Parenchyma cell respiration and survival in secondary xylem: does metabolic activity decline with cell age?

R. Spicer1 & N. M. Holbrook2

1Rowland Institute at Harvard University, 100 Edwin H. Land Boulevard, Cambridge, MA 02142, USA and 2Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA

ABSTRACT

Sapwood respiration often declines towards the sapwood/heartwood boundary, but it is not known if parenchyma metabolic activity declines with cell age. We measured sapwood respiration in five temperate species (sapwood age range of 5–64 years) and expressed respiration on a live cell basis by quantifying living parenchyma. We found no effect of parenchyma age on respiration in two conifers (Pinus strobus, Tsuga canadensis), both of which had significant amounts of dead parenchyma in the sapwood. In angiosperms (Acer rubrum, Fraxinus americana, Quercus rubra), both bulk tissue and live cell respiration were reduced by about one-half in the oldest relative to the youngest sapwood, and all sapwood parenchyma remained alive. Conifers and angiosperms had similar bulk tissue respiration despite a smaller proportion of parenchyma in conifers (5% versus 15–25% in angiosperms), such that conifer parenchyma respired at rates about three times those of angiosperms. The fact that 5-year-old parenchyma cells respired at the same rate as 25-year-old cells in conifers suggests that there is no inherent or intrinsic decline in respiration as a result of cellular ageing. In contrast, it is not known whether differences observed in cellular respiration rates of angiosperms are a function of age per se, or whether active regulation of metabolic rate or positional effects (e.g. proximity to resources and/or hormones) could be the cause of reduced respiration in older sapwood.

Key-words: ageing; heartwood; parenchyma; respiration; sapwood; senescence.

INTRODUCTION

Parenchyma cells within secondary xylem can be extremely long-lived. Individual cells may live for 2–200 years with longevities that are specific to a species but are also shaped by the environment. Surrounded by a matrix of non-living cells that function in water transport and mechanical support, parenchyma cells form an important point of exchange between the symplast and apoplast in woody tissue, functioning in carbohydrate storage, transport and wound response. It is the death of these cells that defines (and arguably drives) heartwood formation, a form of tissue senescence during which the oldest, non-functional xylem is compartmentalized in the centre of the stem. The cause of parenchyma cell death is not known, but evidence for decreased metabolic activity in the innermost sapwood has led to a view of parenchyma ageing as a gradual, passive decline in metabolism that terminates in cell death.

Multiple reports suggest that sapwood respiration declines towards the sapwood/heartwood boundary (Goodwin & Goddard 1940; Higuchi, Shimada & Watanabe 1967; Pruyn, Gartner & Harmon 2002a,b; Pruyn, Harmon & Gartner 2003; Pruyn, Gartner & Harmon 2005), although there have been reports of no change (Bowman et al. 2005) or even an increase in respiration in the ‘transition zone’, a narrow region between the sapwood and heartwood (Shain & MacKay 1973; Bowman et al. 2005). The vast majority of these studies have been in conifers, and reports on angiosperms are mixed as to whether respiration is reduced in inner sapwood (Goodwin et al. 1940; Higuchi et al. 1967; Pruyn et al. 2003). At the cellular level, parenchyma nuclei shrink (Yang 1993) and the number of mitochondria may decline with tissue age (Frey-Wysling & Bozhard 1959), both of which suggest a reduction in metabolic activity. In contrast, the ability of xylem parenchyma to de-differentiate and form callus tissue is unaffected by age in some Pinus species (Allen & Hiatt 1994), and there is evidence that certain Krebs cycle enzymes (malic and succinate dehydrogenase) and glucose-6-phosphate dehydrogenase are more active in the innermost sapwood of Pinus radiata (Shain et al. 1973; Hillys 1987; Hauch & Magel 1998). This latter enzyme catalyzes the first reaction of the oxidative pentose phosphate pathway, which supplies intermediates for phenolic synthesis, an activity that characterizes heartwood formation in many species.

