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Respiratory potential in sapwood of old versus young ponderosa pine trees in the Pacific Northwest

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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 determine respiratory trends in 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 one to four vertical positions in boles of +200-, +50- and +15-year-old *Pinus ponderosa* Dougl. ex Laws. trees. Cores were divided into five segments corresponding to radial depths of inner bark; outer, middle and inner sapwood; and heartwood. Data suggested that core segment CO_2 production was an indicator of its respiratory activity, and that potential artifacts caused by wounding and extraction were minimal. On a dry mass basis, respiratory potential of inner bark was 3–15 times greater than that of sapwood at all heights for all ages ($P < 0.0001$). Within sapwood at all heights and in all ages of trees, outer sapwood had a 30–60% higher respiratory potential than middle or inner sapwood ($P < 0.005$). Heartwood had only 2–10% of 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 sapwood 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 clearly demonstrate that the resulting trends within and among trees are determined by the way in which the data are expressed. Although these data are based on core segments rather than in vivo measurements, we conclude that the relative differences are probably valid even if the absolute differences are not.

Keywords: cambium, phloem, stem respiration, tree age, tree size.

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, crucial details needed for understanding stem respiration are still lacking. For example, at the individual-tree level, 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 for tissues extracted just above ground level in stems of *Pinus radiata* D. Don (Shain and Mackay 1973), *Fraxinus nigra* L., *Acer rubra* L. (Goodwin and Goddard 1940), and *Picea abies* L. Karst. 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) measured temperature variation among various heights and radial depths in *Picea abies* and observed within-stem temperature variations that resulted in scaling errors between 2 and 72% for a single sunny day and 2 and 58% for a whole year, assuming a constant temperature–respiration relationship.

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 were often 50% lower than temperature-predicted respiration rates. Martin et al. (1994) provides three main possibilities to explain this discrepancy. First, increased transpiration rates on hot, sunny afternoons may lead to water deficit and stress, thus decreasing respiration (Lavigne 1987, Kakubari 1988). Second, most respiration-evolved CO_2 is dissolved and carried away by the xylem transpirational stream (Negisi 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). Third,

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 in sapwood respiration in 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* Dougl. ex Laws. trees. We refer to the reported values as respiratory potential rather than respiration rates, because the conditions of our measurements on these excised samples probably differ from those in the tree. Our objective 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.

Materials and methods

Species and site characteristics

We collected samples from +15-, +50- and +200-year-old ponderosa pine trees located just east of the Cascade Range in central Oregon, near Gilchrist (43°28′ N, 121°41′ W) at elevation 1355 m. Unless indicated otherwise, most samples were collected in early March of 1999 (+200) and 2000 (+15, +50) from trees before bud break and the onset of wood production, which occur in early to mid-June in this region. All cores were collected either before (March) or after the end of the growing season (October or February) to ensure that measured respiration rates represented only maintenance respiration (McCree 1970, Thornley 1970). We avoided growth respiration because it is more likely to depend on hormonal or other stimuli, and carbohydrate supply from outside the immediate xylem stores.

Tree felling and sampling

All trees sampled were free of broken tops, stem deformities and disease. Six trees were chosen from each of three age classes (+200-, +50- and +15-year-old trees), and stem diameter at a height of 1 m was recorded (Table 1). After felling the +200-year-old trees, 20-cm-tall stem disks were sawn 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 1).

Disks for respiration measurements were transported to the laboratory wrapped in extra-strength black garbage bags lined with moist paper towel to reduce desiccation. Stem disks were stored at 4 °C. Within 1 week of harvest, three 12-mm 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-old trees, and from Node 15 in the +15-year-old trees. Cores were wrapped in plastic bags and transferred on ice 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 taken to the laboratory. After kiln drying, radial 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, mean annual ring widths for the last 15 years were measured in the lowest disk for each age class, for use in diameter and stem biomass growth calculations.

