (25°C) on other dates (Figure 5.2a). Such peaks were not evident on the corresponding dates for R_m (Figure 5.2b). Similar discrepancies occur for ponderosa pine, where peaks of R_f (25°C) in

TABLE 5.4. Respiratory Q_{10}s for *Pseudotusga menziesii* (PSME – Riddle, OR) and *Pinus ponderosa* (PIPO – Gilchrist, OR) for 2 – 3 temperature ranges in September and March. LSMEANS and 95% confidence intervals from strip plot analysis in PROC MIXED (n = 5 trees). Significant differences from Fisher's Protected Least Squares Differences ($P < 0.05$). For all comparisons each species (PSME or PIPO) was analyzed separately. Lower case letters represent differences among tissue radial positions within each row. Capital letters represent differences among temperature ranges and sampling dates within each column.

Species		Respiratory Q_{10}			
Date	Range (°C)	Inner Bark	Outer Sapwood	Middle Sapwood	Inner Sapwood
PSME					
0999	5 – 15	^{a}3.7 (2.8, 4.8)^{AB}	^{b}8.6 (7.0, 10.3)^{A}	--	^{b}7.5 (6.0, 9.1)^{A}
	15 – 25	^{a}1.7 (1.1, 2.5)^{CD}	^{a}1.9 (1.2, 2.6)^{B}	--	^{a}2.0 (1.3, 2.8)^{B}
	25 – 35	^{a}1.4 (0.9,2.1)^{D}	^{a}1.6 (1.0, 2.2)^{B}	--	^{a}1.8 (1.1, 2.4)^{B}
0300					
	5 – 15	^{a}4.1 (3.2, 5.1)^{A}	^{b}7.1 (5.8, 8.4)^{A}	--	^{b}7.7 (6.3, 9.1)^{A}
	15 – 25	^{a}2.5 (1.8, 3.4)^{BC}	^{b}1.5 (0.9, 2.2)^{B}	--	^{ab}1.7 (1.1, 2.5)^{B}
	25 – 35	--	--	--	--
PIPO					
0999	5 – 15	^{a}4.9 (3.6, 6.8)^{A}	^{a}3.9 (2.9, 5.2)^{A}	^{a}3.7 (2.8, 5.0)^{A}	^{a}3.3 (2.4, 4.4)^{A}
	15 – 25	^{a}1.7 (1.3, 2.4)^{B}	^{a}1.6 (1.2, 2.2)^{B}	^{a}1.8 (1.4, 2.4)^{B}	^{a}1.6 (1.2, 2.2)^{B}
	25 – 35	^{a}1.2 (0.9, 1.7)^{C}	^{a}1.6 (1.2, 2.2)^{B}	^{a}1.5 (1.2, 2.1)^{B}	^{a}1.8 (1.3,2.4)^{B}
0300					
	5 – 15	^{a}2.2 (1.6, 2.9)^{B}	^{a}2.9 (2.2, 3.9)^{A}	^{a}2.8 (2.1, 3.7)^{A}	^{a}2.7 (2.1, 3.7)^{A}
	15 – 25	^{a}1.5 (1.1, 2.0)^{BC}	^{a}1.4 (1.0, 1.8)^{B}	^{a}1.4 (1.1, 1.9)^{B}	^{a}1.5 (1.1, 2.0)^{B}
	25 – 35	--	--	--	--

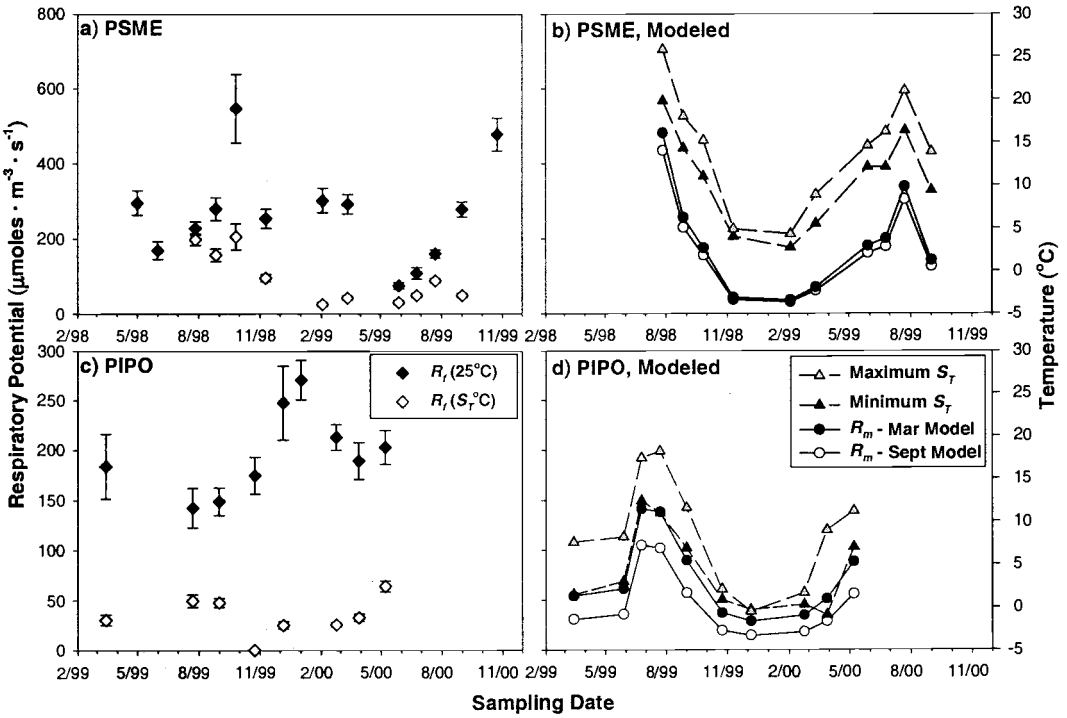

FIGURE 5.2. Seasonal flux of measured and modeled respiratory potential averaged for a volume of live wood (inner bark and sapwood) at 1 m from the ground for *Pseudotsuga menziesii* (PSME – Riddle, OR) and *Pinus ponderosa* (PIPO – Gilchrist, OR). In panels (a) and (c), R_f was calculated at laboratory temperature (25°C) and adjusted to sapwood field temperature (S_T °C) using equation (1). In panels (b) and (d) R_m was estimated using equation (2) (species-specific parameters in Table 2), using either September or March as the date reference in the equation. Minimum and maximum daily sapwood field temperature (S_T) are also in panels (b) and (d).

December (1999) and January (2000) were 20% higher than R_f (25°C) on other dates (Figure 5.2c). Again, such peaks and were not evident for R_m (Figure 5.2d). However, when laboratory respiratory potential was adjusted to sapwood temperature (R_f at S_T °C, Equation 1) and compared to R_m, the trends more closely approximated one another. For example, Douglas-fir R_f (S_T°C) and R_m both showed higher levels of respiratory potential in late August through early October (Figures 5.2a, b). Ponderosa pine showed similar peaks in respiratory potential for R_f (S_T°C) and R_m in late August through September and again in early May (Figures 5.2c, d).

Seasonal trends in R_f (25°C) were significantly different between Douglas-fir and ponderosa pine, the peaks in respiratory potential occurred in September – October for Douglas-fir and in December – January for ponderosa pine (Figures 5.2a, c). Thus, the effects of species, sampling date, and their interaction were all significant ($P < 0.0001$). These same effects were all significant for the seasonal trends in R_f (S_T °C) for the two species. However, the peaks of the two species were slightly more synchronized by sampling date. For example, both species showed peaks in R_f (S_T °C) in late August through September. Overall, respiratory potential per volume of live wood of Douglas-fir was nearly always twice as high as that of ponderosa pine.

5.5. DISCUSSION

Curvature in the respiratory response at higher temperatures (> 25°C) for Douglas-fir at the Corvallis site and for both species at the other sites (data not shown) suggested the approach of an optimal temperature range for enzymatic activity, where enzyme or substrate availability, and not temperature, was the limiting factor of respiration rate. Between 30 – 40°C for Douglas-fir (Corvallis), the onset of declining respiratory potential was likely the result of enzymes denaturing under higher temperatures. The result of the cubic or quadratic equations not accurately describing the relationship between respiratory potential and temperature from 5 – 55°C suggested that the ascending and descending responses should be modeled separately. The effectiveness of this modeling strategy was confirmed by the strong fit of a quadratic equation to the respiratory response from 5 – 35°C for both species.

The difference in the temperature-independent, seasonal flux of respiratory potential (R_f at 25°C) between Douglas-fir and ponderosa pine suggested different strategies of sapwood metabolism for the two species. The higher respiration rates (R_f at 25C) for Douglas-fir in autumn (September and August) as compared to the other sampling dates were potentially related to the seasonal fluctuation of sugars and starches in the sapwood tissues. High levels of starch were recorded in *Picea abies* stem wood in autumn and spring, whereas sugar levels were high in winter (Höll 1985). Such chemical variations in sapwood have been related to seasonal

changes in physiological behavior. For example, photosynthates are accumulated during favorable periods, relocated from leaves to sapwood and roots as dormant season approaches, stored throughout dormancy, and mobilized for re-use in growth and reproduction when the appropriate time arrives (Sauter and van Cleve 1994). In contrast, ponderosa pine showed the highest respiration rates (R_f at 25°C) during December and January as compared to the other sampling dates, which may have been related to living cells of the sapwood metabolically creating a winter build-up of pressure to prevent embolism (air blockages) from spreading throughout the water transport pathways (Améglio et al. 2001). Support for this theory was provided in stems of *Acer* spp., where living cells of the sapwood hydrolyzed starch to sugars at low, nonfreezing temperatures (Marvin et al. 1967).

Further research is needed to ascertain the physiological mechanisms controlling the temperature-independent, seasonal fluxes in stem respiration identified for Douglas-fir and ponderosa pine. However, the flux differences between the species provide potential explanations about species-specific functional roles of sapwood. The possible winter pressurizing role for ponderosa pine sapwood supports Rudman's (1966) idea that wider sapwood may be maintained by species to serve various metabolic requirements. Ponderosa pine may have adapted to survive in extreme climates in terms of moisture availability, whereas Douglas-fir is better suited to climates where the risk of winter-induced embolism is not as imminent.

