

# Within-stem oxygen concentration and sap flow in four temperate tree species: does long-lived xylem parenchyma experience hypoxia?

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## ABSTRACT

Oxygen levels as low as 1–5% (gaseous mole fraction) occur in secondary xylem, but it is not known if there is a consistent pattern of decline in O<sub>2</sub> from the cambium toward the pith, or whether parenchyma cells experience hypoxic conditions deep within the stem. We developed a system for repeated *in situ* measurement of O<sub>2</sub> at different depths within stems of *Acer rubrum*, *Fraxinus americana*, *Tsuga canadensis*, and *Quercus rubra*. In summer during active transpiration, O<sub>2</sub> declined from the cambium toward the heartwood boundary in *F. americana*, *T. canadensis* and *Q. rubra*, but remained constant in *A. rubrum*. Average sapwood O<sub>2</sub> was about 10%, with the lowest values observed in the innermost sapwood around 3–5%. Before spring leaf flush, O<sub>2</sub> content in the outer sapwood was reduced in *Q. rubra* and *T. canadensis* relative to summer, and was occasionally lower than in the inner sapwood. Sapwood respiration in *T. canadensis* was constant above 5% O<sub>2</sub>, but reduced by about 65% at 1% O<sub>2</sub>. In *F. americana*, sapwood respiration was constant above 10% O<sub>2</sub> but reduced by 25% at 5% O<sub>2</sub>, and by 75% at 1% O<sub>2</sub>, the most extreme inhibition observed. However, when prolonged (72 h) exposure to 1%, 5% and 10% O<sub>2</sub> was followed by re-equilibration to 10% O<sub>2</sub>, no inhibition was found. Given the minor (and reversible) effect of low O<sub>2</sub> on parenchyma metabolism at levels common in the inner sapwood, it is unlikely that O<sub>2</sub> content severely limits parenchyma respiration or leads to parenchyma cell death during sapwood senescence. Within-stem O<sub>2</sub> levels may instead be most relevant to metabolism in the cambial zone and phloem, for which sapwood could serve as a significant source of O<sub>2</sub>.

**Key-words:** heartwood; hypoxia; oxygen; respiration; sap flow; sapwood; xylem parenchyma.

## INTRODUCTION

The gas composition inside large woody stems differs significantly from that of the ambient atmosphere because of cellular respiration in the xylem, phloem and cambium,

and the slow rate of gas diffusion through stem tissue. Secondary xylem is typically low in O<sub>2</sub> and enriched in CO<sub>2</sub>, both of which show seasonal and diurnal variation. Reported values for O<sub>2</sub> within the xylem during the growing season range from 1 to 5% (mole fraction expressed as a percent) (Eklund 1993, 2000; Gansert, Burgdorf & Losch 2001; Mancuso & Marras 2003) to 12–19% (del Hierro *et al.* 2002; Pruyn, Gartner & Harmon 2002b), with variation due to differences in time of year, species and ages studied, and position within the stem. Diurnal variation in O<sub>2</sub> within xylem is also significant, but small, with reports ranging from 2 to 5% (that is, %O<sub>2</sub> at the same position may fluctuate between two and five percentage points daily; Gansert *et al.* 2001; del Hierro *et al.* 2002; Mancuso & Marras 2003). This variation is presumably caused by the net effect of changes in parenchyma respiration with temperature and changes in the rate of O<sub>2</sub> supply by the transpiration stream (e.g. del Hierro *et al.* 2002). Although radial diffusion of O<sub>2</sub> through the bark occurs, its contribution to the total supply of O<sub>2</sub> relative to that of the transpiration stream is likely smaller than once thought (Mancuso & Marras 2003).

Secondary xylem contains 5–35% parenchyma by volume depending on species (Panshin & de Zeeuw 1980), with conifers ranging from about 5–8% and angiosperms from 10 to 35%. These cells live for anywhere from two to 150 years, also depending on species, and may exist at depths of 20 cm or more within the stem. It is the death of xylem parenchyma in the innermost sapwood that defines and drives the formation of heartwood (i.e. heartwood no longer contains living parenchyma cells; Chattaway 1952; Frey-Wyssling & Bosshard 1959), and there is often a decline in sapwood respiration from the cambium toward the sapwood/heartwood boundary (Goodwin & Goddard 1940; Pruyn, Gartner & Harmon 2002a; Pruyn *et al.* 2002b). Most measurements of xylem O<sub>2</sub> have been made in small stems or at shallow radial depths (~1 cm), and it is not known how O<sub>2</sub> levels change across the sapwood in large stems, or how relevant these levels are to parenchyma physiology.

Increasing evidence suggests that the transpiration stream is a major source of O<sub>2</sub> in the xylem (Gansert *et al.* 2001; del Hierro *et al.* 2002; Mancuso & Marras 2003). However, in many species there are regions of secondary

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xylem that contain living cells but no longer conduct water. Sapwood contains living parenchyma cells by definition (Panshin & de Zeeuw 1980), but in many trees only the outermost sapwood is active in water transport (Phillips, Oren & Zimmermann 1996; James *et al.* 2002). In conifers, in which the innermost sapwood remains conductive, water transport may cease shortly before parenchyma cell death during heartwood formation (Nobuchi & Harada 1983). Oxygen has been shown to decline to about 6% near the sapwood/heartwood boundary in *Pseudotsuga menziesii* (Pruyn *et al.* 2002b), but it is not known if hypoxic or anoxic conditions occur in the innermost sapwood of other species, or if xylem parenchyma respiration is inhibited at O<sub>2</sub> levels common within stems. Despite the current gaps in our understanding of the relationship between within-stem O<sub>2</sub> and parenchyma physiology, it has been suggested that O<sub>2</sub> depletion is responsible for the decline in respiration and ultimate death of xylem parenchyma cells during the transition from sapwood to heartwood (Panshin & de Zeeuw 1980). One study also suggests that anoxia, generated by flushing with either CO<sub>2</sub> or N<sub>2</sub>, can accelerate the rate of heartwood formation (Eklund & Klintborg 2000).