Stem and bark biomass growth and leaf area calculations

We calculated stem wood and bark biomass growth (kg year^{-1}) for each tree based on 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^{-1}, Table 1). To estimate leaf area, we divided each tree into one to four sections (i.e., +15-year-old trees, one section; +50-year-old trees, two sections; and +200-year-old trees, four sections). We hand-clipped 25% (one of every four leaf bundles) of the leaves (including attached woody material) from large crown sections (Nodes 50–220), and 100% of the leaves from small sections (Node 15), and recorded the fresh mass of the clipped material (B.L. Gartner, J.C. Domec, M. Pruyn and R. Spicer, unpublished data). We took subsamples of the clipped material and recorded the fresh mass. We then dried the samples, separated leaves from woody twigs and branches and recorded dry mass for each. We also collected 10 needle fascicles from the clipped material and stored them at –20 °C in the laboratory, until we were ready to record leaf dimensions to calculate leaf area. We then dried the leaves and recorded dry mass. Based on the proportions of leaf area to dry leaf mass to fresh leaf mass, we calculated total leaf area for each tree.

Respiration measurements

All cores were analyzed within 1 week of sampling. Respiration rates of isolated tissues have been shown to remain constant for up to 14 days in two hardwoods (Goodwin and Goddard 1940), and in *Pinus radiata* (Shain and Mackay 1973) and *Pseudotsuga menziesii* (Mirb.) Franco (M.L. Pruyn, unpublished data). Twenty-four hours before measurement, cores were cut longitudinally and divided 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

Table 1. Mean tree age, height, diameter, total leaf area and growth rates of ponderosa pine trees. For age, height, diameter and total leaf area, values are means ± standard error ($n = 6$). For growth rates, values are least square means and 95% confidence intervals from a one-way analysis of variance. Within a row, different letters indicate significant differences among means (Fisher's protected least significant difference procedure, $P < 0.05$).

Parameter	Tree age class (year)		
	+200	+50	+15
Age (year)			
Mean	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)			
Mean	62 ± 2	27 ± 2	10 ± 0.5
Range	53–67	21–35	9–12
Total leaf area (m^2)			
Mean per tree	540 ± 35	71 ± 16	11 ± 2
Growth rates over last 15 years			
Height (cm $year^{-1}$): Node 15 to treetop	15 ± 3 ab	21 ± 3 a	11 ± 3 b
Diameter 1 m from ground (cm $year^{-1}$)	0.21 ± 0.02 a	0.15 ± 0.02 a	0.20 ± 0.02 a
Stem wood biomass (kg $year^{-1}$)	18 (16, 20) a	1.6 (1.0, 2.4) b	0.21 (0.04, 0.54) c
Stem bark biomass (kg $year^{-1}$)	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 m^{-2} $year^{-1}$)	0.042 ± 0.007 a	0.038 ± 0.007 a	0.031 ± 0.007 a

the center of the sapwood. Heartwood samples (< 10–15 growth rings in length) were taken from tissue located one ring inside (towards the pith) 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 overnight at 25 °C to allow metabolic activity of the core segments to stabilize, thus minimizing the potential effect of accelerated respiration in response to tissue damage sustained during core extraction (Goodwin and Goddard 1940, Hari et al. 1991, Levy et al. 1999).

Immediately before measurement, core segments were reweighed and placed in vials that were sealed with gas-tight rubber septa. To determine the rate of CO_2 production, CO_2 concentration ([CO_2]) within the vials was measured with a Hewlett-Packard (5700A) gas chromatograph (GC) immediately after closing the vials and again after an incubation period. For any given experiment, an incubation period of 6 or 20 h was used, with the longer incubation necessary to accommodate the processing of large numbers of samples. The GC used He as the carrier and a thermal conductivity detector. The GC was calibrated with a standard gas mix of 1% CO_2 and 20% O_2 in N_2. The rate of CO_2 production by core segments, referred to here as respiratory potential, was used as a measure of tissue metabolism. The respiratory potential k (nmol CO_2 g^{-1} dry mass s^{-1}) of core segments was calculated as:

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

where ΔCO_2 is net percent increase in [CO_2] during the incubation period, M_{OD} is oven dry mass (g) of the core segment, T is incubation period (s), V_H is volume of headspace ($2.5e^{-05}$ m^3 – core sample volume), 40.91 is the constant for converting CO_2 molar volume (ml) to moles at 25 °C and 101.3 kPa, and 10^9 is the conversion factor for moles to nmoles. 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 based on Archimedes' principle (ASTM 1998). Dry mass was determined after drying at 60 °C for 48 h.