5.6. ACKNOWLEDGEMENTS

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6. WITHIN-STEM RESPIRATORY GRADIENTS IN RELATION TO RAY ANATOMY AND RESERVE MATERIALS IN TWO CONIFEROUS TREE SPECIES OF CONTRASTING SAPWOOD WIDTH

6.1. ABSTRACT

Two coniferous tree species of contrasting sapwood thickness (*Pinus ponderosa* L., ponderosa pine and *Pseudotsuga menziesii* Mirb., Douglas-fir) were compared with regard to stem respiration and wood chemical and cellular composition to determine whether there is a relationship between sapwood width and stem storage capability. An increment core-based, laboratory method under controlled temperature was used to measure tissue-level respiration (termed respiratory potential) in mature stems (> 100-years-old) of both species. In contrast to the bark to pith trend of decreasing core-based respiratory potentials that was evident at four vertical positions within the stems of both species, ray cell size, frequency, and volumetric proportion remained relatively constant from bark to pith. The basis on which respiratory potential was calculated (i.e. core volume or mass, live bole volume, ray parenchyma volume, or tissue nitrogen content) affected the comparisons between the two species. For example, respiratory potential on a core volume basis was similar between the two species, but on a live bole volume basis respiratory potential was 30 – 40% lower in ponderosa pine versus Douglas-fir. The lower live bole-volume-based respiratory potential of ponderosa pine corresponded to 10 – 25% lower total nonstructural carbohydrate concentrations in the sapwood of this species versus that of Douglas-fir. The lack

of correspondence between ray cell characteristics and tissue chemistry and core-based respiratory potentials suggested that sapwood activity are not driven by quantity of storage sites or tissue-level substrate concentrations. However, the positive correlation between respiratory potential on a live bole volume basis and sapwood concentrations of total nonstructural carbohydrates suggested that the relationship between sapwood storage capacity and its related metabolism are more obvious on the organ (live bole segment) or whole-tree level.

Keywords: stem respiration, sapwood width, ray parenchyma anatomy, nitrogen, total nonstructural carbohydrates, Pinus ponderosa, Pseudotsuga menziesii.

6.2. INTRODUCTION

Biomass allocation toward sapwood production and heartwood formation in trees is subject to biophysical constraints, which may lend to an overall 'design criterion' for the amount of sapwood maintained (Mecuccini et al. 1997, Gartner 2000). For example, a tree forming and maintaining a large as opposed to a small sapwood volume provides additional cells for storage and water transport, yet requires increased metabolic support. Trees' design criteria for sapwood amounts may be related to the hydraulic, mechanical, and/or metabolic functional roles of sapwood. The significance of the hydraulic and mechanical roles for sapwood has received considerable attention (e.g., Gartner 1995, Mencuccini et al. 1997, and Domec and Gartner 2002). In contrast, the potential relationship between sapwood

amounts and the provision of stem storage sites and metabolic support has not been thoroughly examined (Gartner et al. 2000).

The storage potential of the tree is largely determined by the frequency and size of parenchyma cells in the sapwood because they are the sites for the storage and metabolism of photosynthetic reserves in the stem, which constitutes a large proportion of the tree's total storage volume (i.e. branches, stem, and roots). In conifers, the proportion of the sapwood that is parenchyma varies between 5 and 12% (Panshin and DeZeeuw 1980). Within Douglas-fir stems, ray frequency (number of rays / mm^2 tangential area) has been shown to be relatively constant from pith to bark, increasingly significantly only in the few rings closest to the pith (Gartner 2000). Other studies on conifers have shown a decline in ray frequency toward the bark and an increase in the size of individual rays (Bannan 1937, 1954, Gregory and Romberger 1975, Lev-Yadun 1998). From tree base to treetop ray frequency, ray height (Jaccard 1915) and individual ray size (Gartner 2000) may show little variation, or in some cases ray frequency may slightly decrease (Bannan 1965) or increase (Gartner 2000).

Despite the apparent uniformity in ray parenchyma size and abundance, sapwood metabolism is likely to vary with stem position. In *Pinus sylvestris* the metabolic substrates (nonstructural carbohydrates: starch, fructosans and soluble sugars) decreased from the outer sapwood toward the sapwood/heartwood transition zone (Saranpää and Höll 1989). The amount of fatty acids in sapwood triacylglycerols was drastically reduced at the sapwood/heartwood boundary

compared to the sapwood, and starch disappeared from ray parenchyma cells during heartwood formation (Saranpää and Höll 1989). These differences in sapwood substrate composition and abundance indicate differences in activity and vitality among sapwood parenchyma cells residing in the sapwood. On a dry mass basis, respiratory potential of *Pinus ponderosa* inner bark was 3 – 15 times greater than the neighboring outer sapwood, which was in turn 30 – 60% higher than middle or inner sapwood, depending on height within the tree and tree age compared (Pruyn et al. 2002 a). In the same study, sapwood rings produced in the same calendar year released over 50% more CO_2 at treetops than at bases. Similar within-tree patterns were found within stems of *Pseudotsuga menziesii* (Pruyn et al. 2002 b). Additionally, ray cell nuclear morphology changed from outer to inner sapwood in various conifer species, indicating decreased ray vigor (Frey-Wyssling and Bossard 1959, Yang 1993, Gartner et al. 2000). Ray vigor was also found to vary vertically within the stem, being highest at the base of the live crown and decreasing from there toward both the tree base and treetop (Yang et al. 1994).

Ponderosa pine (*Pinus ponderosa*) has wide sapwood (often about 15 to 20 cm in the radial direction with 50 to 200 annual rings) whereas Douglas-fir (*Pseudotsuga menziesii*) has quite narrow sapwood (often about 5 cm in the radial direction with 15 to 40 rings). If sapwood amount is an indicator of a tree's need/use of sapwood storage sites for photosynthetic reserves, there should be differences between the stem physiologies of the two species that reflect their storage roles (or lack thereof). In the current study, ponderosa pine and Douglas-fir

were compared in terms of stem respiration, ray parenchyma anatomy characteristics, and tissue chemistry content. The following hypotheses were tested with the objective of learning if there was evidence that sapwood width and volume were regulated by the dynamics between storage and metabolism of photosynthetic products (metabolic efficiency) in the sapwood. 1) Trends in core-based respiratory potentials (mass or volume based) of the sapwood are similar between the two species and thus do not reflect inter-species differences in metabolic strategy of the sapwood. 2) Core-based respiratory potential (mass or volume based) of the inner bark are higher in ponderosa pine than Douglas-fir, suggesting inter-species differences in the metabolic role of the inner bark. 3) Organ (live bole) based rates of stem respiratory potential are higher in Douglas-fir than ponderosa pine, suggesting that inter-species differences in sapwood storage capacity and its related metabolism are more obvious on the organ or whole-tree level. 4) Tissue chemistry (nitrogen and nonstructural carbohydrate content) and not ray cell size or frequency, reflect the differences in core-segment-based respiratory potential within and between species and across seasons and age classes (i.e. high protein and carbohydrate levels – high respiratory potentials).

6.3. MATERIALS AND METHODS

6.3.1. Study areas and species characteristics

The ponderosa pine (*Pinus ponderosa* L.) trees were located just east of the Cascade Range in central Oregon, near Gilchrist (N43° 28' W121° 41') at elevation 1355 m. Samples were collected from 220+ year-old ponderosa pine trees in early March and September of 1999 and from 50+ and 15+ year-olds in March 2000. All age classes of ponderosa pine trees sampled in March were examined in a previous study (Pruyn et al. 2002 a). Respiration data from that previous study was used in the current study to make comparisons to Douglas-fir. Additionally, core samples from that previous study were used for tissue chemical analyses in the current study. The Douglas-fir trees, *Pseudotsuga menziesii* (Mirb.), were located just east of the Coast Range in southern Oregon, near Riddle (N42° 57' W123° 22', elevation 215 m). Samples were collected from 100+ year-old Douglas-fir trees in September of 1998 and from 10+ year-olds in March of 1999. The size and age characteristics of the trees are presented in Table 6.1.

TABLE 6.1. Age and size characteristics for mature and young ponderosa pine (PIPO) and Douglas-fir (PIPO) trees sampled in March or September. Means ($n = 6$) $\pm$ SE.

Ponderosa pine (PIPO)	Mature (220 Y, Mar)	Mature (240 Y, Sept)	Young (50 Y, Mar)	Young (15 Y, Mar)
Age (Y = years old)	225 ± 27	243 ± 10	72 ± 5	31 ± 3
Stem diameter, breast height (mm)	622.8 ± 19.2	703.8 ± 19.8	268.0 ± 18.9	101.2 ± 4.9
Tree Height (m)	33.3 ± 0.4	33.7 ± 0.4	12.4 ± 0.9	2.9 ± 0.01
Height to base of live crown (m)	13.3 ± 1.0	16.3 ± 0.9	1.6 ± 0.2	(< 0.1 m from ground)
Sapwood area at base (cm^2)	2390 ± 114	2622 ± 162	374.9 ± 71.1	55.7 ± 6.2
Douglas-fir (PSME)		Mature (100 Y, Sept)		Young (10 Y, Mar)
Age (year)	--	101 ± 1	--	10 ± 0.3
Stem diameter, breast height (mm)	--	606.8 ± 16.4	--	35.1 ± 3.5
Tree Height (m)	--	43.6 ± 1.1	--	5.6 ± 1.8
Height to base of live crown (m)	--	27.3 ± 1.3	--	(< 0.1 m from ground)
Sapwood area at base (cm^2)	--	651.9 ± 77.2	--	40.9 ± 7.1

The March sampling was prior to bud break and wood production, whereas September was afterwards, thus enabling a seasonal comparison. Also, measurements were not taken during the growing season (March – September) to ensure that measured respiration rates represented only maintenance respiration and not growth respiration, which is more likely to depend on hormonal or other stimuli, and carbohydrate supply from outside the immediate xylem stores (McCree 1970, Thornley 1970).

6.3.2. Tree felling and sampling

Twenty trees were selected randomly for each species and age class from their applicable sites. All trees were free of broken tops, stem deformities, or visible disease. Six trees (from the randomly selected 20) were chosen for each species/age combination, and the diameter at 1m was recorded (Table 6.1). After felling the 220+ year-old ponderosa pine trees, we sawed 20-cm-tall stem disks from stems at node 220, and just above (to avoid branch whorls within the crown) nodes 65, 50, and 15 (years from the treetop). The same method was used for 100+ year-old Douglas-fir trees, except that tree disks were sampled from nodes 100, 95, 35, and 15. Tree height measurements were taken from tree base to each node, base of the live crown (first stem position above ground level with three live branches), and to treetops (Table 6.1).

Disks for respiration measurements were transported to the laboratory wrapped in extra-strength black garbage bags with moist paper toweling inside to

reduce desiccation, then stored in these bags at 4°C. Within one week after harvesting, three 12mm diameter increment cores were extracted from each node, wrapped in plastic bags and returned to cold storage. For 50+ and 15+ year-old ponderosa pine and 10+ Douglas-fir trees, cores were extracted directly from the felled stems in the field. Three cores each were sampled from nodes 50 and 15 in the 50+ year-olds, from node 15 in the 15+ year-olds and from node 5 in the 10+ year-olds. Cores were wrapped in plastic bags and stored on ice until returned to the laboratory, where they were stored at 4°C.