Although most evidence points towards a reduction in parenchyma cell metabolism with age, there are logical reasons to expect that this may not be true. First, given that (1) the majority of work demonstrating a reduction in respiration with tissue age is in conifers, and (2) that conifers appear to have a gradual loss of living cells with tissue age (i.e. there is evidence for a gradual increase in the number of dead parenchyma towards the sapwood/heartwood boundary; Nobuchi & Harada 1983; Yang 1993; Gartner, Baker & Spicer 2000; Nakaba et al. 2006), it may be...
that this metabolic decline is eliminated when respiration is expressed on a per live cell volume basis. Although attempts have been made to relate respiration rate to parenchyma volume (Ryan 1990; Stockfors & Linder 1998; Pruyn et al. 2005), there has never been an explicit accounting of the live cell fraction. Secondly, parenchyma cells play an active role in the transition from sapwood to heartwood: they synthesize complex polyphenolic compounds (Necˇesaný 1973; Magel 2000), produce cellulose material to form tyloses and tylosoids (blocking angiosperm vessels and conifer resin canals, respectively; Chattaway 1949; Chafe 1973; Saitoh, Ohtani & Fukazawa 1993), secrete gums (Chattaway 1949) and, in some cases, deposit lignin in their cell walls (Balatinecz & Kennedy 1967; Yamamoto 1982). An irreversible decline in metabolic rate with cell age would run counter to the acquisition of new physiological roles at this late stage in development.

Our main objective in this study was to test the hypothesis that parenchyma in secondary xylem respires at the same rate, regardless of age, by expressing respiration on a live cell volume basis. Secondary objectives were to ask whether species that differ widely in sapwood quantity and physiology also differ in sapwood respiration rate, and whether tissue-level respiration is simply a function of the volume proportion of parenchyma. By expressing respiration on a live cell volume basis, we were also able to compare respiration rates of individual parenchyma cells to those of other plant cell types. Finally, by using mature trees, we were able to eliminate several developmental and environmental variables that might have confounded previous results.

MATERIALS AND METHODS

Plant material

For sapwood respiration measurements, mature trees of two conifer [Tsuga canadensis (L.) Carr. and Pinus strobus L.] and three angiosperm (Acer rubrum L., Fraxinus americana L., Quercus rubra L.) species were randomly selected within a 5 ha tract of natural forest in Harvard Forest, Petersham, MA, USA (42.5°N, 72°W, 220 m elevation). Sapwood was sampled with a 12 mm increment borer at 1.4 m above ground, deep enough to reach the darker-coloured heartwood. Five trees per species were sampled in mid-July 2003 and again in mid-June 2004 for a total of 10 trees per species. Stem diameter and sapwood age and depth were recorded and did not differ between sampling dates (Table 1).

The phloem and cambium were removed from each core with a razor blade immediately following extraction. Increment cores were then wrapped in moist paper towel and stored in a cooler (~5 °C) for transport to the laboratory.

Respiration measurements

Fresh tissue was sampled from the outermost (youngest) and innermost (oldest) sapwood by removing two approximately 1 cm³ cylinders from each increment core. The outermost position was adjacent to but did not include the cambial zone (i.e. the cambium and developing xylem were removed with a razor blade). The innermost position was adjacent to but did not include any heartwood. The ‘white ring’ (i.e. transitional ring between sapwood and heartwood in conifers; Nobuchi et al. 1983) in Pinus strobus and Tsuga canadensis, when present, was also excluded from the innermost sample. Distance and ring number from the cambium were recorded for the outer and inner ends of each sample, and the mean of these two ends was used to define each sample’s age and position (Table 2).

 Sapwood samples were equilibrated to 10% O₂ (v/v) before respiration measurements to eliminate potential effects of different native, internal gas compositions between inner and outer sapwood. Ten per cent O₂ was chosen as a realistic midrange for the species studied (Spicer & Holbrook 2005) and to minimize equilibration times. Each sample was weighed, then wrapped in a 2 × 5 cm strip of dampened cheesecloth and placed in an open 10 mL glass vial. The open vials were enclosed in a gas-impermeable glove bag and flushed repeatedly with 10% O₂ (balance N₂). Glove bags were then stored at 4 °C for a total of 36 h with additional repeated flushing of 10% O₂ at 12 and 24 h. Prior tests had shown that 36 h was sufficient to fully equilibrate the samples so that their internal gas compositions matched that of treatment gas, while cold storage suppressed respiration and extended the

Table 1. Outer diameter*, depth and age of sapwood (mean ± SE, n = 10) of trees sampled from Harvard Forest, Petersham MA