Testing of methods

Because of the invasive nature of our sampling method, four tests were conducted to examine potential problems. To determine whether the 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 (Nodes 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. Vial [CO_2] was analyzed by GC immediately, and at 11, 22 and 46 h. Respiratory potential on a mass basis was then calculated for each incubation period (i.e., 0–11, 11–22 and 20–32 h).

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

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

where k_2 and k_1 are 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/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-old trees from the above harvest, one 8-mm core from each of the +200-year-old trees, and one 12-mm core from each of the +15-year-old trees. Cores were prepared and analyzed as described. Only inner bark, and 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. The incubation period was 6 h.

To determine whether the observed CO_2 production was a result of diffusion of residual, stored CO_2 within cores, respiratory potential in cores treated with and without chloropicrin (a fumigant that kills live cells, often used as an insecticide) were compared 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 (1 ml) was pipetted into a vial placed in the bottom of the desiccator. Vacuum grease was used to seal the desiccator lid. Untreated core segments were stored similarly with the exception of chloropicrin. Core segments were stored for 18 h in a fume hood at 23 °C, and then placed in sterile vials in a sterile laminar-flow hood, septum-sealed and analyzed as described above. Immediately following the analysis, core segments were submerged in 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 not release CO_2 and would not stain red, whereas controls would release CO_2 and stain red. Carbon dioxide released by chloropicrin-treated core segments that did not stain red would indicate that stored CO_2 was released from live segments.

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 the 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). The volume of each radial position 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 compare our core-based measurements of respiratory potential across the three age classes in this study, and with 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 to Node 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 with 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 man-

ner, we calculated weighted respiratory potentials for whole trees.

Statistical analysis

All data were analyzed with Statistical Analysis Systems software, Release 7.0 (SAS Institute, Cary, NC). 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. We report means ± standard error, except transformed means, where confidence intervals are used.

Repeated measures analysis 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 was used to describe the relationship between temperature and respiratory potential. To compare Q_{10} values among the four radial positions at different temperature ranges, least squares means were calculated 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 with Fisher's protected least significant difference 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 used to generate least square means and make comparisons with Fisher's protected least significant difference procedure 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 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 for leaf area calculations, standard errors were not pooled at each step of the calculation. For simplicity, they were 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 analysis of variance was performed to generate least square means, with the understanding that year sampled may have been a confounding variable. Specific pair-wise comparisons among age class means were conducted with Fisher's protected least significant difference procedure.

Results and discussion

Effects of incubation time

The rate of CO_2 production (respiratory potential) for inner bark was constant over incubation times of 11, 22 and 46 h at all vertical positions (Figure 1a). 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 (Figures 1b–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 increasing incubation time at all vertical positions, except Node 15, where it was fairly constant (Figures 1b–d). 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 a result of the [CO_2] history of the plant, whereas direct effects result from the [CO_2] at the time of respiration measurement (Amthor 1991). Our ponderosa pine sapwood results indicated that respiration rates began to increase when the [CO_2] within the vials reached 1–5%. Indirect effects of increased rates of apparent respiration have been observed, primarily in crop plants, leaves (e.g., Azcón-Bieto et al. 1983) and tree roots (e.g., McDowell et al. 1999). Indirect effects are hypothesized to result from high concentrations of nonstructural carbohydrates in tissues 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 nonstructural carbohydrates (Lev-Yadun and Aloni 1995), the indirect effects of [CO_2] reported for storage organs and roots may also explain our findings. Clearly, further investigation is needed to better understand responses of stem wood respiration to changes in intercellular [CO_2].

Temperature effects

To regress respiratory potential on temperature, respiratory potential was log-transformed to correct for nonconstant variance in the residual plots. Because the response variable was still nonlinear and nonconstant 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 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 signif-