A second, small disk (< 5 cm tall) was taken from each vertical position of all trees and returned to the laboratory. After kiln drying, radii measurements and ring counts from pith to the distal edge of each tissue (outer and inner bark, sapwood, and heartwood) were recorded from the small disks taken at each stem height, for all age classes.

6.3.3. Respiratory measurements

All extracted cores were analyzed within one week of sampling, using a previously developed protocol (Pruyn et al. 2002 a, b). Twenty-four hours prior to measurement, cores were divided into three segments: inner bark (phloem); outer, middle, and inner sapwood; and heartwood. Any green (photosynthetic phelloderm) tissue that was visible just underneath the bark surface on the inner bark samples from younger tissues was removed with a razor blade. Sapwood was defined as the woody tissue extending from the outermost growth ring to the

innermost growth ring before the heartwood. Transition zone rings (one or two lighter colored rings at the sapwood / heartwood boundary) were not included in the samples. Outer, middle, and inner sapwood samples were obtained by dividing sapwood into three equal radial lengths. The exception was in cores extracted from 220+ ponderosa pine trees, where sapwood width was greater than 100 growth rings. In this case, core segments of 10 – 15 growth rings in length were taken from the outer and inner sapwood boundaries and from the center of the sapwood. Heartwood samples (< 10 – 15 growth rings in length) were taken one ring interior (towards pith) of the transition zone rings. Node 15 of 15+ year-old ponderosa pine trees and node 5 of 10+ year-old Douglas-fir trees did not have heartwood. Number of rings per segment was recorded, so that a mean age could be determined for each segment. These segments were weighed, wrapped tightly in plastic, and then stored at 25°C overnight to allow metabolic activity in core segments to stabilize (Goodwin and Goddard 1940, Levy et al. 1999).

Immediately prior to measurement, core segments were re-weighed and placed in vials, which were then sealed with gas-tight rubber septa. To determine a rate of CO_2 production, the CO_2 concentration within vials was measured with a Hewlett-Packard (5700A) gas chromatograph (GC, Hewlett Packard, Rt. 41, Avondale PA 19311, USA) immediately after closing the vials and again after an incubation period. Core segments were incubated at 25°C for six hours (unless indicated otherwise) between GC measurements. Details of GC analysis and calculation of respiratory potential (nmoles $CO_2 \cdot s^{-1}$, on a core volume, core mass

or moles nitrogen basis) are in Pruyn et al. (2002 a, b). Immediately following the GC analysis, core segments were weighed a third time. Changes in the three successive wet masses verified that water loss was low (1 – 3%) between sampling and measurement. Fresh volume of core segments was estimated as the water displaced when samples were submerged (D2395, ASTM 2001). Dry masses were determined after oven drying at 60°C for 48 hours. From this point onward, the reported values were referred to as respiratory potential, rather than respiration rate, because measurement conditions of these excised samples were probably different from those within the tree.

6.3.4. Scaling core segment respiratory potential to the organ level

To account for the differences in sapwood thickness between ponderosa pine and Douglas-fir trees, core segment volumes were scaled to a volume of a cylinder of stem, standardized as 12 mm tall for each vertical position (ponderosa pine: node 220 – 15, Douglas-fir: node 100 – 15). The volume of each radial position for each node was calculated by subtracting consecutive cylinders (e.g., inner bark – sapwood = inner bark). Each radial position's volume for each node was then multiplied by the respective respiratory potential on a core segment volume basis to calculate its net CO_2 production. Weighted respiratory potentials for each node were calculated by summing the volumetric rates of each radial position and dividing by each node's live bole volume (inner bark + sapwood). Thus, the weighted respiratory potential for each node is a function of the sum of

the net CO_2 production of stem segments from each radial position, divided by the sum of the stem segment volumes from each radial position (live bole volume).

6.3.5. Ray size and distribution

One oven-dried core segment of the three replicates from each radial and vertical stem position was randomly selected for microscopy analysis for the six trees each of mature ponderosa pine (220+ years-old) and Douglas-fir (100+) trees from the September harvests. Tangential sections were made with the microtome in the middle of the earlywood from two – four growth rings throughout the core segment. Only two rings were sampled from core segments from younger tissues because there were fewer growth rings per segment. Eight tangential sections were made per core segment (two – four per ring, depending on number of rings sampled), stained in safranin, and permanently mounted. Ray area, frequency and volume (a proportion) were estimated using an image analysis system and following the protocol of Gartner et al. (2000).

A compound microscope (Nikon Labophot-2, Nikon Microscopy, Melville, NY, USA) with a 10X objective lens and a color video camera (Sony CCD/RGB, Sony Electronics Inc., Park Ridge NJ, USA) were used to view the slides. The microscopic image of the tangential section was projected onto a color monitor (33 cm diagonal distance, Sony Trinitron, Sony Electronics Inc., Park Ridge NJ, USA) and onto a computer monitor (Apple Macintosh Quadra 800) by way of a digitizing card. One – two fields of view (1.00 mm × 0.80 mm each) were randomly selected

from each tangential section made, for a total of eight fields per core segment. Using NIH image v. 1.60 (Rasband 1996) software, all rays within each field of view were highlighted using the program's paint tool. Entire rays (both simple and fusiform) were highlighted, including cell walls, ray tracheids, and for fusiform rays, epithelial cells. The resin canal itself was excluded from fusiform rays.

Average ray area was calculated as well as ray frequency (the number of rays per mm^2 tangential section). Additionally, ray volume (proportion of tangential area occupied by rays) was calculated as the sum of all ray areas divided by the sum of the area viewed. The assumption was that the same ray proportion would be found at any nearby depth, implying that the relative area measured was a good estimate of the volumetric proportion tissue that was ray. Mean respiratory potential on a core volume basis was divided by mean proportion of ray volume for each for each vertical and radial stem position of mature, September harvested, ponderosa pine and Douglas-fir (Table 6.2 and Figure 6.1). This value was then plotted according to growth ring number from bark for each species.

6.3.6. Chemical Analyses

Core segments were ground to pass a 20 – mesh screen and then analyzed for protein using a LECO CHN-1000 Analyzer (LECO, 3000 Lakeview Ave., St. Joseph, MN 49085-2396, USA) that measured tissue total carbon content (%) and total nitrogen content (%). The samples were combusted in a chamber and converted into gases, which passes through infrared cells to determine carbon and

also through a thermal conductivity cell to determine nitrogen. Ground core samples were also analyzed for total nonstructural carbohydrate content (%) via a process that involved a complete hydrolysis of extracted carbohydrates to reducing sugars using a 0.2 N sulfuric acid solution. The reducing power of the neutralized hydrolysate was then determined by titration with standardized sodium thiosulfate. Included in total nonstructural carbohydrate content were the sugars, dextrin, starch and fructusan, but not the structural carbohydrates such as hemicellulose and cellulose (AOAC 1965). All chemistry analyses were conducted by the Nutritional Analysis Laboratory in the Rangeland Ecosystem Science Department at Colorado State University (Natural Resource Bldg. Rm. 240, Fort Collins, CO 80523-1478).

To determine if there was an effect of the length of time elapsed before cell death on core segment chemical content, ponderosa pine cores that were tested for respiratory potential as described above (delayed death – controls) were compared to cores that had been dropped immediately into liquid nitrogen after coring (immediate death by liquid N_2). This comparison tested whether core segments expended a significant portion of their reserves (i.e. starch, sugar, and protein) during the measurement process of core segment respiratory potential. Comparisons between delayed and immediate death treatments were made at each of the three different vertical (node 220, node 65, and node 15) and radial (inner bark, outer sapwood, and inner sapwood) stem positions for the six trees. For the delayed death treatment, samples for the analysis were the oven-dried core segments from the respiratory potential analysis. Each sample was a combination

of two (or three for inner bark) of the three replicates from each stem position. For the immediate death treatment, a separate core was extracted from each stem vertical position, divided according to tissue radial position as described above, wrapped in aluminum foil and dropped into liquid N_2. The cores were stored at -20°C until they were prepared for grinding by oven-drying them in paper envelopes at 65°C for 48 hours.

The remaining comparisons of tissue chemistry were conducted within and among the trees described in Table 6.1. Core segments for these analyses were exposed to the delayed death treatment from above, thus the analyzed tissue was from the oven-dried core segments from the respiratory potential analyses. To calculate the moles of nitrogen in each sample, core segment dry mass was multiplied by the percentage of nitrogen of the sample and divided by the atomic weight of nitrogen. This number was then used to calculate respiratory potential on a moles nitrogen basis (μmoles CO_2 · moles N · s^{-1}), which was plotted against total nonstructural carbohydrate content (%) for each sample. Each radial and vertical position (e.g. inner bark at node 15) was plotted separately because of the lack of independence among stem radial and vertical positions within each tree. To observe (non-statistically) if there was an effect of stem radial or vertical position on the relationship between nitrogen-based respiratory potential and total nonstructural carbohydrate content, an average was calculated for each stem position and then re-plotted.

Respiratory potential was calculated on nitrogen basis rather than a core mass or core volume basis when examining its relationship to total nonstructural carbohydrate content of the core because nitrogen content is more representative of the proteins available for the respiratory reaction. In contrast, core mass and core volume include living and dead cell material. Thus, the objective was to determine whether there was a relationship between core respiratory potential on a nitrogen basis and available substrate (total nonstructural carbohydrates).

6.3.7. Statistical Analyses

All data were analyzed in Statistical Analysis Systems software, release 8.0 (SAS Institute Inc. 1998). The Shapiro-Wilk *W*-test was used to determine whether the response variables were distributed normally. A transformation (square-root or natural log) was performed when necessary to meet assumptions of normality and constant variance. Least squares means (LSMEANS), generated from the various SAS procedures described below, are reported $\pm$ pooled SE, or confidence intervals for transformed variables. Within a specific table or figure, if confidence intervals were required for one variable, they were presented for all.

To compare respiratory potential on a core-volume basis between ponderosa pine and Douglas-fir trees, least squares means (LSMEANS) were generated using PROC MIXED, with a randomized block design and strip-plot (split-block) treatments (Little and Hills 1978, Milliken and Johnson 1984). Trees were blocks and the effects of tissue radial position, species, and their interaction were tested

separately for each stem vertical position (node). Pair-wise comparisons (*t*-tests) among tissue radial positions and between species were conducted using Fisher's Protected Least Significant Difference (FPLSD) procedure (Fisher 1966). Of the multiple comparisons, only the between-species comparisons were reported for each tissue radial position at each node.

A strip-plot analysis was also used to generate LSMEANS and make comparisons (using FPLSD) of volume-based, inner bark respiratory potential among stem vertical positions within trees. The effects of tissue radial position, vertical position, and their interaction were tested separately for each species. Weighted rates of respiratory potential per unit live bole volume were compared between species using a strip-plot analysis and FPLSD. Comparisons of tissue chemical contents between pre-drying treatments, species, or seasons and among stem radial and vertical positions, or age classes were made by generating LSMEANS via the strip-plot analysis and using FPLSD.