The main objective of this study was to determine whether or not O<sub>2</sub> levels in the innermost sapwood, in which parenchyma cells die during the formation of heartwood, decline to levels low enough to either severely limit respiration or cause cell death. A novel technique is described that allows for repeated 'point' measurements of O<sub>2</sub> within the xylem of multiple individuals over a period of one year or more. *In situ* O<sub>2</sub> levels at different radial depths are reported for four species representing a diverse range of xylem anatomies and sapwood longevities. Secondary objectives were to relate within-stem spatial patterns of cell death and sap flow to observed O<sub>2</sub> levels, and to determine the sensitivity of parenchyma respiration to low O<sub>2</sub>.

## MATERIALS AND METHODS

### Plants and field site

Six individuals each of *Fraxinus americana*, *Acer rubrum*, *Quercus rubra*, and *Tsuga canadensis* were randomly selected from a five hectare tract of natural forest in Harvard Forest, Petersham, MA, USA (42.5°N lat, 72°W long, 220 m elev). Mean temperatures at Harvard Forest are 20 °C in July, -7 °C in January, with 110 cm annual precipitation distributed evenly throughout the year. Trees were cored with a 5-mm borer at 1.4 m above-ground along two radii to determine the locations and ages of sapwood and heartwood (Table 1). All six trees were used for measurement of O<sub>2</sub> content at different radial depths. Three of the six trees per species were used for sap flow measurements. For sapwood respiration measurements, large diameter (12 mm) cores were extracted from 20 additional, randomly selected trees each of *T. canadensis* and *F. americana*.

**Table 1.** Diameter outside bark, age, and number of years and width of sapwood (SW; based on vital staining of live cells with triphenyl-tetrazolium chloride) for study trees

	d.b.h. (cm)	Age (year)	SW rings (year)	SW width (cm)
<i>Fraxinus americana</i>	34.7 ± 2.5	68 ± 5	45 ± 2	7.9 ± 0.9
<i>Acer rubrum</i>	33.7 ± 1.7	49 ± 3	38 ± 3	10.1 ± 1.0
<i>Tsuga canadensis</i>	39.2 ± 4.0	60 ± 2	25 ± 2	5.8 ± 0.8
<i>Quercus rubra</i>	33.1 ± 5.8	57 ± 6	8 ± 1	1.8 ± 0.3

All measurements were made at 1.4 m height. Mean (± SE) shown for *n* = 6 trees per species.

### Oxygen probe tube construction and installation

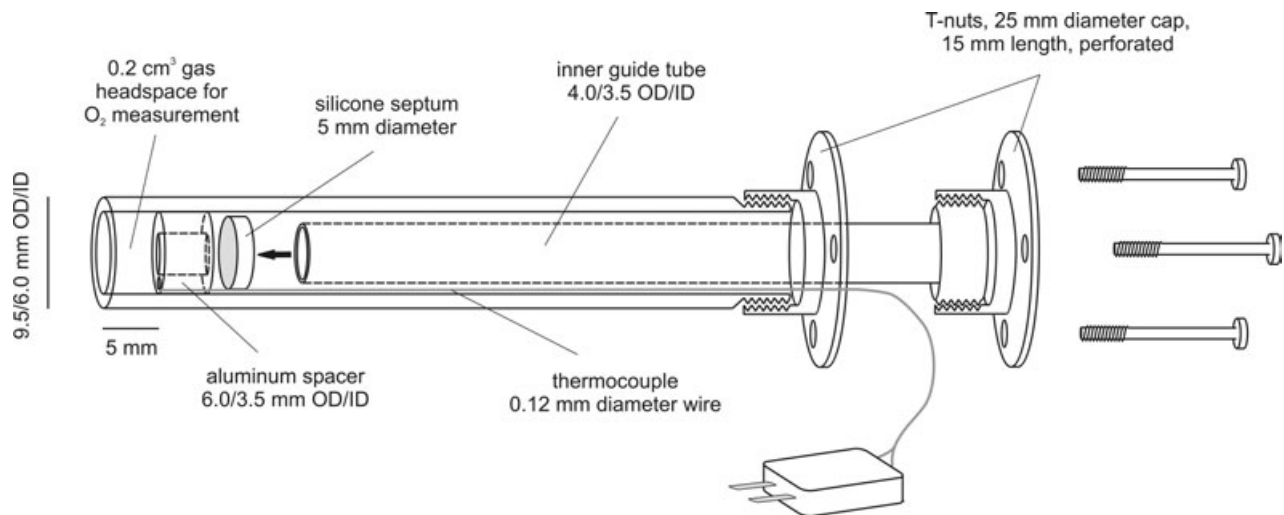
Metal tube assemblies were permanently installed in each of the 24 trees (six per species) used for O<sub>2</sub> measurements. Stainless steel tube assemblies (Fig. 1) allowed for a small (about 0.2 cm<sup>3</sup>) gas headspace within the tree to be accessed periodically with a needle-tipped fibre-optic O<sub>2</sub> sensing probe through a layered silicone rubber septum. A gas tight seal was formed by compressing the septum against an aluminium spacer with a smaller diameter tube, which served as a guide for the O<sub>2</sub> probe during measurements (i.e. the probe was threaded through the inner guide tube to pierce the septum). Tubes ranged in length from 5 to 18 cm, depending on the depth of insertion into the tree. The septum could be replaced and resealed after repeated use. Because it is necessary to correct all O<sub>2</sub> measurements for temperature, a thermocouple junction was sealed in the innermost end of the tube assembly, adjacent to the headspace, with the leads extending to a thermocouple connector outside the tree.