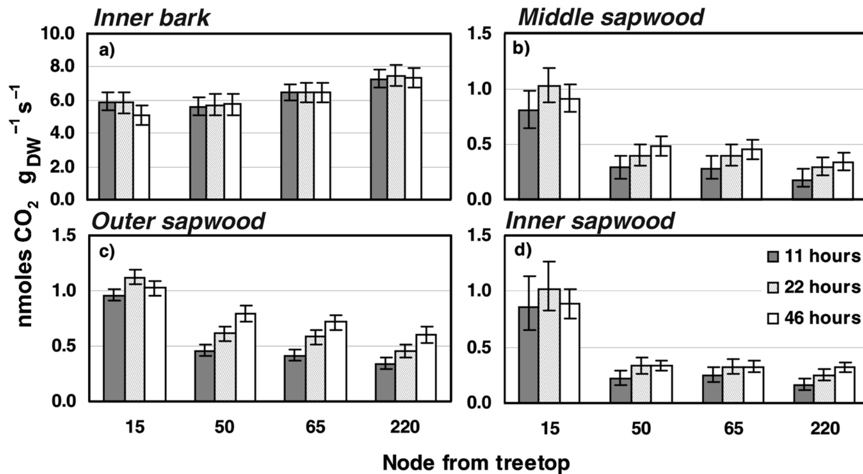

Figure 1. Core segment respiratory potential (nmol CO_2 g_{DW}^{-1} s^{-1}) at 25 °C with respect to incubation time for each radial position at each vertical position (note difference in *y*-axis scale for inner bark). Different bar shades represent different incubation periods. Time intervals between gas chromatography readings are not equal. Values are means ± standard error (inner bark and outer sapwood), or confidence intervals (middle and inner sapwood), *n* = 6.

icant to the fitted quadratic equation (*P* = .06), indicating that the intercept was probably near zero (Table 2). However, because we measured respiratory potential at 0 °C, we did not drop β_o from the equation.

Inner bark and sapwood both responded to increasing temperature by increasing their respiratory potentials (Figures 2a–d, Table 2). This response has often been attributed to increases in respiratory enzyme activities with increasing temperature (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 for respiration rate. To account for correlation among the four radial positions when comparing core respiratory responses to temperature, we calculated Q_{10} values 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 between temperature range and tissue radial position, each had significant effects on Q_{10} ($P < 0.0001$). There was a significant interaction term largely because inner bark Q_{10} dropped from about 2.6 to 1.5 between the ranges of 10–15 °C and 15–25 °C, whereas sapwood Q_{10} dropped from about 6.0 to 1.4 between the ranges of 5–10 °C and 10–15 °C (Figure 2). Decreasing Q_{10} with increasing temperature is in accordance with the findings of Larcher (1983), who concluded that plant Q_{10} values approach 2.0 at 5–25 °C, increase to > 3.0 below 5 °C, and drop to < 1.5 above 25–30 °C. This temperature dependence of Q_{10} is due to a shift in activation energy of respiratory enzymes (Lyons 1973). This shift occurred at a higher temperature range for inner bark than for sapwood in our study. Effect of tissue radial position alone was insignificant ($P = 0.8$) because at any given temperature range, sapwood Q_{10} was fairly uniform among the three radial positions (Figures 2b–d). This uniformity of sapwood Q_{10} (and inner bark at 15–25 °C, Figure 2a) was notable because cambial age ranged from 1 to +100 years from bark to the sapwood–heartwood boundary. Thus, respiratory enzymes in the live stem wood responded to temperature similarly, regardless of tissue age.

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. Respiratory potential was significantly higher in large inner sapwood segments than in small inner sapwood segments of +200-year-old trees ($P = 0.03$) and significantly lower in large inner bark segments than in small inner bark segments of +15-year-old trees ($P = 0.05$, Table 3). Large core segments had 40–70% of the surface/volume ratio of small segments, so if wounding increased respiration, one would have expected higher respiratory potential in small segments than in large segments, which is contrary to our results (except inner bark of +15-year-old trees).

Table 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). Values are estimates ± standard error, and significance to equation is indicated by asterisks: * = $P < 0.05$; ** = $P < 0.01$ and *** = $P < 0.001$.