6.4. RESULTS

6.4.1. Respiratory potential comparisons between the two species

For the September harvests, respiratory potential on a core volume basis (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$) of inner bark core segments of ponderosa pine was 1.5 to 3 times higher than Douglas-fir at all stem vertical positions with the exception of node 19 (ponderosa pine) / node 15 (Douglas-fir), where ponderosa pine inner bark

was 30% lower than in Douglas-fir (Table 6.2). In contrast to the inner bark, respiratory potential of sapwood core segments on a volume basis at each stem vertical and radial position was similar between the two species (Figure 6.1). When there were significant differences, there was no obvious trend of ponderosa pine core segments respiring higher or lower than Douglas-fir.

TABLE 6.2. Respiratory potential of inner bark core segments (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$) of mature ponderosa pine and Douglas-fir trees sampled in September (dry season). Least squares means and confidence intervals ($n = 6$ trees). Significant differences among means from Fisher's protected least squares differences ($P < 0.05$). For each node (r, row), lower case letters indicate differences between species. For each species (c, column), capital letters indicate differences among nodes.

	Core Respiratory Potential (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$)					
Node from Treetop	Ponderosa pine	r	c	Douglas-fir	r	c
Node 240 (PIPO)/Node 100 (PSME)	2.0 (1.6, 2.6)	a	A	0.94 (0.7, 1.2)	b	AB
Node 100/95	1.6 (1.5, 1.7)	a	AB	0.76 (0.7, 0.8)	b	A
Node 40/35	1.7 (1.6, 1.9)	a	AB	1.2 (1.1, 1.4)	b	B
Node 19/15	1.5 (1.3, 1.6)	a	B	2.2 (2.0, 2.4)	b	C

These trends in volumetric respiratory potential between species differed from those for mass-based respiratory potential (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$). Inner bark respiratory potential on a mass basis of mature ponderosa pine was three to four times higher than that of mature Douglas-fir at all stem vertical positions, except at node 19 (ponderosa pine) / node 15 (Douglas-fir) where the two species respired at equal rates (data not shown). At a specific stem vertical and radial position, sapwood respiratory potential on a mass basis of ponderosa pine was

FIGURE 6.1. Respiratory potential on a core volume basis (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$) versus approximate number of growth rings inward from bark of mature ponderosa pine (PIPO, 220+ years-old) and Douglas-fir (PSME, 100+ years-old) at four different stem heights (nodes from treetop) sampled in September (dry season). Respiratory potentials are LSMEANS (strip-plot model in PROC MIXED) and number of growth rings are means, with 95% confidence intervals in both the x- and y-axial directions ($n = 6$ trees). For each node, marked pairs (*) of like-shapes indicate significant differences between species ($P < 0.05$, Fisher's protected least squares differences). a) Stem node 240 (PIPO)/node 100 (PSME) from treetop. b) Stem node 100 (PIPO)/node 95 (PSME). c) Stem node 40 (PIPO)/node 35 (PSME). d) Stem node 19 (PIPO)/node 15 (PSME).

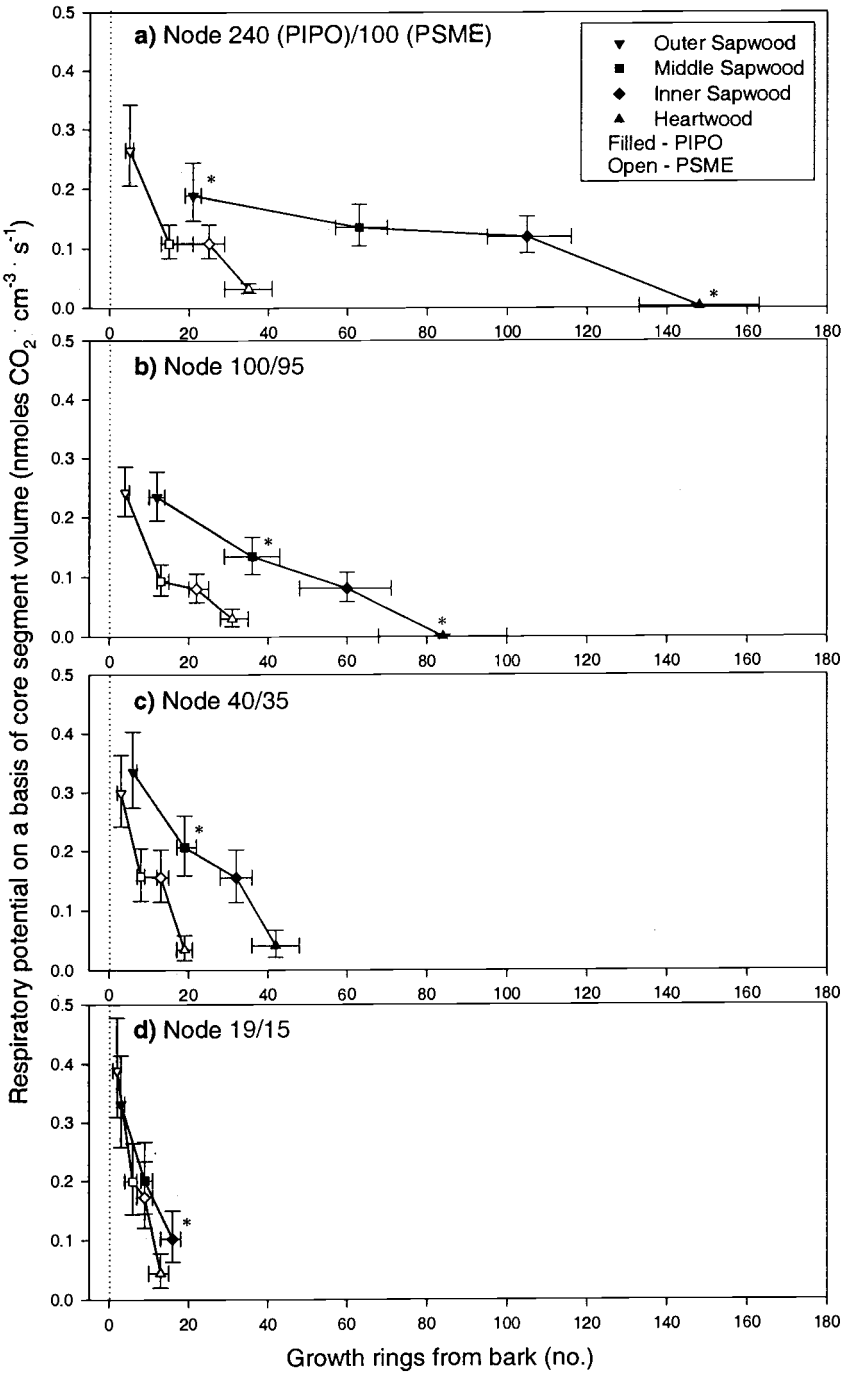
a) Node 240 (PIPO)/100 (PSME)
b) Node 100/95
c) Node 40/35
d) Node 19/15
Outer Sapwood
Middle Sapwood
Inner Sapwood
Heartwood
Filled - PIPO
Open - PSME
Respiratory potential on a basis of core segment volume (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$)
Growth rings from bark (no.)

FIGURE 6.1.

frequently one to two times higher than that of Douglas-fir (data not shown). Trends between the two species in core volume based respiratory potential differed from those for live-bole-segment volume (inner bark + sapwood) based, weighted respiratory potential at each node. Ponderosa pine weighted respiratory potential (on a live bole volume basis) was 40% lower than Douglas-fir at the tree base (node 240, ponderosa pine /node 100, Douglas-fir) and 30% lower near the tree top (node 19, ponderosa pine /node 15, Dougals-fir) (Figure 6.2 a), whereas the core volume based rates for inner bark were higher in ponderosa pine than Douglas-fir and for sapwood were mostly equal between the two species. On average, sapwood cross-sectional area at each node of the mature (220+ years-old) ponderosa pine trees was approximately two to four times higher than each corresponding node of the mature (100+ years-old) Douglas-fir (Figure 6.2 b).

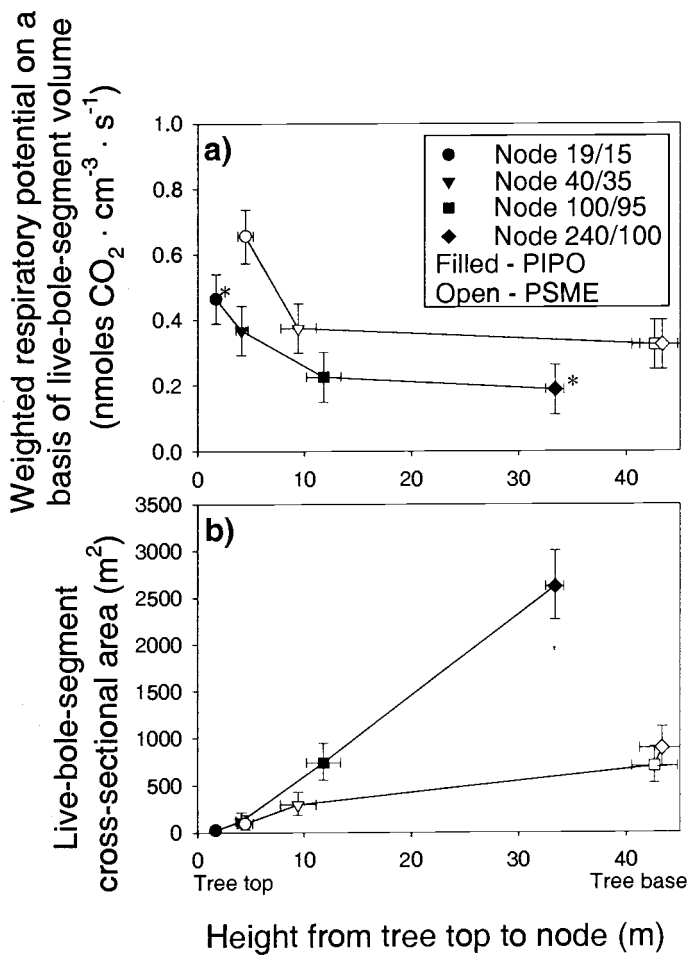

FIGURE 6.2. (a) Weighted respiratory potential on a live-bole-segment volume basis (inner bark plus sapwood, nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$) and (b) live-bole-segment cross-sectional area (m^2) versus stem height from tree base to node from tree top (m) of mature ponderosa pine (PIPO, 220+ years-old) and Douglas-fir (PSME, 100+ years-old) sampled in September (dry season). Respiratory potentials are LSMEANS (strip-plot model in PROC MIXED) and tree heights are means, with 95% confidence intervals in both the x- and y-axial directions ($n = 6$ trees). For each node, marked pairs (*) of like-shapes indicate significant differences between species ($P < 0.05$, Fisher's protected least squares differences).