Tubes were installed in all trees in April 2002 before the start of cambial activity. Holes 9 mm in diameter (0.5 mm smaller than the tube diameter) were drilled to three or four radial depths per tree (Table 2), all at 1.4 m height, spaced evenly about the circumference of the tree and placed randomly with respect to compass direction. Tubes were installed in the heartwood and innermost and outer-

**Table 2.** Maximum depth (cm inside cambium) of O<sub>2</sub> probe tube assemblies (mean ± SE; *n* = 6 trees)

	Outer	Middle	Inner	HW
<i>Fraxinus americana</i>	0.5	3.8 ± 0.5	7.0 ± 0.9	10.7 ± 0.9
<i>Acer rubrum</i>	0.5	5.0 ± 0.5	9.0 ± 1.0	12.6 ± 0.5
<i>Tsuga canadensis</i>	0.5	3.8 <sup>a</sup>	4.5 ± 0.6	10.6 ± 1.1
<i>Quercus rubra</i>	0.5	–	1.3 ± 0.3	8.4 ± 1.5

Inner positions were placed approximately 5 mm outside the sapwood/heartwood (SW/HW) boundary, with middle positions placed equidistant between the outer and inner positions in species with wide sapwood, and in one individual of *T. canadensis*<sup>a</sup>. Sap flow probes were installed to the same maximum depths except for the outer position, where probes were installed to 1 cm, so that the entire heated/sensing region was in the sapwood.



**Figure 1.** Oxygen tube assembly. A removable inner guide tube compresses a silicone septum against the aluminum spacer to form a gas-tight seal, allowing the headspace to come into equilibrium with the local internal gas composition in the stem. Oxygen measurements are made by threading the needle-tipped  $O_2$  probe through the inner guide tube and penetrating the septum. The aluminium spacer is glued in place with a thermocouple junction exposed to the gas headspace and wire leads running to a connector outside of the tree. Small bolts were used to tension the inner guide tube against the stationary outer tube to compress the septum, which could be removed and replaced after repeated use.

most sapwood in all four species, and midway between the two sapwood positions in *F. americana* and *A. rubrum*, both of which have relatively wide sapwood. Tubes were coated outside along their length with all-weather epoxy and hammered into the drilled holes to the desired depths, leaving about 3 cm of the tube extending out of the tree. A final bead of epoxy was placed in a circle around the point of insertion against the bark. When not in use, the opening to each inner guide tube was plugged with a small rubber stopper and the entire tube assembly extending from the tree was enclosed in a 3-mil plastic bag for protection from rain and animals.

### Oxygen measurements

Oxygen measurements were made on all trees during June 2002, when leaves were fully expanded and there was active transpiration, and April 2003, before leaf flush. Oxygen was measured in the headspace behind the septum with a needle-tipped (21 gauge) fibre-optic probe and spectrometer-based sensing system (Ocean Optics, Inc., Dunedin, FL, USA). The probe was calibrated in the laboratory with humidified gas standards, and temperature calibrated using gas standards incubated in a water bath over the range of 15–35 °C. The spectrometer was carried in the field in an insulated cooler and connected to a laptop computer. Readings of ambient air  $O_2$  (corrected for temperature) between within-stem  $O_2$  readings allowed for frequent checks of the calibration. Within-stem  $O_2$  readings stabilized within 15–20 s of insertion through the septum, and repeated insertions gave readings within  $\pm 0.1\%$  (mole fraction; e.g. between 7.8 and 8.0%  $O_2$ ). Temperature within the headspace was recorded for each  $O_2$  measurement for later corrections.

Each tree was measured for  $O_2$  on multiple (3–5) days, typically between 1000 and 1500 h, and the mean over several days was taken. Diurnal variation was estimated both by making point measurements every 5 h on the same tree, and by continuously monitoring a single position within the sapwood. Both measurements suggested that maximum diurnal variation was about 3% (i.e. the same position might read between 6 and 9% over a 24-h period) in regions of active sap flow, which agrees with other published reports (Gansert *et al.* 2001; del Hierro *et al.* 2002; Mancuso & Marras 2003). Tests for gas leakage around the tube were performed in the laboratory on fresh stem segments, sealed at both ends with wax, with a PVC chamber sealed against the bark and around the tube through which 100%  $O_2$  was continually flushed. There was no evidence of increased rates of diffusion of  $O_2$  into the stem (as measured with the fibre-optic  $O_2$  probe) as a result of  $O_2$  flushing.

### Sap flow measurements

Granier sap flow sensors of variable lengths were constructed following a modification of the methods of James *et al.* (2002). Briefly, 12-mm-long segments of 2.4 mm diameter aluminium tubing were crimped onto the ends of lengths of PEEK tubing, so that 10 mm served as the heated/sensing length. A 0.8-m length of resistance wire (120  $\Omega$  total resistance) was used to generate 0.15 W of power per probe by supplying a constant current of 35 mA with a voltage regulator and potentiometer.

Probes were installed in June 2002 in three of the six trees used for  $O_2$  measurements, with the maximum (innermost) radial depth matching the maximum depth of the  $O_2$  probe guide tubes (Table 2). Probes were installed at the same depth and height as each  $O_2$  tube, but between 5 and 10 cm

away horizontally. Constant-current heater circuits were powered by deep cycle batteries in the field, and data was collected with a Campbell datalogger/multiplexer every 10 or 15 min. Because of the total number of probes used and distances between trees in the field, not all probes were monitored at the same time. The standard Granier calibration (Granier 1987) was used to convert the temperature difference between the heated and unheated probes to sap flux density ( $\text{g m}^{-2} \text{s}^{-1}$ ) assuming a constant velocity along the 1 cm sensor length. This calibration has been validated (Clearwater *et al.* 1999) and tested both for this probe design (James *et al.* 2002) and for the species included in this study (Catovsky, Holbrook & Bazzaz 2002). Unheated data was collected for several days for each probe after installation to check for intrinsic diurnal trends in temperature difference. In all cases the unheated diurnal variation was less than 1% of the diurnal variation for heated probes.

### Sapwood tissue respiration

The effect of low O<sub>2</sub> on sapwood respiration was tested by measuring rates of O<sub>2</sub> consumption by fresh sapwood following equilibration to known O<sub>2</sub> concentrations. Four large increment cores (12 mm diameter) were extracted from each of 10 individuals of *F. americana* and *T. canadensis* in July 2004. These species were chosen to include a conifer and angiosperm with significant declines in O<sub>2</sub> across the sapwood. Segments about 1 cm<sup>3</sup> were cut from the outer sapwood (excluding the cambial zone), weighed, wrapped in moist cheesecloth, and placed in open glass vials (6 mL). One of each of the four cores per tree was randomly assigned to a gas treatment: 1, 5, 10 and 15% O<sub>2</sub> (balance N<sub>2</sub>). Open vials were placed in disposable plastic glove bags and flushed repeatedly (five or six times) with the treatment gas, sealed, and placed in cold (4 °C) storage. Vials were incubated under the treatment gas for 36 h with flushes at 12 and 24 h. Prior tests had shown that 36 h was sufficient to fully equilibrate the samples so that their internal gas composition matched that of treatment gas, while cold storage suppressed respiration and extended the length of time respiration rates (after return to 20 °C) remained stable. At the end of the equilibration period, the vials were sealed (following a final flush) with aluminium crimp-top lids with a central butyl rubber septa. Sealed vials were then transferred to a water bath where they were incubated at 20 °C throughout the measurement period.

