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Comparative sectoriality in temperate hardwoods: hydraulics and xylem anatomy

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In wood, lateral transport of water and minerals occurs readily in 'integrated' trees but is more restricted in 'sectored species'. Dye distribution and a novel hydraulic technique are used to quantify species-specific differences in sectoriality in three temperate hardwoods, *Betula papyrifera*, *Acer saccharum* and *Quercus rubra*. Sectoriality was related to key elements of xylem structure: intervacular pitting, vessel diameter and vessel grouping. Perfusion of 0.5% safranin through isolated roots showed root-to-branch dye transport was most extensive in *B. papyrifera* and least extensive in *Q. rubra*. To test sectoriality using hydraulics, 20 mM KCl solution was pushed at 0.1 MPa through 5-cm wood segments, before and after occluding the direct axial outlet with glue, with flow rate measured in grams of solution expelled over time. Direct (axial) conductance ($\text{g MPa}^{-1} \text{s}^{-1}$) through unglued outlets was compared with indirect (tangential) conductance around occluded outlets. Species with high indirect/direct conductance ratios (Integration Index) are the most integrated. Integration Index ranged from 0.26 in *B. papyrifera* to 0.02 in *Q. rubra*. Macerates showed that *B. papyrifera* has much greater percentage of cell wall area covered with intervacular pits than does *A. saccharum* or *Q. rubra*. Vessel grouping was closest in *B. papyrifera* and vessels were most isolated in *Q. rubra*. Widest diameter vessels occurred in *Q. rubra*, where they concentrated in springwood. Intervacular pitting, vessel diameter and grouping are wood traits that contribute to the continuum of sectoriality in trees, and may influence the ability of tree species to dominate in homogeneous or in patchy environments. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, 150, 61–71.

ADDITIONAL KEYWORDS: bordered pits – diffuse porous – functional variation – hydraulic conductance – intervacular pits – ring porous.

INTRODUCTION

A sessile and autotrophic lifestyle dictates that plants exploit their fixed environmental space to the fullest. Plants are well known for their ability to respond to both spatial and temporal heterogeneity (patchiness) in resource availability (Terashima, 1992; Robinson, 1994; Pearcy, Gross & He, 1997). For example, roots of grasses growing in nutrient-poor dunes elongate, branch and live longer when they encounter resource-rich pockets, such as buried nests of turtle eggs (Stegmann, Primack & Ellmore, 1988). This results in a surge of mineral uptake into the shoots, as measured by gamma-emitting tracers in intact plants. Other

reports show that species differ in their ability to transport resources captured by one part of the plant to other parts (Watson & Casper, 1984; Marshall, 1996; Orians *et al.*, 2004).

Once captured, water and minerals are transported to distant organs. They may then spread throughout the plant, or they may concentrate into a portion, or sector, of the shoot. Vascular connections between roots and sectors of the shoot provide a structural mechanism that concentrates resources into sectors of the plant rather than spreading them throughout. Vascular restrictions may affect plant growth, morphology and chemistry. Split-root experiments with tomato have demonstrated that roots grown in nutrient-rich soil patches preferentially supply orthostichous leaves and branches (Orians, Ardón & Mohammad, 2002).

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Differential sectoriality in herbaceous plants is mediated by primary xylem grouped in bundles that branch and fuse around leaf gaps (Price, Hutchings & Marshall, 1996; Orians, Pomerleau & Ricco, 2000). Sectoriality in woody plants is mediated by vessel-to-vessel connectivity (Orians, Babst & Zanne, 2005a).

In long-lived trees, a sectorized anatomy may be expressed in several ways. Artisanal potters making traditional ash glazes (Tichane, 1987) recognize that mineral content (hence colour) of oak ash glazes made from different branches of the same tree can differ by as much as 30% (Y. and S. Rybczynski, pers. comm.). For oak, this suggests that certain branches make up mineral-rich sectors. In maple trees, leaf colour change in autumn occurs first in a subset of branches on any given tree (Fig. 1). Elsewhere, a sectorized morphology is indicated by patchy dieback patterns within

the crown (Davis *et al.*, 2002) or shown experimentally by restricted spread of dyes injected into the trunk (Kozłowski & Winget, 1963; Shigo, 1985; Larson, Doubt & Matthes-Sears, 1994; Tyree & Zimmermann, 2002). These results suggest that sectorized transport generates intracrown variation in traits, although somatic mutations may also play a role.

Differences in sectoriality among trees have been demonstrated by split-root experiments (Orians *et al.*, 2004). Uptake patterns of dye and mineral isotopes showed that *Betula* spp. are more integrated than *Acer* spp. or *Populus* spp. Split-root experiments are, however, time-intensive and costly. A more rapid technique could facilitate exploration into how differential sectoriality affects plant responses to their environment, the distribution of plant species and plant-herbivore interactions (Orians & Jones, 2001).



Figure 1. Autumn leaf colour change in *Acer saccharum* growing on Tufts University campus in Medford, Massachusetts. Autumn coloration develops in a subset of branches (sectors) earlier than in others, indicating differences in tissue quality between sectors of the crown.

In this study we complement traditional dye-uptake studies with a novel hydraulic technique that allows us to quantify differences in sectoriality in the wood of three temperate hardwood trees (*Betula papyrifera*, *Acer saccharum* and *Quercus rubra*). We measure direct axial conductance and indirect tangential conductance (persisting after the direct pathway was blocked). A comparison of indirect to direct conductances (Integration Index) quantifies the relative ease of tangential spread in wood, allowing a continuous spectrum of integrated–sectored transport. We develop this hydraulic approach as a rapid tool for quantifying sectoriality in trees.