6.4.2. Relationship between ray anatomy and respiratory potential

Average ray area (μm^2), ray frequency (no./mm^2), and ray volume (% of the volume) were relatively uniform with respect to growth ring number from bark and vertical position on the stem for both species of mature ponderosa pine (Figure 6.3 a, b, c) and Douglas-fir trees (Figure 6.4 a, b, c) harvested in September. Exceptions to this trend in ponderosa pine were when ray frequency tended to be higher at node 19 than at the other nodes, and at when ray volume tended to be higher in older tissues at nodes 19 and 50 (closer to pith, Figure 6.3 c). The trend of increasing ray volume from bark to pith of ponderosa pine at node 19 was the reverse of its trend in core volume-based respiratory potential (Figure 6.1 d). Douglas-fir also showed higher frequencies of rays at node 15 than at the other nodes (Figure 6.4 a), but the lower average ray area canceled out this effect (Figure 6.4 b), resulting in a constant proportion of ray volume with respect to growth ring and stem node.

Respiratory potential per unit ray volume decreased with increasing growth ring number for both ponderosa pine (Figures 6.3 d) and Douglas-fir (Figure 6.4 d). Respiratory potential per unit ray volume and growth ring number was similar between the two species, with respiratory potential being near 6.0 (nmoles $CO_2 \cdot$ cm^{-3} ray volume $\cdot$ s^{-1}) near the bark and approaching or equaling zero in the heartwood. The decline in respiratory potential (before reaching zero) for ponderosa pine spanned more than 100 growth rings (Figure 6.3 d), whereas it only

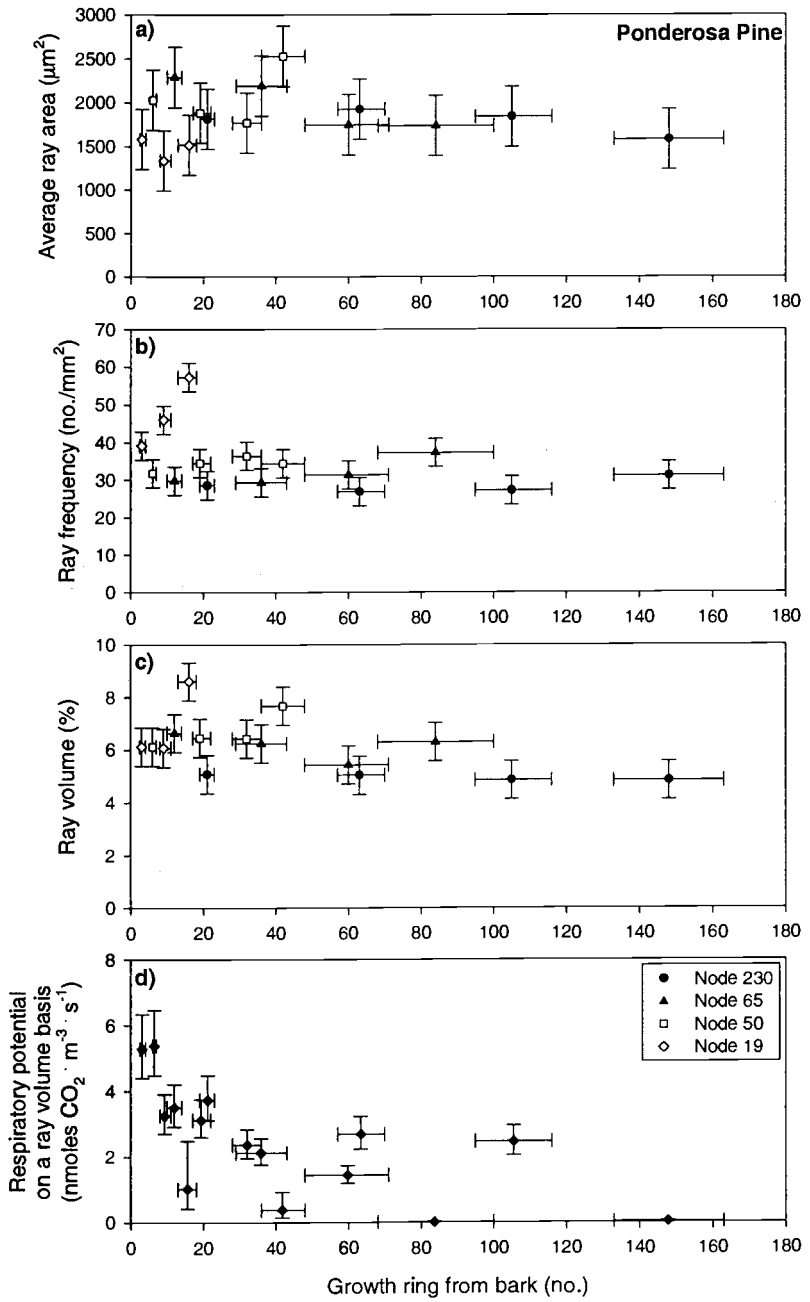

FIGURE 6.3. Parenchyma characteristics versus approximate number of growth rings inward from bark of mature (220+ years-old) ponderosa pine trees ($n = 6$ trees) at four different stem heights (nodes from treetop) sampled in September (dry season). Stems were sampled and analyzed for ray characteristics in the tangential plane. a) Respiratory potential on a ray volume basis (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$). b) Average ray area ($\mu m^2$). c) Ray frequency (no./mm^2). d) Ray volume (ray area/total area, %).

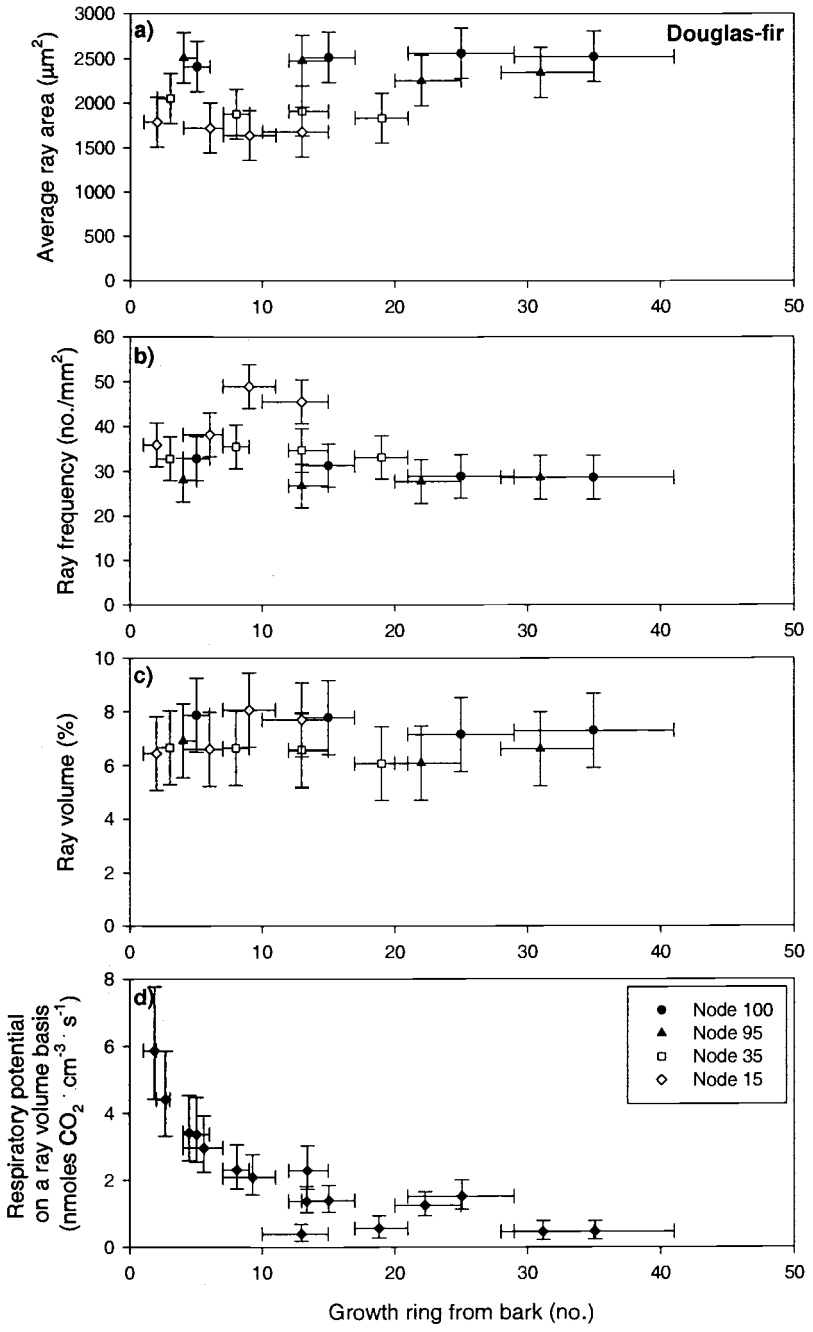

FIGURE 6.4. Parenchyma characteristics versus approximate number of growth rings inward from bark of mature (100+ years-old) Douglas-fir trees (n = 6 trees) at four different stem heights (nodes from treetop) sampled in September (dry season). Stems were sampled and analyzed for ray characteristics in the tangential plane. a) Respiratory potential on a ray volume basis (nmoles $CO_2 \cdot cm^{-3} \cdot s^{-1}$). b) Average ray area ($\mu m^2$). c) Ray frequency (no./mm^2). d) Ray volume (ray area/total area, %).

spanned 35 growth rings for Douglas-fir (Figure 6.4 d), a result of ponderosa pine sapwood being more than 50 rings wider than in Douglas-fir.

6.4.3. Relationship between tissue chemistry and respiratory potential

There was no effect of delayed versus immediate cell death treatment on the carbon content of inner bark core segments from the mature ponderosa pine trees. In contrast, there were significant differences between treatments in the sapwood; immediate death samples had 2 – 4% higher carbon contents than did delayed death samples (Table 6.3). There were also no significant differences in the nitrogen content of inner bark samples between treatments, whereas sapwood nitrogen content tended to be lower in immediate than delayed death in most samples and was significantly lower in three of the samples (immediate death 23 – 36% lower than delayed death, Table 6.3). Immediate cell death had the most drastic effects on core segment nonstructural carbohydrate content. For example, inner bark total nonstructural carbohydrate content of immediate death samples was 50 – 60% lower than delayed death samples and sapwood content of immediate death samples was 30 – 80% higher than delayed death samples (Table 6.3).