<table>
<thead>
<tr>
<th>Species</th>
<th>Diameter (cm)</th>
<th>SW depth (cm)</th>
<th>SW age (rings)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± Range</td>
<td>Mean ± Range</td>
<td></td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>33.1 ± 2.1</td>
<td>8.3 ± 1.1</td>
<td>43 ± 4 29 – 60</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>34.4 ± 2.0</td>
<td>7.5 ± 1.2</td>
<td>48 ± 3 34 – 64</td>
</tr>
<tr>
<td>Pinus strobus</td>
<td>35.4 ± 1.5</td>
<td>3.5 ± 0.5</td>
<td>21 ± 2 12 – 29</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>36.0 ± 1.2</td>
<td>1.5 ± 0.2</td>
<td>8 ± 1 5 – 13</td>
</tr>
<tr>
<td>Tsuga canadensis</td>
<td>34.7 ± 1.7</td>
<td>4.1 ± 0.4</td>
<td>28 ± 4 17 – 47</td>
</tr>
</tbody>
</table>

* Diameter measured over-bark at 1.4 m above ground. SW, sapwood.
length of time respiration rates (after return to 20 °C) remained stable. Although high levels of CO₂ may exist within the stem, CO₂ was not included in the measurement gas because good estimates of realistic levels in situ were not available, and because different-aged sapwood responded similarly to CO₂ (Spicer & Holbrook 2007).

Following equilibration and a final flush of 10% O₂, the vials were sealed with crimp-top lids each containing a butyl rubber septum. 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 4–6 h for 24–36 h by penetrating the septum with a needle-tipped fiber optic O₂ probe (USB2000 spectrometer and FOXY probe, Ocean Optics, Inc., Dunedin, FL, USA). The probe, which operates using principles of fluorescence quenching in the presence of O₂, was calibrated with humidified O₂ standards at 20 °C. The slope of the linear portion of the curve (typically the first 12–24 h) was taken as the rate of O₂ consumption and converted to mol hr⁻¹ by calculating the total volume of gas in the vial. At the end of this period, O₂ levels were between 3 and 7% depending on the rate of O₂ consumption. Samples were then removed from vials, measured for fresh volume by displacement, and vacuum infiltrated with a cold, phosphate-buffered (pH 7.2) 3.5% paraformaldehyde fixative.

**Parenchyma volume estimates**

The proportion of volume occupied by living parenchyma was determined for each sample through image analysis of light and fluorescence microscopy sections. Tangential sections (20 μm) were cut from both ends of each sample with a sliding microtome, stained in 1% aqueous safranin-o, mounted and viewed with a 10× objective. Ten randomly located images (0.66 mm² area) were captured for each tangential section for a total of 20 images per sample (10 images from each end) and processed in ImageJ (http://rsb.info.nih.gov/ij/). Rays were manually highlighted with a paint tool, and the proportion of tangential surface occupied by ray was measured (Fig. 1a,b). This proportion of area was assumed to equal the proportion of volume, as rays were often continuous radially through the sample, and both ends were measured. The mean value for all 20 images

<table>
<thead>
<tr>
<th>Species</th>
<th>Outer sapwood</th>
<th>Inner sapwood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Distance</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>4.7 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>4.8 ± 0.7</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td><em>Pinus strobus</em></td>
<td>4.1 ± 0.7</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>1.7 ± 0.2</td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>3.1 ± 0.4</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

**Table 2.** Age (ring number) and distance (mm) from the cambium for inner and outer sapwood positions (mean ± SE, n = 9 or 10)
was taken to characterize the proportion of ray parenchyma for each sample.

In addition to ray parenchyma, both Fraxinus americana and Quercus rubra contain significant volumes of axial parenchyma (axial parenchyma is absent from Acer rubrum and T. canadensis). Axial parenchyma was quantified for transverse sections (20 µm) taken from both the upper and lower surfaces of F. americana and Q. rubra samples, stained for starch (indicative of parenchyma) with 1% aqueous potassium iodide, and viewed with a 20× objective. Additional transverse sections were stained with safranin-o. Ten randomly located images (0.17 mm² area) were captured per section for a total of 20 images per sample. As described above, parenchyma was manually highlighted with a paint tool (Fig. 1c,d); the proportion of surface area occupied by parenchyma was measured, and the mean of all 20 images was used to characterize the sample. Resin canal epithelial cell volume in P. strobus was estimated by measuring the average transverse area of individual resin canals (cell area only, 10 resin canals) and counting resin canals on two transverse surfaces per sample. Proportion volume was calculated assuming that canals ran through the length of the sample.