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

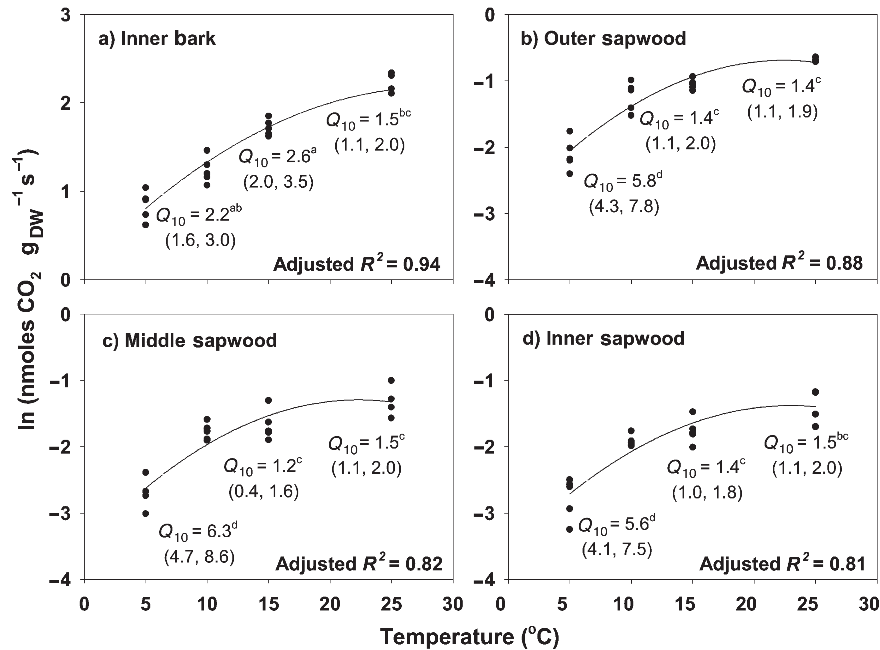

Figure 2. Relationship between temperature (°C) and natural log of stem respiratory potential (nmol CO_2 g_{DW}^{-1} s^{-1}) of core segments extracted at breast height from +200-year-old trees. Relationships are shown for (a) inner bark, (b) outer sapwood, (c) middle sapwood and (d) inner sapwood (note 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^2 values 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, $P < 0.05$).

Effects of chloropicrin treatment

Chloropicrin-treated core segments (inner bark, outer and inner sapwood) released < 1% of the CO_2 of controls in both +200- and +50-year-old trees, indicating that there was little stored CO_2 in segments (Figure 3). Additionally, chloropicrin-treated core segments formed no red color when treated with triphenyl tetrazolium chloride (TTC) solution, whereas untreated segments were a deep red color except at the 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 to diffusion of stored CO_2. Further, because TTC staining was fairly uniform throughout most of the core segments, there was no evidence that coring caused a wound response by killing cells on segment surfaces, e.g., due to heat at the core borer.

Growth rates in young versus old trees

Height growth over the last 15 years in +200-year-old trees was not significantly different from that in +50- or +15-year-old trees (P = 0.1); however, it was significantly greater in +50-year-old trees than in +15-year-old trees (Table 1, P =

Table 3. Effect of core diameter on core segment respiratory potential at 25 °C, extracted from breast height of +200- and +15-year-old trees. Values are means ± standard error (n = 6). For each tree age class and radial position, significant differences from paired *t*-tests between small and large cores are indicated by an asterisk: * = P < 0.05

Core size (mm)	Respiratory potential by radial position (nmol CO_2 g_{DW}^{-1} s^{-1})		
	Inner bark	Outer sapwood	Inner sapwood
Old (+200 years) trees			
8	7.4 ± 0.7	0.40 ± 0.03	0.21 ± 0.02*
5	5.1 ± 1.0	0.36 ± 0.03	0.12 ± 0.02*
Young (+15 years) trees			
12	6.8 ± 0.4	0.60 ± 0.06	–
5	8.0 ± 0.7	0.59 ± 0.04	–

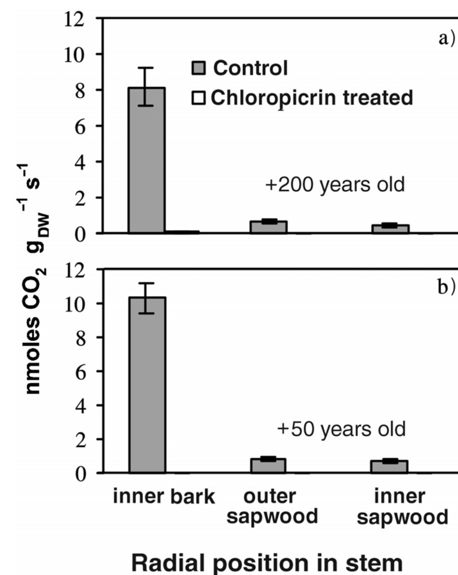

Figure 3. Effect of chloropicrin on respiratory potential (nmoles CO_2 g_{DW}^{-1} s^{-1}) at 25 °C of core segments extracted at breast height from +200- and +50-year-old stems. Values are means ± standard error, n = 6.