TABLE 6.3. Effect of delayed versus immediate cell death (control versus liquid N_2) on the chemical composition of mature ponderosa pine core tissue sampled in September (dry season). Least squares means and confidence intervals (n = 6 trees, except liquid-N_2-treated inner bark samples where n = 2). Each radial position was analyzed separately. For each row, each pair (none versus liquid N_2) of marked (in bold) means for each tissue chemical-content was significantly different at ($P <$ 0.05, Fisher's protected least squares differences). Unmarked means are not different.

Stem Vertical and Radial Position	Total Carbon (%)		Total Nitrogen (%)		Total Nonstructural Carbohydrates (%)	
	Delayed Death (Control)	Immediate Death (Liquid N_2)	Delayed Death (Control)	Immediate Death (Liquid N_2)	Delayed Death (Control)	Immediate Death (Liquid N_2)
Node 220						
inner bark	49.7 ± 0.7	49.8 ± 1.1	0.38 ± 0.02	0.39 ± 0.03	**18.6 ± 1.0**	**7.3 ± 1.6**
outer sapwood	**47.8 ± 0.1**	**49.3 ± 0.1**	0.12 ± 0.01	0.09 ± 0.01	2.5 ± 0.4	3.6 ± 0.4
inner sapwood	**47.9 ± 0.2**	**49.2 ± 0.2**	**0.11 ± 0.01**	**0.07 ± 0.01**	**2.2 ± 0.2**	**3.7 ± 0.2**
Node 65						
inner bark	49.3 ± 0.7	49.3 ± 1.1	0.37 ± 0.02	0.31 ± 0.03	13.9 ± 1.0	7.0 ± 1.6
outer sapwood	**48.2 ± 0.1**	**49.3 ± 0.1**	**0.15 ± 0.01**	**0.11 ± 0.01**	**2.1 ± 0.4**	**3.8 ± 0.4**
inner sapwood	**48.0 ± 0.2**	**49.5 ± 0.2**	**0.13 ± 0.01**	**0.10 ± 0.01**	2.6 ± 0.2	2.9 ± 0.2
Node 15						
inner bark	50.7 ± 0.7	51.7 ± 1.1	0.33 ± 0.02	0.28 ± 0.03	**15.8 ± 1.0**	**7.7 ± 1.6**
outer sapwood	**48.1 ± 0.1**	**49.5 ± 0.1**	0.16 ± 0.01	0.16 ± 0.01	**3.3 ± 0.4**	**4.7 ± 0.4**
inner sapwood	**47.9 ± 0.2**	**49.4 ± 0.2**	0.14 ± 0.01	0.13 ± 0.01	**3.5 ± 0.2**	**4.7 ± 0.2**

Carbon content was fairly constant within stems, across age classes, and between species or seasons, ranging from 47 – 52% (data not shown). For inner bark, total nitrogen content at the tree base was 27% higher for mature ponderosa pine than for mature Douglas-fir, and the reverse was true at node 19 (ponderosa pine) /node 15 (Douglas-fir) (Table 6.4). However, for sapwood there were no significant differences between the nitrogen content of the two species. Thus, the effect of species and the interaction of species by tissue were not significant for core segment nitrogen content ($P > 0.2$).

For inner bark, total-nonstructural carbohydrate content of the inner bark was 1.2 – 2.2 times higher in ponderosa pine than Douglas-fir. In contrast, for sapwood total nonstructural carbohydrate content was 10 – 25% lower in ponderosa pine than that in Douglas-fir, with the exception of the outer sapwood at node 15 where the contents were equal (Table 6.4). Although the effect of species was not significant for total nonstructural carbohydrate content ($P = 0.1$), the interaction of species by tissue was significant ($P < 0.0001$). Ponderosa pine inner bark nitrogen and total nonstructural carbohydrate content decreased from tree base to treetop, whereas it increased in Douglas-fir. In contrast, both species' sapwood nitrogen and total nonstructural carbohydrate content increased from tree base to treetop. Thus, the interaction of species by tissue by node was significant for nitrogen and total nonstructural carbohydrate contents ($P < 0.0001$).

March-harvested (wet season) mature ponderosa pine core segments showed no significant differences in nitrogen content from those harvested in

TABLE 6.4. Comparison of core tissue chemical composition between mature ponderosa pine and mature Douglas-fir both sampled in September (end of dry season). Least squares means and confidence intervals (n = 6 trees). Significant differences among means from Fisher's protected least squares differences ($P < 0.05$). For each tissue radial position (r, row), different lowercase letters represent differences between species. For each species (c, column), different uppercase letters represent differences among tissue and vertical positions.

	Total Nitrogen (%)						Total Nonstructural Carbohydrates (%)					
Stem Vertical and Radial Position	Ponderosa Pine	r	c	Douglas-fir	r	c	Ponderosa Pine	r	c	Douglas-fir	r	c
Tree Base												
inner bark	0.38 (0.35, 0.41)	a	A	0.30 (0.27, 0.32)	b	A	19 (17, 20)	a	A	8.8 (8.1, 9.6)	b	A
outer sapwood	0.12 (0.09, 0.15)	a	B	0.13 (0.10, 0.16)	a	B	2.5 (2.3, 2.7)	a	B	2.8 (2.6. 3.1)	b	B
inner sapwood	0.11 (0.08, 0.14)	a	B	0.12 (0.09, 0.15)	a	B	2.4 (2.2, 2.7)	a	B	3.2 (3.0, 3.5)	b	C
Node 15												
inner bark	0.33 (0.30, 0.36)	a	C	0.44 (0.41, 0.47)	b	C	16 (14, 17)	a	C	13 (12, 14)	b	D
outer sapwood	0.16 (0.13, 0.19)	a	D	0.17 (0.15, 0.21)	a	D	3.3 (3.0, 3.6)	a	D	3.3 (3.0, 3.5)	a	C
inner sapwood	0.14 (0.11, 0.17)	a	BD	0.15 (0.12, 0.18)	a	BD	3.5 (3.2, 3.8)	a	D	3.9 (3.6, 4.3)	b	E

September (dry season), with the exception of inner bark at node 15, which was 39% higher during the wet than the dry season (Table 6.5). Total nonstructural carbohydrate content decreased significantly from wet to dry season (wet 20 – 70% > dry) at all radial and vertical stem positions with the exception of inner sapwood at node 15, which remained the same (Table 6.5). Respiratory potential on a core segment mass basis (nmoles $CO_2 \cdot g(DW)^{-1} \cdot s^{-1}$) also decreased significantly from wet to dry (wet 1.2 – 3.7 × > dry) for inner sapwood at node 65 and at all tissue radial positions at node 15 (Table 6.5). For most other stem radial and vertical positions, mass-base respiratory potential stayed the same, but for inner sapwood at node 220 and outer sapwood at node 65, it increased.

With regard to chemical and respiratory differences among tissue radial positions, both March and September sampled trees showed significantly higher nitrogen, total nonstructural carbohydrate contents, and core mass-based respiratory potential in the inner bark as compared to the sapwood (Table 6.5). Thus, the effect of tissue radial position was significant for both chemical contents and respiratory potential for both harvest seasons ($P < 0.05$). The treetop to tree base trends in tissue chemistry and core segment respiratory potential were also generally similar to one another. Inner bark nitrogen and nonstructural carbohydrate contents and respiratory potential decreased from tree base to treetop (except node 15 in March), whereas in the sapwood, they increased or stayed the same. However, the significance of these trends differed slightly between harvest seasons. For example, the effect of vertical position on tissue nitrogen content was significant in

TABLE 6.5. Effect of season sampled (wet versus dry), and of stem radial and vertical positions on the chemical composition and respiratory potential of mature ponderosa pine core tissue. Least squares means and confidence intervals ($n = 6$ trees). Significant differences among means from Fisher's protected least squares differences ($P < 0.05$). Differences between seasons were analyzed separately from differences among stem positions. For each response variable, different lowercase letters represent differences between seasons within each row (r). Different uppercase letters represent differences among stem radial and vertical positions within each column (c).

TABLE 6.5.

Stem Vertical and Radial Position	March (Wet)	r	c	September (Dry)	r	c
	Total Nitrogen (%)					
Node 220						
inner bark	0.39 (0.35, 0.43)	a	A	0.38 (0.35, 0.41)	a	A
outer sapwood	0.12 (0.09, 0.15)	a	B	0.12 (0.09, 0.15)	a	B
inner sapwood	0.11 (0.08, 0.14)	a	B	0.11 (0.08, 0.14)	a	B
Node 65						
inner bark	0.33 (0.30, 0.35)	a	C	0.37 (0.34, 0.40)	a	AC
outer sapwood	0.14 (0.11, 0.16)	a	BE	0.15 (0.12, 0.18)	a	BD
inner sapwood	0.11 (0.08, 0.14)	a	B	0.13 (0.11, 0.16)	a	BD
Node 15						
inner bark	0.46 (0.42, 0.49)	a	D	0.33 (0.30, 0.36)	b	C
outer sapwood	0.16 (0.13, 0.19)	a	E	0.16 (0.13, 0.19)	a	D
inner sapwood	0.16 (0.13, 0.19)	a	E	0.14 (0.11, 0.17)	a	BD
	Total Nonstructural Carbohydrates (%)					
Node 220						
inner bark	22 (19, 24)	a	AC	19 (17, 20.2)	b	A
outer sapwood	3.4 (3.1, 3.7)	a	B	2.5 (2.3, 2.7)	b	B
inner sapwood	3.5 (3.2, 3.8)	a	BD	2.4 (2.2, 2.7)	b	B
Node 65						
inner bark	23 (21, 25)	a	A	14 (13, 15)	b	C
outer sapwood	3.6 (3.3, 3.9)	a	BD	2.1 (1.9, 2.2)	b	D
inner sapwood	3.4 (3.2, 3.7)	a	B	2.6 (2.4, 2.9)	b	B
Node 15						
inner bark	19 (17, 21)	a	C	16 (14, 17)	b	E
outer sapwood	3.8 (3.5, 4.1)	a	D	3.3 (3.0, 3.6)	b	F
inner sapwood	3.4 (3.1, 3.6)	a	B	3.5 (3.2, 3.8)	a	F
	Core Respiratory Potential ($nmoles\ CO_2 \cdot gDW^{-1} \cdot s^{-1}$)					
Node 220						
inner bark	7.3 (6.2, 8.6)	a	A	7.3 (6.2, 8.7)	a	A
outer sapwood	0.45 (0.38, 0.53)	a	B	0.45 (0.38, 0.54)	a	B
inner sapwood	0.21 (0.18,0.25)	a	C	0.29 (0.25, 0.34)	b	C
Node 65						
inner bark	6.2 (5.3, 7.4)	a	A	5.7 (4.8, 6.8)	a	A
outer sapwood	0.52 (0.44, 0.62)	a	B	0.68 (0.57, 0.80)	b	D
inner sapwood	0.28 (0.24, 0.33)	a	C	0.24 (0.20, 0.28)	b	C
Node 15						
inner bark	5.9 (5.0, 7.0)	a	A	4.3 (3.7, 5.1)	b	E
outer sapwood	1.3 (1.1, 1.5)	a	D	0.98 (0.82, 1.2)	b	F
inner sapwood	1.0 (0.8, 1.2)	a	E	0.27 (0.22, 0.32)	b	C

March ($P < 0.0008$), but not in September ($P = 0.6$). Further, the same effect on total nonstructural carbohydrate content was not significant in March ($P = 0.2$), but was in September ($P < 0.0001$). In contrast, the effect of vertical position on core segment respiratory potential was significant in both March and September ($P < 0.05$). An interesting difference between the seasons was that the September-harvested, inner sapwood respiratory potential was not higher at node 15 as compared to the lower nodes. Additionally, the bark to pith trends in tissue chemistry and respiratory potential differed among the three stem vertical-positions for both harvest seasons, as was indicated by the significant interaction between stem radial and vertical positions ($P < 0.03$).