Oxygen was measured in each vial every 4 to 6 h for 24–36 h by penetrating the septum with a needle-tipped O<sub>2</sub> probe, which was calibrated with humidified standards at 20 °C. The slope of the linear portion of the curve (typically the first 24 h) was taken as the rate of O<sub>2</sub> consumption and converted to mol O<sub>2</sub> h<sup>-1</sup> by calculating the total volume of gas in the vial. Samples were then removed from vials, measured for fresh volume by volume displacement, dried in an oven at 105 °C for 36 h, and weighed. Respiration was expressed on a per fresh tissue volume basis (i.e. mol O<sub>2</sub> cm<sup>-3</sup> h<sup>-1</sup>). Similar to other published results (Pruyn *et al.* 2002a,b) tests showed that respiration was unchanged

following 2 weeks of cold storage. Fresh weight, fresh volume and dry weight were used to calculate the total fresh tissue volume occupied by air, water and cell wall material by assuming a constant density for oven-dried solid cell wall material of 1.5 g cm<sup>-3</sup> (Panshin & de Zeeuw 1980).

The ability of parenchyma cells to recover from prolonged hypoxia was tested in a separate experiment using identical methods with the following exceptions. Outer sapwood segments from an additional 10 individuals each of *F. americana* and *T. canadensis* (three cores per tree, each randomly assigned to one of three treatments) were first incubated at 1, 5 and 10% O<sub>2</sub> for 72 h at room temperature to allow for normal respiration under prolonged exposure to the treatment gases. All samples were then transferred to 10% O<sub>2</sub> and allowed to re-equilibrate in cold storage (4 °C) for 36 h. Vials were then sealed, incubated at 20 °C in a water bath, and respiration rates were measured (all starting at 10% O<sub>2</sub>) as described above.

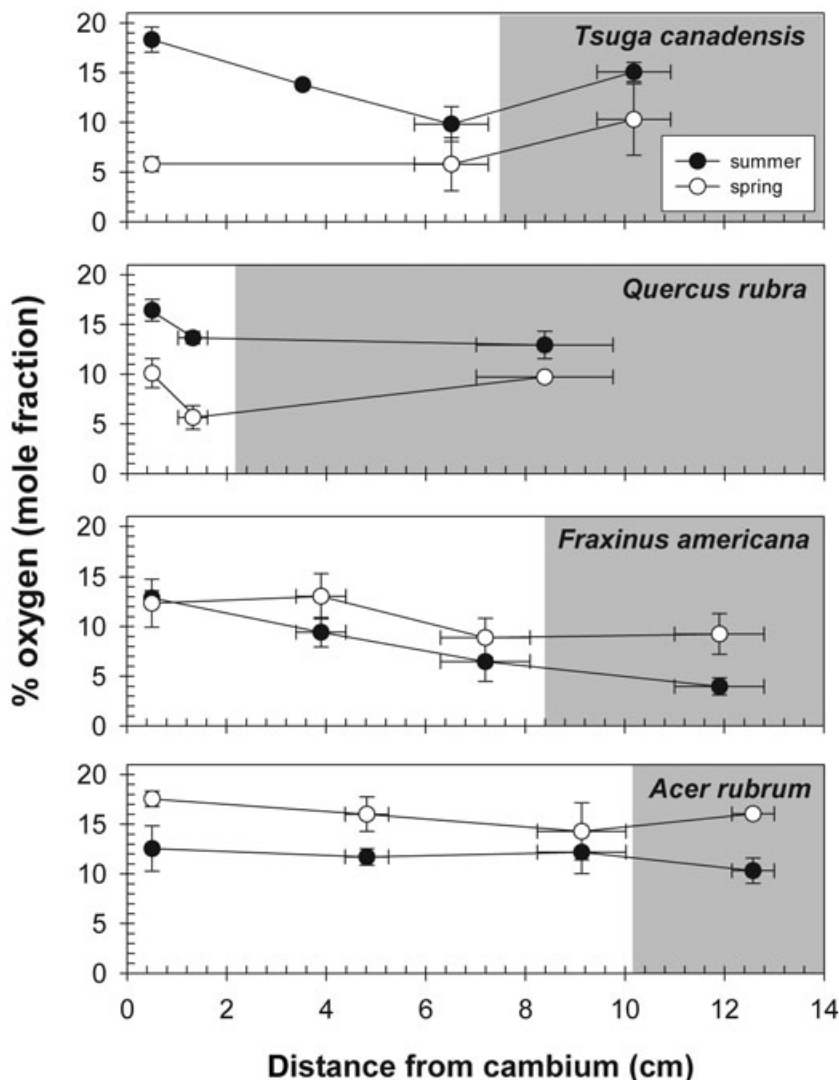
### Statistical analyses

Tissue respiration data were tested in an ANCOVA using the surface area of each sample as a covariate, due to the effect of surface area on rates of diffusion. The difference between species in the relative proportions of tissue occupied by cell wall, air and water was tested in an ANOVA. In both cases, tree within species was used as a blocking factor. The effects of radial position, season, and their interaction on *in situ* O<sub>2</sub> content were tested within each species in an ANOVA with tree as a blocking factor. A mixed model analysis, which would account for the hierarchical structure in the O<sub>2</sub> data (radial position within tree, tree within species, and repeated measures between seasons), was not performed due to the number of missing values (i.e. for any given radial position, only four of the six trees may be represented, and these trees likely differ between seasons), and the fact that species differed in the total number of radial positions. In all cases, significant main effects were compared with a Fisher LSD correction for multiple comparisons; significant interaction terms were compared using contrasts and Bonferroni-corrected *P*-values to limit the experiment-wise error rate (Sokal & Rohlf 1995).