**Live parenchyma estimates**

Radial tissue sections were used to determine the presence and absence of nuclei to quantify the proportion of parenchyma that was alive. Preliminary work using vital stains (triphenyl-tetrazolium chloride, which when reduced produces coloured, insoluble formazans) and a fluorescent nucleic acid stain 4′,6-diamidino-2-phenylindole (DAPI) had shown that the presence of a nucleus is a sufficient indicator of cell vitality (Fig. 2). Radial sections (20 µm) of all five species were rinsed in phosphate-buffered saline (PBS) (pH 7.5), incubated in 1% (w/v) Triton-X in PBS overnight, and stained with 0.001% DAPI in PBS. Sections were mounted in 4:1 glycerol : PBS and viewed with a 4× (P. strobus and T. canadensis) or 10× (A. rubrum, F. americana, Q. rubra) objective on an epifluorescent microscope (365ex λ/450em λ).

For each annual ring within a sample, the total number of rays and the number of rays containing nuclei were tallied. Rays of angiosperms (A. rubrum, F. americana, Q. rubra) retained nuclei until their abrupt disappearance at the sapwood/heartwood boundary. In contrast, conifer species (P. strobus and T. canadensis) showed dead rays (those completely lacking nuclei) in the outer sapwood, with increasing frequency towards the inner sapwood. The proportion of live rays was calculated for each annual ring, and a mean value (proportion live ray) was determined for each section by weighting the contribution of each ring according to its radial width.

**Statistical analyses**

The effects of species and radial position on sapwood respiration were tested in a repeated measures analysis of variance (ANOVA) that included sampling year as a block, species as a ‘between subject’ factor, and radial position as a ‘within subject’ factor (i.e. radial position was spatially repeated within each tree, the subject). Differences between radial positions within each species were tested with paired t-tests (two-tailed). Orthogonal contrasts were used to test a priori comparisons where possible. Multiple a priori comparisons (e.g. those among interaction terms) were tested with Bonferroni-adjusted P-values to minimize the experiment-wise error rate (Sokal & Rohlf 1995). Unplanned multiple comparisons were made with Fisher’s least significant difference (LSD) corrected P-values.

**RESULTS**

The effect of radial position was highly significant (Table 3) such that the youngest, outermost sapwood respired at a higher rate than the oldest, innermost sapwood in all species except P. strobus when expressed on a per tissue volume basis (Fig. 3a, Table 4). Sampling date was a significant blocking factor (Table 3) such that respiration rates were slightly higher, and the magnitude of the difference between inner and outer sapwood slightly greater in 2003 relative to 2004. Most pairwise species comparisons were significant for the outer sapwood (Pinus versus Tsuga and Fraxinus versus Acer were exceptions), whereas only Fraxinus and Quercus differed significantly for the inner sapwood (P = 0.02, Fisher’s LSD).

There were large species differences in parenchyma content and vitality (Fig. 4, Tables 3 & 5). Conifers had less
than one-third the parenchyma volume of angiosperms ($P < 0.0001$, orthogonal contrast). Angiosperms differed in both the total amount and composition of parenchyma such that *A. rubrum*, which lacks axial parenchyma, had the smallest total proportion of parenchyma (Fig. 4). *Fraxinus* had the highest proportions of both of axial and total parenchyma volume. Angiosperms showed no evidence of parenchyma death, either ray or axial, throughout the sapwood (Fig. 5). In contrast, both conifers showed measurable proportions of dead ray parenchyma starting in the outer sapwood and increasing towards the sapwood/heartwood boundary (Fig. 6). Both species had a greater proportion of living parenchyma in the outer sapwood (Table 3; paired *t*-test *P*-values < 0.01). *Tsuga* had a smaller proportion of living parenchyma in the inner but not the outer sapwood relative to *Pinus* ($P = 0.0003$ and 0.5, respectively, orthogonal contrasts). In no case was there any difference in total (live plus dead) parenchyma volume between outer and inner sapwood (Table 3).