There were no significant differences in core segment nitrogen content among the three age classes of ponderosa pine sampled in March with the exception of inner bark at node 15 of the 50 year-old trees, which was $\geq 25\%$ lower than either the 15 or 220+ year-olds (Table 6.6). Total nonstructural carbohydrate content of the inner bark of 220+ year-old trees was 26% higher at node 50 than that of the 50+ year-olds (Table 6.6). In contrast, sapwood at node 15 of the younger, 15+ year-old trees had the highest total nonstructural carbohydrate content, followed by the 220+ year-olds, and finally the 50+ year-olds had the lowest content.

TABLE 6.6. Comparison of core tissue chemical composition among three age classes of ponderosa pine trees sampled in March (wet season). Least Squares Means (n = 6 trees). For each radial position (column), significant differences among tree ages marked by paired symbols (in bold) for node 50, or by different letters for node 15 ($P < 0.05$, Fisher's protected least squares differences). Unmarked means are not different.

Ponderosa Pine Tissue Chemistry		**Stem Radial Positions**		
Node from treetop	Tree Age	Inner bark	Outer sapwood	Inner Sapwood
Total Nitrogen (%)				
Node 50	50	0.30 (0.28, 0.33)	0.13 (0.10, 0.15)	0.10 (0.08, 0.13)
Node 50	240	0.33 (0.30, 0.35)	0.14 (0.11, 0.16)	0.11 (0.09, 0.14)
Node 15	15	0.44 (0.41, 0.47)[a]	0.15 (0.12, 0.18)	0.13 (0.10, 0.16)
Node 15	50	0.33 (0.30, 0.37)[b]	0.16 (0.12, 0.19)	0.15 (0.12, 0.19)
Node 15	240	0.45 (0.41, 0.50)[a]	0.16 (0.13, 0.19)	0.16 (0.13, 0.19)
Total Nonstructural Carbohydrates (%)				
Node 50	50	**18.3 (17.4, 19.2)**	3.2 (2.8, 3.6)	4.0 (3.5, 4.4)
Node 50	240	**23.1 (22.1, 24.1)**	3.6 (3.2, 4.0)	3.4 (3.1, 3.8)
Node 15	15	17.9 (16.4, 19.4)	4.4 (4.0, 4.8)[a]	4.0 (3.7, 4.4)[a]
Node 15	50	16.7 (15.3, 18.1)	3.3 (3.0, 3.6)[b]	3.1 (2.9, 3.4)[b]
Node 15	240	19.2 (17.1, 21.7)	3.8 (3.5, 4.1)[c]	3.4 (3.1, 3.6)[b]

The trend of lower nitrogen and total nonstructural carbohydrates of the inner bark of Douglas-fir compared to ponderosa pine corresponded to pine's lower nitrogen-based respiratory potentials (nmoles $CO_2 \cdot$ moles $N^{-1} \cdot s^{-1}$) (Figure 6.5 a, c). The season-sampled and the age class had little effect on the relationship between respiratory potential on a moles nitrogen basis and total nonstructural carbohydrate content for any of the stem radial or vertical positions (Figure 6.5). Averages from each panel of Figure 6.5 suggested a positive relationship between nitrogen-based respiratory potential and total nonstructural carbohydrate content of core segments (Figure 6.6). Inner bark segments had higher values than sapwood, and sapwood segments near treetops tended to have higher values than those near tree bases.

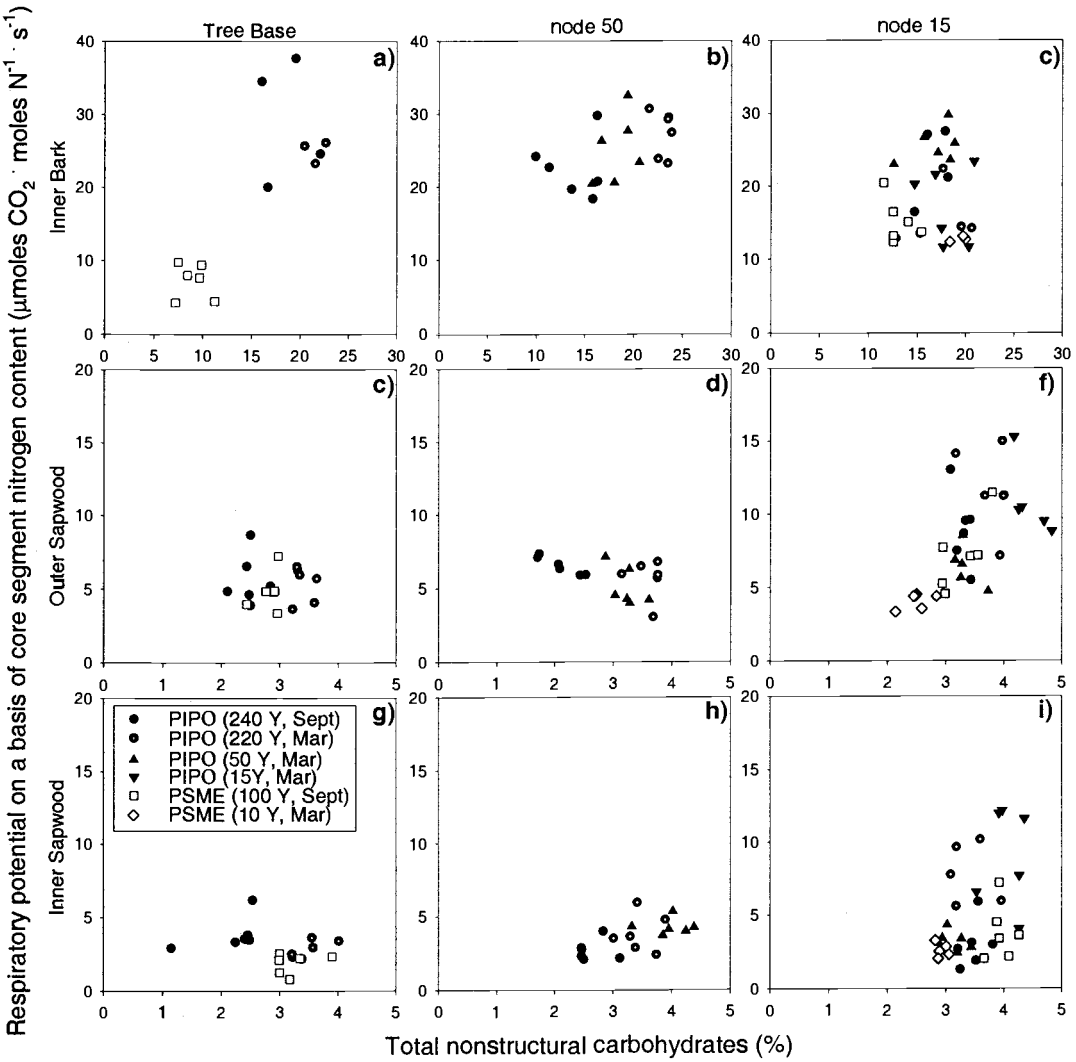

FIGURE 6.5. Respiratory potential on a basis of core tissue nitrogen content (μmoles CO_2 · moles N^{-1} · s^{-1}) versus total nonstructural carbohydrate content of core tissue (%) of ponderosa pine and Douglas-fir from 2 – 3 different age classes and 2 different seasons. Each column of panels represents a different stem vertical position, tree base (a, c, g), node 50 from treetop (b, d, h), and node 15 from treetop (c, f, i, except 10+ year-old PSME, which were from node 5). Each row of panels represents a different stem radial position, inner bark (a, b, c), outer sapwood (c, d, f), and inner sapwood (g, h, i). Abbreviations: Y = years-old, Mar = March, and Sept = September. Note: the y-axis of the inner bark panels are not of the same scale as that of outer or inner sapwood.

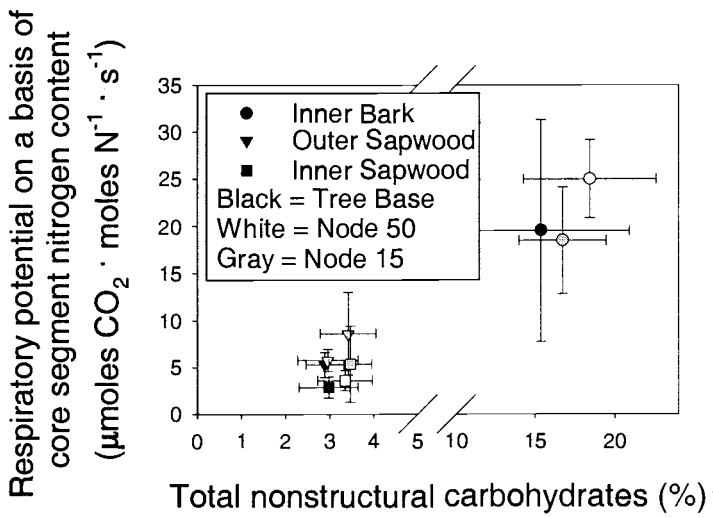

FIGURE 6.6. Respiratory potential on a basis of core tissue nitrogen content (μmoles CO_2 · moles N^{-1} · s^{-1}) versus total nonstructural carbohydrate content of core tissue (%) of ponderosa pine and Douglas-fir from 2 – 3 different age classes and 2 different seasons. Each point represents a mean ± standard deviation from each panel of Figure 6.5.

6.5. DISCUSSION

As hypothesized, both species showed the same pattern and similar values of respiratory potential on a core volume-basis for mature trees, with highest values at the inner bark, and values decreasing toward the sapwood/heartwood border. These results of the current study, in the dry season are the same as those found during the wet season (March, Pruyn et al. 2002 a, b). In contrast to the first hypothesis from the current study, ponderosa pine had higher core mass-based respiratory potential than did Douglas-fir. This may have resulted from the lower

density of ponderosa pine wood (0.38 – 0.40 g · cm^{-3}) as compared to Douglas-fir (0.40 – 0.45 g · cm^{-3}, Panshin and DeZeeuw 1980).