## RESULTS

### Oxygen contents

Oxygen levels generally declined from the outer toward the inner sapwood in *Q. rubra* and *F. americana*, but remained constant in *A. rubrum* (Fig. 2). The inner sapwood had significantly lower O<sub>2</sub> than the outer sapwood in both seasons in *F. americana* and *Q. rubra* (Fisher adjusted *P*-values = 0.03 and 0.06, respectively) but only in the summer in *T. canadensis* (adjusted contrast *P*-value < 0.005). The effect of radial position depended on the season only in *T. canadensis* (i.e. the radial position × season interaction was significant; *P*-value = 0.04). The lowest O<sub>2</sub> levels recorded in the



**Figure 2.** Oxygen content (mole fraction expressed as percentage) at different radial depths within the stem during summer (leaves fully expanded) and spring (before leaf flush); mean  $\pm$  SE,  $n = 6$  trees. Shaded region represents heartwood as determined by vital staining with triphenyl-tetrazolium chloride. Oxygen was measured *in situ* with a fibre optic needle-tipped and spectrometer through permanently installed tubes.

innermost sapwood were about 3%, and these were observed in *F. americana*, *T. canadensis*, and *Q. rubra*, although rarely in each species.

Oxygen levels measured before leaf flush in the spring were significantly lower than those measured in the summer throughout the sapwood in *Q. rubra* ( $P$ -value  $< 0.0001$ ), but only in the outermost sapwood in *T. canadensis* (adjusted contrast  $P$ -value  $< 0.005$ ), and were unchanged in the heartwood. In contrast, spring  $O_2$  levels were higher than in summer in *A. rubrum* ( $P$ -value = 0.003) with no effect of radial position ( $P$ -value = 0.5). In *Fraxinus*, only radial position had a significant effect on  $O_2$  ( $P$ -value = 0.04), with higher  $O_2$  in the outer sapwood relative to both inner sapwood and heartwood (Fisher-adjusted  $P$ -values = 0.03 and 0.01, respectively).

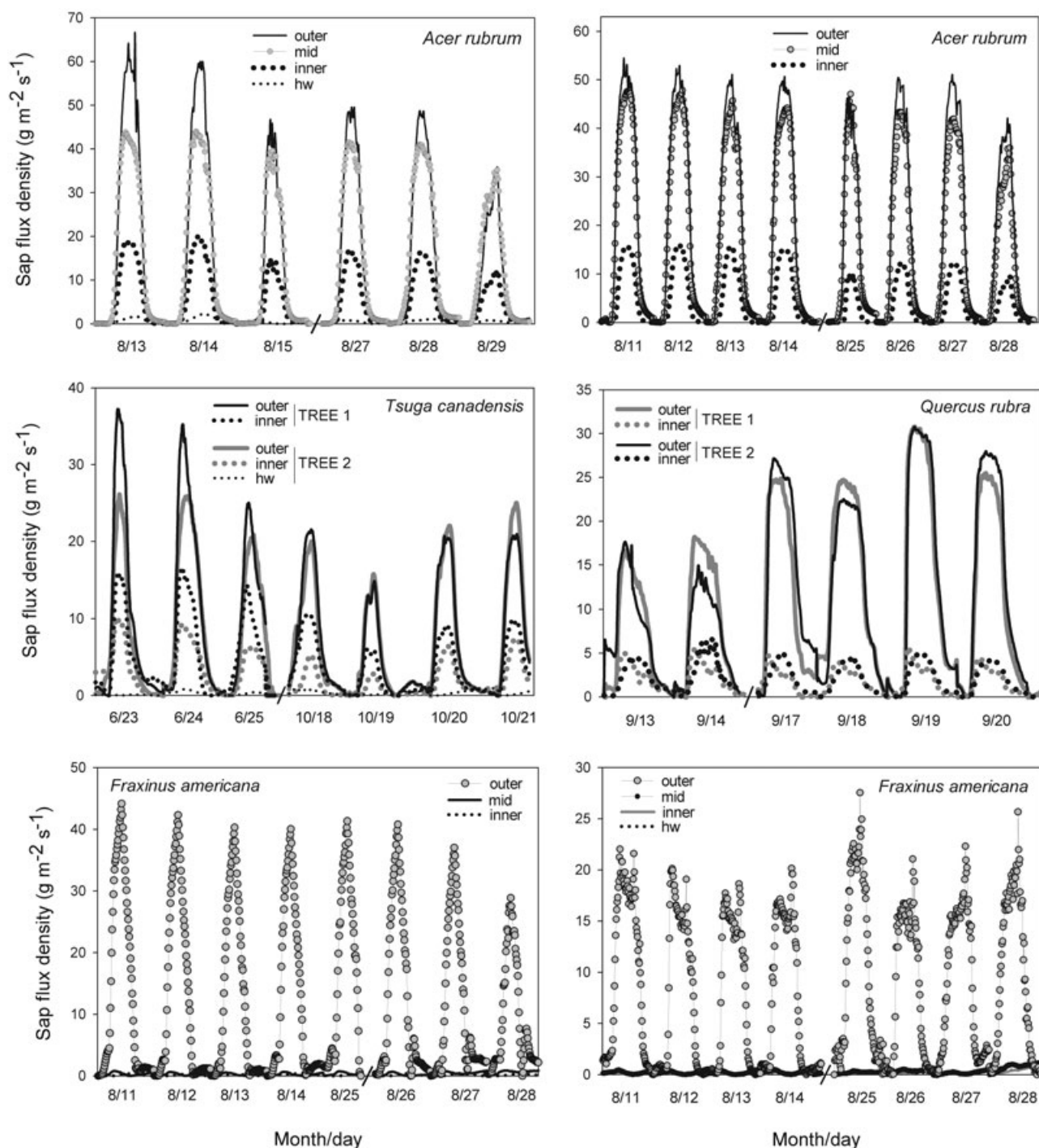
### Sap flow

Species varied considerably in the extent to which the inner sapwood was functional in water transport (Fig. 3). *A. rubrum*, *T. canadensis*, and *Q. rubra* all had significant flow

in the innermost sapwood ( $31 \pm 3$ ,  $37 \pm 7$  and  $25 \pm 6\%$  of outer flow, respectively; mean  $\pm$  SE across 6 d,  $n = 3$  trees). In *A. rubrum* the middle sapwood was nearly as conductive as the outer sapwood ( $87 \pm 6\%$  of outer flow rates), whereas in *F. americana* only the outer sapwood was conductive ( $< 1\%$  of outer flow in both middle and inner positions). In no case was heartwood conductive.