When respiration was expressed on a per live cell volume basis, there were large species differences (Table 3) such that conifers had almost three times the respiration rate of angiosperms (Fig. 3b, contrast $P < 0.001$). The effect of radial position on live cell respiration depended on species (Table 3, interaction term marginally significant with $P = 0.07$). Parenchyma in the outermost sapwood respired

### Table 3. Repeated measures analysis of variance (ANOVA) testing effects of species and radial position on respiration per tissue volume ($R_{\text{tiss}}$), respiration per live parenchyma volume ($R_{\text{live}}$) and proportion of tissue volume occupied by parenchyma ($V_{\text{par}}$)

<table>
<thead>
<tr>
<th>Model term</th>
<th>d.f.</th>
<th>$R_{\text{tiss}}$</th>
<th>$R_{\text{live}}$</th>
<th>$V_{\text{par}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sampling date</td>
<td>1</td>
<td>0.005</td>
<td>0.005</td>
<td>0.6</td>
</tr>
<tr>
<td>Species</td>
<td>4</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radial position</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Radial position × sampling date</td>
<td>1</td>
<td>0.01</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Radial position × species</td>
<td>4</td>
<td>&lt;0.0001</td>
<td>0.07</td>
<td>0.4</td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sampling date is included as a blocking factor, and radial position is treated as a spatially repeated measure within each stem.

d.f., degrees of freedom.
at almost twice the rate as that of inner sapwood for all three angiosperms (Fig. 3b), whereas for conifers, there was no significant difference between the two radial positions (Fig. 3b, Table 4).

DISCUSSION

We found little evidence for a decline in parenchyma cell respiration that is inherent to the process of cellular ageing. First, there was no decline in parenchyma respiration with age in the conifers *T. canadensis* and *P. strobus*, despite maximum cell ages of about 30 and 20 years, respectively. Only *Tsuga* showed reduced respiration with age on a per tissue volume basis, and this was eliminated once the proportion of dead cells was taken into account. Second, although the angiosperms (*A. rubrum*, *F. americana* and *Q. rubra*) had reduced respiration in older tissue – a difference that was unaffected by correcting for live cell volume because all sapwood parenchyma remained alive – this does not necessarily reflect an inherent or intrinsic decline with age because age and position within the stem are confounded. In *Quercus* and *Acer*, parenchyma in the innermost sapwood respired at the same rate despite a difference in age of 35 years. One would have to invoke dramatic species differences in cellular ageing trends to explain this result. Although species-specific

<table>
<thead>
<tr>
<th>Species</th>
<th>$R_{\text{tiss}}$</th>
<th>$R_{\text{live}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer rubrum</em></td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Pinus strobus</em></td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>0.0003</td>
<td>0.0004</td>
</tr>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>0.007</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Note that given a total of five comparisons, the Bonferroni-adjusted $P$-value required for significance is 0.01 (Sokal et al. 1995).

### Table 5. Percent ray parenchyma living in outer versus inner sapwood samples (mean proportion of rays retaining nuclei ± SE, $n = 9$ or 10)

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent live parenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Pinus strobus</em></td>
<td>93 ± 3</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>90 ± 4</td>
</tr>
</tbody>
</table>

Differences between inner and outer positions were significant for both *Pinus* and *Tsuga* ($P = 0.01$ and 0.001, respectively, two-tailed paired $t$-test).

Figure 5. Radial tissue sections of the outer and inner sapwood (SW) of angiosperms showing fluorescent DAPI-stained nuclei. The outermost SW (1-year-old annual ring) is shown for (a) *Fraxinus americana*, (b) *Quercus rubra* and (c) *Acer rubrum*. The annual ring adjacent to the heartwood (HW) is shown for *Fraxinus* (d,e), *Acer* (f) and *Quercus* (g–i). The abrupt loss of nuclei at the SW/HW boundary is shown for *Quercus* (i). Tissue ages and depths within the stem for the innermost SW shown here are 54 years and 14 cm (*Fraxinus*), 40 years and 7 cm (*Acer*), 7 years and 3 cm (*Quercus*). Scale bar = 50 $\mu$m.
cellular ageing is certainly possible, it seems unlikely given the range of maximum parenchyma ages found both within a species (30+ year range within this study alone) and within an individual (e.g. 15+ year differences in sapwood age between the stem base and top have been observed; Spicer & Gartner 2001; Domec, Pruyn & Gartner 2005). Similarly, substrate limitation is unlikely to cause the observed declines in respiration because parenchyma cells often store large quantities of starch (sometimes lipids), even in the innermost sapwood (Fischer & Höll 1992; Magel, Jay-Allemand & Ziegler 1994; Höll 2000). Many of the innermost sapwood samples in this study showed parenchyma filled with amyloplasts.