0.01). Diameter growth (at 1 m height) over the last 15 years was not significantly different among the three age classes (Table 1, $P = 0.1$). Stem wood and bark biomass growth was significantly greater in +200-year-old trees than in +50- or +15-year-old trees, and was also greater in +50- than in +15-year-old trees (Table 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 1). These results contrast with those recorded for *Pinus ponderosa* from a nearby site in Black Butte, OR (Ryan et al. 2000), where biomass growth per unit leaf area was 2–10 times greater in young trees (10–80 years old) than in old trees (390 years old). Growth rates of young pines from the former study were 2–6 times greater than those of the young pines in our study, whereas growth rates of old pines from the former study were 1–2 times those of the old pines discussed here. Thus, our young pines may have been suppressed, and the three age classes may not represent a true chronosequence for pines.

Radial trends in core segment respiratory potential

In all tree age classes and at all vertical positions on stems, rate of CO_2 production (nmol CO_2 g_{DW}^{-1} 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 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 the sapwood–heartwood boundary have been reported for O_2 uptake (Goodwin and Goddard 1940, Shain and Mackay 1973), as well as for CO_2 production (Møller and Müller 1938) in isolated stem tissue (in vitro) from various tree species. Our values for CO_2 production (Table 4) were 2–6 times greater than those for O_2 uptake in the earlier studies. Possible explanations for this discrepancy include 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 middle sapwood rate was also significantly higher than inner sapwood rate (Table 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 or dormancy, or both, of metabolic activity in sapwood parenchyma cells. This interpretation is supported by the finding that ray cell nuclear morphology changes from outer to inner sapwood in various conifer species, thus indicating decreased ray vigor (Frey-Wyssling and Bosshard 1959, Yang 1993, Gartner et al. 2000). Also,

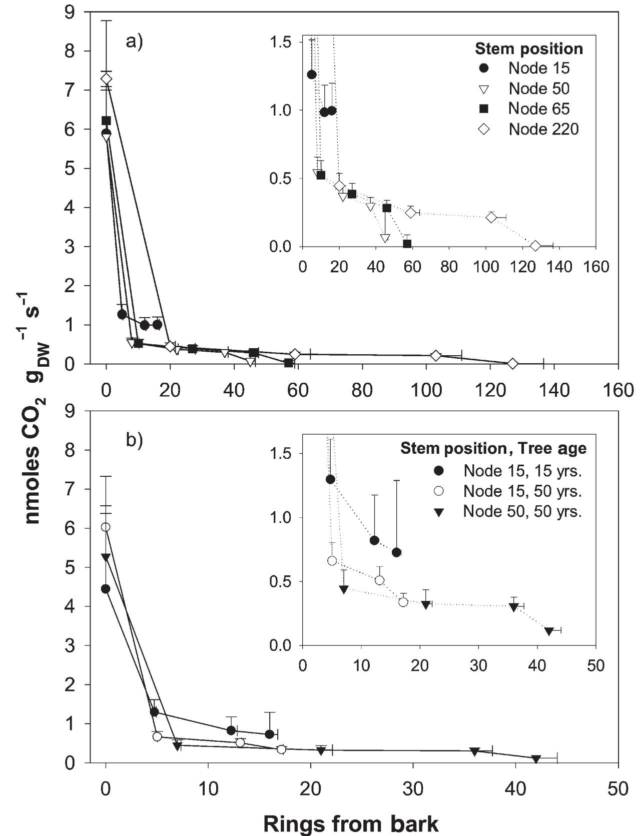

Figure 4. Relationship between tissue age and respiratory potential (nmol CO_2 g_{DW}^{-1} s^{-1}) of core segments extracted from one to four vertical positions in (a) +200-, (b) +50- and +15-year-old stems. For each stem position, the highest point represents inner bark activity, followed by outer, middle and inner sapwood and heartwood. Cores from Node 15 had no heartwood. Insets provide detailed views of sapwood and heartwood trends. For clarity, only (+) confidence intervals appear in graphs. Means and confidence intervals ($n = 6$) are given in Table 4.