### Respiration and tissue air/water content

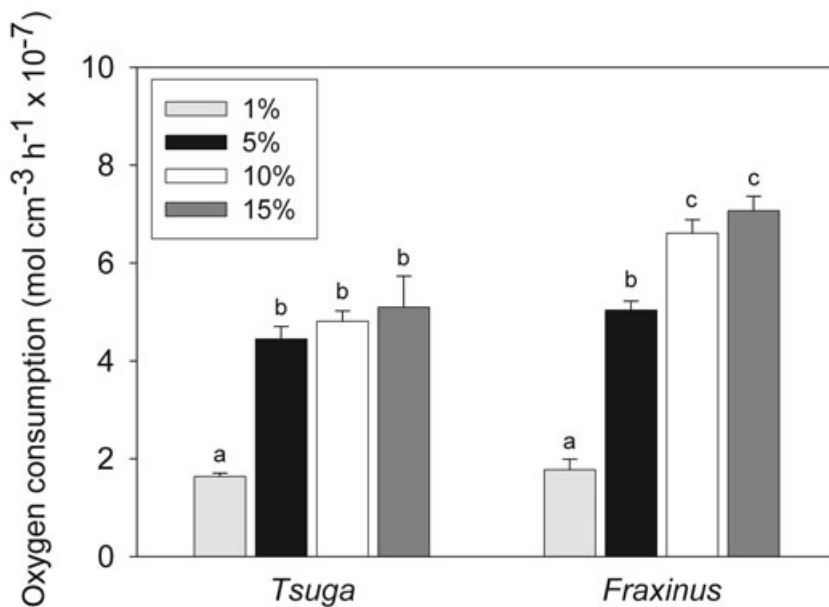
The effect of  $O_2$  level on sapwood respiration was strongly dependent on species (i.e. there was a significant species  $\times$  treatment interaction,  $P$ -value  $< 0.0001$ ), although respiration in *F. americana* and *T. canadensis* was unchanged at  $O_2$  levels above 10 and 5%  $O_2$ , respectively (Fig. 4). In *F. americana*, respiration was reduced by less than one-quarter between 10 and 5%  $O_2$ , and by about one-third between 5 and 1%  $O_2$ . In *T. canadensis*, only 1%  $O_2$  significantly reduced respiration relative to the maximum rate, but to a lesser extent than in *F. americana*. Sapwood respiration following 72 h of exposure to 1 and 5%  $O_2$ , and



**Figure 3.** Sap flux density ( $\text{g m}^{-2} \text{s}^{-1}$ ) at different radial depths within stems of *Acer rubrum*, *Tsuga canadensis*, *Quercus rubra*, and *Fraxinus americana*. Two individuals are shown for each species. Sap flow was measured at the same radial depths as O<sub>2</sub> measurements using modified Granier sensors with 1-cm-long heated/sensing tips. Outer positions were in the outermost 1 cm of sapwood; inner positions were 5 mm from the sapwood/heartwood boundary; middle (mid) was half way between inner and outer; heartwood (hw) was half way between the sapwood/heartwood boundary and pith.

then a period of equilibration to 10% O<sub>2</sub>, was unchanged in both species relative to the 10% O<sub>2</sub> control (Table 3). In no case did exposure to 1% O<sub>2</sub> for up to 7 d result in significant parenchyma death, as judged by vital staining with triphenyl-tetrazolium chloride.

The two species differed significantly in the proportions of fresh volume occupied by water, cell wall and air (Fig. 5; adjusted  $P$ -value  $< 0.0001$  in all cases). Sapwood of *F. americana* was composed of roughly equal proportions of water, air and cell wall material, with air occupying about  $31 \pm 1\%$



**Figure 4.** Respiration (mol O<sub>2</sub> cm<sup>-3</sup> h<sup>-1</sup> × 10<sup>-7</sup>) of excised sapwood tissue of *Tsuga canadensis* and *Fraxinus americana* following equilibration to four known O<sub>2</sub> concentrations. Within each species, bars with the same lowercase letters were not significantly different (adjusted *P*-values >0.05; contrasts of interaction means from ANCOVA).

(mean ± SE) volume, whereas *T. canadensis* tissue was only about 11 ± 1% air.

## DISCUSSION

Oxygen levels as low as 1–5% (gaseous mole fraction) have been recorded in sapwood, but most of these measurements have been made in young, small-diameter trees (Eklund, Little & Riding 1998; Mancuso & Marras 2003) or at shallow radial depths (e.g. 5–10 mm beneath the cambium; Eklund 1990, 1993; del Hierro *et al.* 2002). Increasing evidence suggests that the transpiration stream is a major source of O<sub>2</sub> in secondary xylem (Gansert *et al.* 2001; del Hierro *et al.* 2002; Gansert 2003; Mancuso & Marras 2003). Given that the cessation of water transport typically precedes the death of parenchyma cells during heartwood formation by one to many years, it is possible that parenchyma cells deep within stems experience hypoxic or even anoxic conditions. We have found that although O<sub>2</sub> levels are often reduced in the inner sapwood, they are not low enough to cause cell death, and would be unlikely to cause a biologically significant reduction in respiration rate except at the lowest O<sub>2</sub> levels. In four temperate species that differ widely in anatomy and sapwood longevity, average sap-