When respiratory potential was scaled up to bole segments, the current research showed that mature ponderosa pine had lower values than did Douglas-fir. This suggested that trees with proportionally larger amounts of live bole had lower per unit respiratory demand than trees with smaller amounts. It seems contradictory that on the core volume basis the respiratory potential of ponderosa pine was often equal to, or sometimes higher than that of Douglas-fir, whereas on a live-bole-segment volume basis, the weighted respiratory potential of ponderosa pine was either equal to or less than Douglas-fir. However, the explanation lies within the calculation of weighted respiratory potential. For any given node, the radial position that has the largest volume is the outer sapwood because (a) the inner bark is considerably thinner than the outer sapwood, and (b) the middle and inner sapwood have the same thickness as the outer sapwood, but are closer to the pith. Thus, in the calculation of weighted respiratory potential, the outer sapwood respiratory potential has the most 'weight', which for ponderosa pine was either lower or equal to Douglas-fir at all four nodes. The conclusion from the comparison between the core based respiratory potentials and the live-bole-segment volume based weighted respiratory potentials was that each scale is important in evaluating the metabolic and storage roles of the sapwood. For example, on the core-level we learned that the sapwood was not homogenous, and on the live-bole-

segment level, that there was a possible inverse relationship between weighted respiratory potential and the corresponding live-bole volume.

The ray parenchyma anatomical characteristics were generally consistent with respect to stem radial (growth ring) and vertical (node) position, which as hypothesized, did not reflect the within-stem respiratory gradients in the mature ponderosa pine or Douglas-fir trees. A notable exception was the high frequency of rays at node 15 as compared to the lower nodes in stems of ponderosa pine, which corresponded to sapwood respiratory potentials (core volume-based) that were 1.3 – 2 times higher near the treetop as compared to the base. The similar ranges in respiratory potential on a ray volume basis from outer to inner sapwood between ponderosa pine and Douglas-fir suggested a bark-to-pith decrease in ray vigor, a trend that has been reported in numerous other studies (Goodwin and Goddard 1940, Frey-Wyssling and Bosshard 1959, Shain and MacKay 1973, Yang 1993, Gartner et al. 2000). However, to more accurately represent live ray cell volume, vital staining techniques should be used in future work, rather than the more general safranin stain from the current study (e.g. triphenyl tetrazolium tri-chloride, Ryan 1990; or Cooamassie Blue, Stockfors and Linder 1998).

The objective of examining the effect of the length of time elapsed before cell death on tissue chemical content was to learn whether the experimental process of measuring respiratory potential depleted core segment chemical composition. Higher nitrogen and total nonstructural carbohydrate content in liquid-N_2-treated (immediate death) core segments as compared to the untreated controls (delayed

death) potentially indicated a depletion effect of the respiratory measurements. In contrast, a decline in the chemical content of immediate death core segments may have resulted from cell wall barriers being ruptured during freezing, subsequently enhancing the volatilization of chemicals during drying. Although often significant, the differences between core segment total carbon and nitrogen contents for the delayed versus immediate death treatments were quite subtle, suggesting that these materials were not depleted during measurement of core respiratory potential. In contrast, differences in nonstructural carbohydrate content between delayed and immediate death core segments were notable and inconsistent in the inner bark and sapwood (i.e. immediate versus delayed death was decreased in inner bark, yet increased in sapwood). As a result of this inconsistent response to the immediate death treatment, core segments were not treated prior to oven drying in the current study. In future work, other rapid-killing treatments (e.g. freeze drying) should be explored to maximally reflect the *in situ* chemical composition of stem wood.

As hypothesized, higher nitrogen and total nonstructural carbohydrate content in the inner bark of ponderosa pine versus that of Douglas-fir corresponded to a higher volume-based or mass-based, core segment respiratory potential, indicating that high enzyme (nitrogen), or high substrate (carbohydrates) concentrations in tissue may drive respiration. Further, inner bark tissue as compared to the sapwood was rich in nitrogen and total nonstructural carbohydrates and also had higher core segment based respiratory potential. However, in the

sapwood, high levels of nitrogen/sugars/starch did not always correspond to higher core-segment-based respiratory potentials. For example, Douglas-fir sapwood had higher total nonstructural carbohydrate contents than ponderosa pine sapwood, but its core volume/mass-based, core segment respiratory potentials were equal to/less than (respectively) pine. Also, higher core-segment-based respiratory potentials in the outer sapwood versus inner sapwood, or near the treetop versus the tree base did not necessarily correspond to higher nitrogen/sugars/starch contents. One explanation for the lack of correspondence between sugars/starch content and core-segment-based respiratory potential may be that all sapwood sugars/starches do not increase or decrease consistently within stems. Saranpää and Holl (1989) found that although glucose, fructose, and sucrose amounts were greatest in *Pinus sylvestris* outer sapwood and gradually decreased toward the innermost sapwood rings, arabinose/galactose remained constant throughout the outer sapwood rings and increased drastically toward the heartwood.

Nonstructural carbohydrate concentrations may be higher in Douglas-fir sapwood than in ponderosa pine because Douglas-fir trees maintain relatively less amounts of sapwood and thus have less space available for storage of reserve materials. In contrast, nonstructural carbohydrates in ponderosa pine may be spread throughout its large sapwood volume, thus resulting in a low per unit concentration. Although higher nonstructural carbohydrate concentrations in Douglas-fir versus ponderosa pine did not correspond to higher respiratory potentials on a core segment basis, higher concentrations in Douglas-fir did

correspond to higher respiratory potentials on a live bole volume basis. This potential correlation suggested a whole-tree level control of stem sapwood amounts and respiratory metabolism, rather than a tissue-based, substrate-driven control. Further research is necessary to determine whether this relationship is robust among other species and age classes. Additionally, the relationship between inner bark and sapwood with regard to their tissue chemistry and respective respiratory potentials should be examined as possible determinants of sapwood amounts and whole-tree level metabolism.

6.6. ACKNOWLEDGEMENTS

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7. CONCLUSION

7.1. SUMMARY

The examination of within-tree patterns of stem respiratory potential revealed decreasing activity from inner bark to pith, with a two to ten fold decrease from inner bark to outer sapwood, and a gradual decrease throughout the sapwood toward the sapwood/heartwood boundary. This trend in respiratory potential was repeated at multiple stem heights, in various age classes and species, and across seasons. Within a specific growth ring (calendar year) stem respiratory potential was uniform among various stem heights, only increasing near treetops. However, this within-stem vertical pattern in respiratory potential varied among stem tissues (i.e. inner bark versus sapwood), species, and age classes. Trends of increased respiratory potential near apical (treetops) and cambial (inner bark) meristems suggested that respiratory activity in parenchyma cells may be driven by their proximity to substrate supply and/or involvement in cell formation and expansion.

Comparisons in respiratory potential among species revealed core-based differences that were transferred to the whole-tree level. Species that maintained lesser relative volumes of live bole had whole-tree respiratory potentials that were higher than species with greater live bole volumes. This trend was evident among five coniferous tree species in this study (*Abies amabilis*, *Pinus ponderosa*, *Pseudotsuga menziesii*, *Thuja plicata*, and *Tsuga heteropylla*), and in two species (*Pinus ponderosa* and *Pseudotsuga menziesii*), this trend was evident from monthly

monitoring over the course of a year. The inverse relationship between whole-tree respiratory potential and relative live bole volume suggests the possibility of a trade-off: trees that maintain large volumes of live wood cannot afford to be as metabolically active (per unit live bole volume) as trees with lesser volumes.

The hypothesis of high whole-tree respiratory potentials corresponding to relatively low sapwood volumes was partially supported by results from tissue chemistry analyses. *Pseudotsuga menziesii* (a species with relatively small sapwood volume) had higher concentrations of nonstructural carbohydrates in its sapwood than *Pinus ponderosa* (a species with relatively large sapwood volume). However, high tissue carbohydrate contents did not always indicate high respiratory potential, especially core-based respiratory potential. Additionally, neither sapwood nitrogen content nor anatomical characteristics were reliable indicators of core-based respiratory potentials. The possibility of a positive correlation between whole-tree respiratory potential and sapwood nonstructural carbohydrate content, combined with the lack of correlation between core-based respiratory potential and tissue carbohydrate content suggested a whole-tree level control of stem sapwood amounts and respiratory metabolism, rather than a tissue-based, substrate-driven control.

7.2. FUTURE RESEARCH

Several avenues for future research were uncovered in this dissertation work. One is to explore more thoroughly the response of decreased respiratory

potential in cores exposed to high carbon dioxide and low oxygen concentrations. This type of work, combined with the use of respiratory inhibitors (e.g., cyanide or SHAM), would help elucidate which pathway of respiration (anaerobic, aerobic, or alternative) is active within stems and how these pathways differ when the surrounding carbon dioxide levels change. Better knowledge of the respiratory pathways deep within sapwood is important to provide a better understanding of how stem tissue would respond to increasing atmospheric CO_2, which is predicted to double during the next century (from current 300 to over 600 ppm). Because sapwood is already under elevated CO_2 concentrations (10,000 – 80,000 ppm), it is difficult to know what level of elevated CO_2 will cause changes within the stem. Thus, experiments are needed to determine how the stem's aeration system (internal CO_2 and O_2 concentrations) responds to changing gaseous conditions in its environment.

Another opportunity for further research is to measure the seasonal changes in *Pinus ponderosa* and *Pseudotsuga menziesii* tissue nonstructural carbohydrate contents, as well as relative water contents to determine whether they correspond to their temperature independent variations in respiratory potential. In this way, one could determine a temperature-independent, physiological basis for seasonal fluxes in respiration. For example, if fluxes in sapwood water content corresponded with seasonal fluxes in respiration, one might infer that respiration is dependent on water availability in the xylem. This conclusion would support the hypothesis that oxygen is supplied to the respiring parenchyma via the transpiration stream.

Additionally, vital staining techniques should be used on sapwood tissues at the various stem radial and vertical positions to complement the ray anatomy work in this dissertation study. In this way percentages of vitality could be applied to the volumetric ray proportions, and then compared to the patterns in respiratory potential.

Finally, ecosystem-level questions with regard to the results from this dissertation should also be explored. The potential inverse relationship between whole-tree respiratory potential and relative live bole volume should be explored further to determine whether it is robust among more species and age classes. Further, the significance of this relationship should be considered with regard to the potential functional roles of tree species to ecosystems. Ecological models should be employed to determine how tree species composition (i.e. those maintaining large versus small amounts of sapwood) affects the uptake and release of carbon in ecosystems.

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