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 they receive reactivation signals from the cambial or apical meristem, or both (Shain and Mackay 1973, Bamber 1976).

Vertical trends in core segment and stem node respiratory potential

Effects of stem vertical position on core segment respiratory potential per unit dry mass were significant in both +200- and +50-year-old trees ($P < 0.0001$ and $P = 0.002$, 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 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 $P = 0.005$, respectively), indicating that rate of decline in activity from the bark to the sapwood–heartwood boundary

Table 4. Core segment respiratory potential at 25 °C versus radial position, vertical position and tree age. Values are least square means and 95% confidence intervals, except for +50-year-old heartwood, where ± standard error are shown (n = 6). Different letters indicate significant differences among means (Fisher's protected least significant difference procedure, $P < 0.05$). Each age class was analyzed separately. Within each age class, heartwood was analyzed independently from inner bark and sapwood.

Tree age (year)	Node	Respiratory potential (nmol CO_2 g_{DW}^{-1} s^{-1})				
		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	6.21a	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)	–

was not the same at all stem vertical positions. The finding that respiratory potential was fairly uniform throughout the +200-year-old stems (Node 220–50) corroborates reports by 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*. 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, the 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. However, it was not significant ($P = 0.5$) in +50-year-old trees (Table 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-old trees, and significantly lower ($P < 0.04$) near tree bases (Node 220) in +200-year-old trees than at either Node 50 or 65 (Table 5). Thus, mass- and volume-based respiratory potentials both increased significantly toward treetops.

The marked increase in respiratory potential near the treetop in ponderosa pine may be explained by the close proximity of Node 15 to the substrate supply, where physiological activities such as growth, substrate metabolism and transport are high. Alternatively, there may have been a higher percentage of ray parenchyma in the sapwood at Node 15 than at 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*. 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 (Haffner et al. 1991).

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 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 of +200- or +15-year-old trees (Table 5, $P < 0.02$). Respiratory potential per unit outer-bark surface area was significantly higher in +200-year-old tree bases than in +50-year-old tree bases (tree base to Node 15, Table 5, $P < 0.0001$). In contrast, respiratory potential per live wood volume was significantly lower in +200-year-old tree bases 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 5, $P = 0.3$). Whole-tree rate of CO_2 production per unit outer-bark surface area was significantly higher in +200-year-old trees than in either +50- or +15-year-old trees ($P < 0.0001$), whereas CO_2 production rate per unit volume (live and stem wood) was significantly lower in +200- and +50-year-old trees than in +15-year-old trees (Table 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 our 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 demonstrates that estimates of whole-tree 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,

Table 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, values are means ± standard error, $n = 6$. For respiratory potential, the least square means ± standard error, or 95% confidence intervals in parentheses ($n = 6$), are from a strip-plot model (by node), or from a one-way analysis of variance model (by segment). Different capital letters within a column (node), or different lowercase letters within a 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.

Parameter, stem segment	Tree age class (year)		
	+200	+50	+15
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	–	–
RESPIRATORY POTENTIAL			
Per outer-bark surface area ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) 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 ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) 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 ($\mu mol\ CO_2\ m^{-3}\ s^{-1}$) 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 ($\mu mol\ CO_2\ m^{-3}\ s^{-1}$) 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 ($\mu mol\ CO_2\ m^{-3}\ s^{-1}$) 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 ($\mu mol\ CO_2\ m^{-3}\ s^{-1}$) 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

young stem segments because the surface area/volume ratio was smaller in +200-year-old trees than in +15-year- old trees (10:1 versus 30:1 m). 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 unit sapwood volume to represent whole-tree level respiration instead of the weighted mean of all nodes overestimated whole-tree respiration by 200%, whereas using only Node 220 respiratory potential underestimated whole-tree respiration by 12% (Table 5). Further research is needed both to ascertain whether similar scaling complexities exist in other species and to incorporate diurnal or seasonal effects. Tissue nitrogen content or parenchyma cell volume should also be explored as a possible basis for expressing respiratory potential.

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