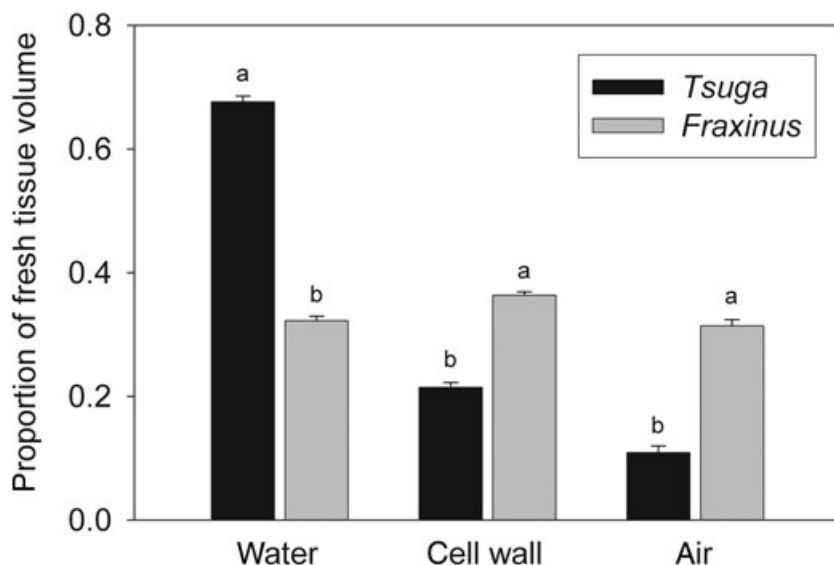
**Table 3.** Sapwood tissue respiration (mol O<sub>2</sub> cm<sup>-3</sup> h<sup>-1</sup> × 10<sup>-7</sup>) measured at 10% O<sub>2</sub> following a 72-h incubation at 20 °C in 1, 5 and 10% O<sub>2</sub> (mean ± SE; *n* = 10 trees)

	1% O <sub>2</sub>	5% O <sub>2</sub>	10% O <sub>2</sub>
<i>Fraxinus americana</i>	5.0 ± 0.3	5.5 ± 0.4	5.5 ± 0.3
<i>Tsuga canadensis</i>	3.6 ± 0.2	3.9 ± 0.3	3.4 ± 0.2

There were no significant differences between gas treatments, nor was there a significant gas × species interaction (ANOVA with tree as block).

wood O<sub>2</sub> was about 10% with the lowest values observed in the innermost sapwood around 3–5%. Sapwood tissue respiration was unaffected by a drop from 15 to 10% O<sub>2</sub> in *F. americana*, and by a drop from 15 to 5% in *T. canadensis*. When measured at 5% O<sub>2</sub>, respiration in *F. americana* was only reduced to 75% of the maximum rate. At 1% O<sub>2</sub>, a value lower than any actually observed in the innermost sapwood, respiration was reduced to just 35 and 25% of the maximum rate for *T. canadensis* and *F. americana*, respectively. Respiration was able to recover to normal rates when measured at 10% O<sub>2</sub> following 72 h at 1 and 5% O<sub>2</sub>, and parenchyma remained alive for up to 7 d under 1% O<sub>2</sub>. Given the relatively minor effect of reduced O<sub>2</sub> on parenchyma metabolism at O<sub>2</sub> levels that appear to be common in the innermost sapwood, and the moderate effect found at the most extreme levels (whose occurrence is likely rare and transient), it is unlikely that O<sub>2</sub> content plays a role in the death of parenchyma cells during sapwood senescence.

*In situ* O<sub>2</sub> measurements in woody stems may instead be most relevant to cellular metabolism in the outermost region of the stem. Our measurements suggest that in spring, when the cambium is active and secondary xylem and phloem begin to differentiate, O<sub>2</sub> levels can be drawn down through a combination of increased consumption and reduced supply (i.e. no active transpiration). We saw a marked decrease in O<sub>2</sub> in the spring (before leaf flush, relative to summer) in *T. canadensis* and *Q. rubra*, both of which had active cambia at the time of measurement (based on appearance and bark slippage). In contrast, *A. rubrum*, a species in which xylem production begins several weeks after leaf flush (Suzuki, Yoda & Suzuki 1996; Lavigne, Little & Riding 2004), had slightly higher O<sub>2</sub> levels throughout the stem in spring relative to summer. The timing of xylem production in particular is related to stem respiration (Lavigne *et al.* 2004) because the cambium produces far more xylem cells than phloem cells. This difference in the rate of



**Figure 5.** Proportion of fresh tissue volume occupied by water, cell wall, and air in *Tsuga canadensis* and *Fraxinus americana*. Within each tissue volume component (e.g. air), bars with the same lowercase letters indicate that the two species were not significantly different (Fisher-adjusted  $P$ -values <0.0001 for all significant differences, ANOVA).

cell production between xylem and phloem may be greatest in spring, at least in conifers (Wilson & Howard 1968). The decrease in O<sub>2</sub> in spring for *T. canadensis* was such that the lowest O<sub>2</sub> levels within the stem occasionally (in some individuals) occurred in the outer sapwood.

Drawdown of O<sub>2</sub> in the outermost sapwood is the result of O<sub>2</sub> consumption by respiring xylem parenchyma, but could also include consumption by cambial tissue and phloem, in which respiration is markedly (2–10 $\times$ ) higher due to the greater proportion of living cells (Goodwin & Goddard 1940; Pruyn *et al.* 2002a,b). Several of our outermost tubes were pushed outward within the stem through the action of cambial activity during the second (2003) spring so that the headspace in the tube was in the cambial zone or phloem, and these positions produced some of the lowest values observed (approximately 1% O<sub>2</sub>; these were excluded from the analysis). This is in keeping with observations of a positive relationship between O<sub>2</sub> levels within the cambium and cambial activity (as measured by cell number; Eklund *et al.* 1998), and of hypoxic conditions developing within the phloem (van Dongen *et al.* 2003). It has also been suggested that the vascular cambium may become anaerobic and undergo fermentative metabolism (Kimmerer & Stringer 1988). In *Q. rubra*, in the absence of transpiration in spring, the greatest reduction in O<sub>2</sub> was in the inner sapwood. This cannot be explained by cambial activity, but could be the result of locally increased sapwood respiration before bud break. There is some evidence that respiration increases during the dormant season in the innermost sapwood (Shain & MacKay 1973). The extent to which sapwood serves as an O<sub>2</sub> source for the cambial zone and phloem will depend on the diffusive properties of the different tissues. The cambium has historically been viewed as a barrier to diffusion of gas *into* the stem (e.g. Hook & Brown 1972), but if the phloem and cambium are strongly hypoxic, then O<sub>2</sub> permeability of the cambium may be more relevant to diffusion from the xylem to the phloem, across the cambium.