An alternative explanation is that parenchyma metabolism is actively regulated and effectively ‘down-regulated’, either with age or, more likely, with position in the stem. The ability of parenchyma cells to de-differentiate and begin dividing at any age (Fisher 1981; Allen et al. 1994), as well as the role of parenchyma in synthesizing secondary compounds and tyloses just prior to heartwood formation, suggest that communication routes exist for regulation. There is also one account of an increase in metabolic activity in the narrow ‘transition zone’ between sapwood and heartwood (Shain et al. 1973). One obvious approach for future work will be to determine radial profiles of metabolic activity at a high spatial resolution across the sapwood (i.e. per annual ring), and at different times of the year. Similarly, one can begin to separate the effects of age and position by measuring respiration rates in tissue of the same age sampled at different heights in the stem – the same annual ring number will be closer to the sapwood/heartwood boundary towards the top of the stem.

Species differences in respiration rate were much larger when expressed on a live cell basis than on a tissue volume basis. These data suggest that bulk tissue respiration cannot be estimated by quantifying parenchyma volume because there may be large species differences in cellular respiration rates. Sapwood, rather than parenchyma, may behave uniformly across species: here, the large parenchyma volume in angiosperms (18–25%) combined with a low cellular respiration rate (about one-third that of conifers) produce tissue respiration rates roughly similar to those of conifers. It will be interesting to see if angiosperms and conifers with the same volume fraction of parenchyma have similar cell-level respiration rates [e.g. Salix and Tilia are reported to have proportions of parenchyma (6–8%) comparable to some

Figure 6. Radial tissue sections of the outer (a,b), middle (c,d) and inner (e,f) sapwood (SW) of Pinus strobus (a,c,e) and Tsuga canadensis (b,d,e) showing DAPI-stained nuclei. Parenchyma death occurs in Pinus in (c) the middle SW, where the upper file of cells in a short ray has died, and (e) the innermost SW, where parenchyma cells fill with polyphenolics at the SW/HW boundary. The middle SW of Tsuga contains both living and dead rays (d), but at the SW/HW boundary (f), all remaining rays die. Outer SW is 1 year old and adjacent to the cambium. Tissue ages/depths within the stem for the middle and inner SW, respectively, are 8 years/2 cm and 16 years/4 cm (Pinus); 22 years/5 cm and 56 years/6 cm (Tsuga). Scale bar = 50 μm.
conifers]. These smaller differences in bulk tissue respiration may be normalized at the whole-tree level by varying volumes of sapwood. There is evidence among conifers that species with narrow sapwood have higher bulk tissue respiration rates than those with wide sapwood (Pruyn et al. 2003). Our data support this idea among angiosperms as well, with the caveat that species differences in tissue respiration are not simply a function of parenchyma volume. *Quercus*, which maintains a very narrow band of sapwood, has the highest rate of respiration (both for total sapwood and for outer sapwood alone) but an intermediate volume of parenchyma.

Our results suggest that bulk sapwood respiration is quite low, roughly an order of magnitude lower than cambial tissue (Goodwin et al. 1940; Higuchi et al. 1967) and half that of secondary phloem and cambial tissue combined (Pruyn et al. 2002a,b; Pruyn et al. 2003; Bowman et al. 2005; Pruyn et al. 2005). By expressing respiration on a live cell volume basis, we were able to ask whether this low tissue respiration rate is simply a function of the small proportion of living cells, or whether parenchyma cells have a low metabolic rate as well. Quantifying live cell volume in plant tissue is rarely done, and there are no published accounts of respiration rates for live cells within a more complex tissue. However, there are two extremes of tissues composed of pure living cells against which we may compare our rates: tubers and meristems. ‘Resting’ (mature, non-growing) potatoes respire at 0.5–1.5 μmol O₂ cm⁻³ h⁻¹ (per fresh volume; Bidwell 1974; Hajirezaei et al. 2003), a rate on par with our lowest angiosperm values (1.4–4.2 μmol O₂ cm⁻³ h⁻¹), but an order of magnitude lower than our range for conifers (11.6–14.5 μmol O₂ cm⁻³ h⁻¹). In contrast, fine root meristems respire at 100–250 μmol O₂ cm⁻³ h⁻¹ (Lambers 1985; Tang & Peters 1995; Bidel et al. 2000; Asplund & Curtis 2001), an order of magnitude higher than our highest conifer values. Parenchyma cells are apparently not in a ‘quiescent’ state, and instead have a metabolic rate slightly above simple storage tissue but well below that of dividing, meristematic tissue.