The combined spatial patterns of O<sub>2</sub> content and sap flow during active transpiration also underscore the importance of gas diffusion into and within the stem. In the summer, *T. canadensis* and *F. americana* show similar rates of O<sub>2</sub> decline toward the sapwood/heartwood boundary, despite very different flow rates in the inner sapwood: in *T. canadensis* flow is only reduced by about 60% (relative to outer sapwood), whereas in *F. americana* there is essentially no flow except in the outermost annual rings. Although sap flow measurements were not concurrent with O<sub>2</sub> measurements (they are separated by about 1 month), there is unlikely to be any change in the relative difference in flow between radial positions within the stem during that time. In ring-porous species, the outermost annual ring is functional before leaf expansion (Suzuki *et al.* 1996), whereas in diffuse-porous and conifer species, the contribution of the outermost annual ring will be small over a 1-cm distance (the probe heated/sensing length). Maintenance of such high levels of O<sub>2</sub> in the absence of flow in *F. americana* is not the result of a lower respiration rate, as tissue respiration is higher in *F. americana* than in *T. canadensis*, even in the inner sapwood (unpublished results for inner position). Instead, a higher air fraction in *F. americana* tissue is likely to facilitate movement of gases within the stem. Given that diffusion of gases is 10 000 times faster in gas than in liquid, the volume fraction of gas will have a profound effect on the diffusive properties of woody tissue.

The effects of low O<sub>2</sub> on plant tissue respiration are well-studied in roots and aquatic plants where flooding creates hypoxic conditions, but vascular tissue has received less attention. We found no respiratory inhibition until O<sub>2</sub> was reduced to below 10% (*F. americana*) or 5% (*T. canadensis*). A 'critical oxygen pressure' between 10 and 5% O<sub>2</sub> is in agreement with others reported in the literature for excised plant tissue in water-saturated air (Saglio, Raymond & Pradet 1983; Saglio *et al.* 1984). When incubated in aqueous solution, respiration shows exponential or logarithmic decline from higher O<sub>2</sub> levels (i.e. inhibition occurs

below 20–30%; Geigenberger *et al.* 2000), but this is likely due to restricted diffusion through an aqueous medium and an unstirred boundary layer (Saglio *et al.* 1984). Respiration in *T. canadensis* was less inhibited by low O<sub>2</sub> than in *F. americana*, which is surprising given that diffusion through *F. americana* tissue is likely to be faster because of the higher air content (i.e. slowed diffusion of O<sub>2</sub> to the active site of cytochrome oxidase means that effectively, respiration is more sensitive to reduced O<sub>2</sub>). It is possible that respiration in *T. canadensis* is inherently more robust against low O<sub>2</sub> conditions (or was in such a state at the time of sample collection), or that the pre-measurement equilibration time served to acclimate *T. canadensis* more than *F. americana* through a shift in metabolic pathways toward O<sub>2</sub> conservation (Xia & Saglio 1992; Andrews *et al.* 1993; Andrews *et al.* 1994; Drew *et al.* 1994; Drew 1997; Geigenberger *et al.* 2000; van Dongen *et al.* 2003).

The system of permanently installed tubes described here allows *in situ* 'point' measurements of gaseous O<sub>2</sub> and is well-suited to large sample sizes, field measurements and long-term studies, unlike current systems, which are designed for continuous data collection at a single position within the stem and the capture of diurnal variation (Gansert *et al.* 2001; del Hierro *et al.* 2002). Diurnal variation of O<sub>2</sub> is likely to be greatest in the outermost sapwood, where variation in both flow rate (driving O<sub>2</sub> supply) and temperature (driving respiration and therefore O<sub>2</sub> demand) is greatest. Although our measurements were not always made when O<sub>2</sub> was at a daily minimum, our overestimation of the minimum O<sub>2</sub> levels experienced in the innermost sapwood is likely to be very small. Diurnal variation reported for the outside of the stem during active transpiration is typically two to four percentage points [e.g. diurnal min–max of 2–6% in *Olea europaea* (Mancuso & Marras 2003) and 3–5% in *Betula pendula* (Gansert *et al.* 2001)]. Reduced or negligible sap flow in the innermost sapwood and reduced temperature variation deep within the stem both should minimize the variation in O<sub>2</sub> near the sapwood/heartwood boundary.

Reported patterns of sapwood O<sub>2</sub> and sap flow suggest that the determinants of spatial patterns of O<sub>2</sub> within secondary xylem are complex. Among the four published studies to date reporting concurrent sap flow and O<sub>2</sub> measurements, two have found daily O<sub>2</sub> maxima to correspond with peak sap flow and stable O<sub>2</sub> minima in the absence of flow (del Hierro *et al.* 2002; Mancuso & Marras 2003), while two have found minima at the end of the light period (Gansert *et al.* 2001; Gansert 2003). Supply of O<sub>2</sub> will be determined by sap flow (and the dissolved O<sub>2</sub> concentration in soil water) and diffusion of O<sub>2</sub> into and within the stem. Demand will be determined by parenchyma respiration, which is in turn determined by temperature, within-stem gas composition, and possibly by parenchyma age. The effect of low O<sub>2</sub> on parenchyma respiration should provide a negative feedback, such that depletion of O<sub>2</sub> leads to decreased consumption. It is not known whether xylem parenchyma show acclimation to hypoxic conditions as in other plant tissues, including down-regulation of glycolysis

and preferential induction of ethanolic rather than lactic acid fermentation under extreme hypoxia or anoxia (Xia & Saglio 1992; Drew *et al.* 1994; Drew 1997; Geigenberger *et al.* 2000; van Dongen *et al.* 2003). A better understanding of how parenchyma cell age, gas composition (both O<sub>2</sub> and CO<sub>2</sub>), and temperature all affect sapwood respiration will be key to determining within-stem patterns of hypoxia and anoxia.

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