Within-stem variation in rates of cellular respiration may reflect changes in parenchyma function with age/position in the stem, or more specifically, with the water-transport capacity of the xylem. Both *Quercus* and *Fraxinus* are ring-porous species and only transport water in the outermost annual ring(s) (Spicer et al. 2005). Axial parenchyma in *Fraxinus* frequently surrounds vessels (more so than in *Quercus*: Fig. 1c,d) and may remain alive for 45+ years after its associated vessels have ceased to conduct water. This may represent a shift towards a resting state in which primary functions are in wound response and carbohydrate storage, rather than in transpiration stream maintenance and modification (Bucci et al. 2003; Salleo et al. 2004; ZwieNiecki et al. 2004). In support of a functional shift, sample age was a far better predictor of parenchyma respiration in *Fraxinus* (linear regression *P* < 0.0001; *R²* = 0.67, data not shown) than it was in *Acer* (*P* = 0.03, *R²* = 0.28, data not shown), which maintains transpiration throughout its sapwood (Spicer et al. 2005).

That rates of parenchyma respiration vary among species may also suggest functional specialization of parenchyma among taxonomic groups. Conifers have narrow (one to two cells wide) rays with a single parenchyma cell type and almost no axial parenchyma. Angiosperms are more diverse in parenchyma composition, with wider rays (2–30 cells wide) composed of several cell types, and large volumes of axial parenchyma arranged in complex patterns, often surrounding and linking vessels. There are almost certain to be functional/physiological differences between ray and axial parenchyma in angiosperms, but it is not known to what extent they differ in rates of respiration. If axial parenchyma respires at a lower rate than ray parenchyma, for instance, it can explain the higher tissue respiration rate of *Quercus* relative to *Fraxinus*.

It remains to be seen if differences between conifers and angiosperms hold with a wider sampling of species, but there may be fundamental differences in the manner in which sapwood senesces in these two taxonomic groups. Parenchyma cell death within the sapwood of conifers may be the result of a cambial process, and unrelated to sapwood ageing and death at the sapwood/heartwood boundary. Ray initials can be lost from the cambium, at least in conifers, and this occurs with increasing frequency as the cambium ages or when growth is slowed (Bannan 1934; Barghoorn 1940). Once a cambial ray initial is lost, that radial file ceases to be continuous with the phloem, and this loss of access to carbohydrates could result in cell death. Ray initials are typically lost from the top and bottom of rays, and short rays may be eliminated altogether (Bannan 1934; Barghoorn 1940; Larson 1994). We observed that ray death in conifer sapwood began with the top and bottom radial cell files (Fig. 6c; see also Nakaba et al. 2006), and that short rays were more likely to die than tall rays, particularly in *Tsuga*. In addition to their outright loss from the cambium, ray initials at the top and bottom of a ray may switch from producing ray parenchyma to producing ray tracheids (Fig. 2; Bannan 1934; Barghoorn 1940; Larson 1994), thereby severing a symplasmic connection to the phloem (ray tracheids die during differentiation). In contrast, parenchyma cell death caused by cambial dynamics is probably insignificant in angiosperms given their wide rays and/or abundant axial parenchyma, which can link radial files.

In summary, we found that long-lived xylem parenchyma cells respire at relatively low rates that vary across species, and that respiration rates do not necessarily decline as cells age. Further work is needed to tease apart the effects of age and position on respiration within tree stems. The fact that trees maintain a fairly species-specific sapwood depth (and thus volume), rather than number of years of sapwood, is suggestive of positional and not age-based controls. This is in keeping with the idea that tree size, and not age, accounts for physiological limits to growth (Koch et al. 2004; Men-cucini et al. 2005). Clearly, there is still much to learn about parenchyma metabolism and function in secondary xylem, and with longevities ranging from 2 to 200 years, these cells may make an interesting model system for cellular ageing and non-replicative senescence.
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