Hydraulic conductance must depend on anatomical features of vessels (Carlquist, 2001). Our second goal is to identify key anatomical traits in secondary xylem that influence the degree of sectoriality in trees. Features, such as extensive sidewall pitting, that promote tangential spread between vessels (Kitin *et al.*, 2004) are likely to be well developed in integrated trees because such vessel structure allows material to flow around the trunk and throughout the crown. On the other hand, sectored wood should contain features that promote axial conductance or resist tangential flow (Jones & Lord, 1982). These could include low intervacular exchange brought on by sparse pitting or by large distances between neighbouring vessels, both of which tend to isolate water-conducting elements, and by large vessel diameter. We report relationships of xylem traits such as pitting, vessel grouping and vessel element diameter to hydraulic measures of sectoriality, allowing us to focus on xylem traits that help explain differences among species.

MATERIAL AND METHODS

STUDY SPECIES

Three species were selected for this study: *Acer saccharum* Marsh., *Betula papyrifera* Marsh. and *Quercus rubra* L. They exemplify contrasting life histories in hardwood forests, and we have found differences in sectoriality in saplings of *Acer* and *Betula* (Orians *et al.*, 2004). In addition, they provide a range of xylem structure with *A. saccharum* and *B. papyrifera* having diffuse-porous wood and *Q. rubra* being ring-porous. This allowed us to relate anatomical features to the hydraulic properties of wood.

Acer saccharum, *B. papyrifera* and *Q. rubra* are all important components of eastern North American forests (McWilliams *et al.*, 2000), often growing in association with each other (Samuelson & Hogan, 2003). They range considerably in shade tolerance and silvical traits. *Acer saccharum* is shade tolerant and a late-successional dominant of mesic sites (Delcourt & Delcourt, 2000; McWilliams *et al.*, 2000; Samuelson & Hogan, 2003). It often occupies cool north-facing

slopes and is replacing pioneer species of oak as it competes against less shade-tolerant hardwoods (Fralish & Franklin, 2002). By contrast, *B. papyrifera* is an early-successional tree, intolerant of shade. It occupies sandy or rocky soils, including those at disturbed sites (Marchand, 1987; Delcourt & Delcourt, 2000; Fralish & Franklin, 2002). Compared with *A. saccharum* and *B. papyrifera*, *Q. rubra* is intermediate in shade tolerance. Although growing in association with *A. saccharum* and *B. papyrifera*, it tends to occupy drier subxeric soils and prevails on south- and west-facing slopes (Delcourt & Delcourt, 2000). *Quercus rubra* becomes a suppressed understorey species when competing against shade-tolerant *A. saccharum* (Fralish & Franklin, 2002).

DYE TRANSPORT IN SAPLINGS

Dye transport was used to test for the ease of resource spreading (degree of integration – sectoriality). Dye was supplied to isolated roots on 1-year saplings of *B. papyrifera*, *A. saccharum* and *Q. rubra*. Saplings were grown in the greenhouse after having over-wintered in pots outdoors. Four saplings of each species were removed from pots with their roots rinsed of soil and then held in a beaker of water. With the shoot clamped to a ring stand for support, a single lateral root was cut under water then immersed for 24 h in a filtered solution of safranin-O (0.5% in deionized water). After 24 h, leaves were removed and oven-dried for 48 h at 60 °C to quantify leaf biomass on each branch, as an indicator of the transpiration sink strength drawing dye into each branch. Dye distribution was noted by peeling bark away from branches to reveal presence of any red-stained wood, and the percentage of stained branches was determined.

HYDRAULICS IN BRANCH SEGMENTS

To compare degree of sectoriality across species, the extent of tangential flow contributing to hydraulic conductance was tested in woody branch segments from mature trees. Branch samples were taken from four individuals of *B. papyrifera*, *A. saccharum* and *Q. rubra* growing in eastern Massachusetts. Samples ranging in thickness from 0.8 to 1.1 cm were harvested in late summer and cut to internode lengths of 5.5 cm. Samples were flushed with and stored in 95% EtOH passed through a 0.45 µm filter (Millipore Corp.). After storage, samples were flushed again with filtered 20 mM KCl. Samples were allowed to remain in 20 mM KCl for at least 3 h at 4 °C after which preserved samples maintain similar conductance to that of fresh samples (A. Zanne, unpubl. data).

To determine both tangential spread and axial flow, flushed woody internodes were trimmed with razor blades to 5 cm. The proximal inflow surface was

plugged with two coats of acrylic glue (Super Bonder Loctite 409 and 712 fixer, Loctite Corp.), except for a 60° pie section of sapwood that was left open (Fig. 2, I). The pathway of axial flow was then visualized by sending safranin-O (0.1% in 20 mM filtered KCl) through the inflow section for 10 min or until 5 mL of stain solution passed through the internode. The stained cells allowed us to visualize the axial flow path and account for any spiralling of flow that occurred over the 5-cm pathway. Internodes were then flushed with 10 mL of filtered 20 mM KCl. Axial conductance was measured by pushing 20 mM filtered KCl through the inflow section at 0.1 MPa, and measuring outflow from the unglued distal surface to a balance (Fig. 2, II). Conductance (K) was determined as, $K = \text{flow rate}/\text{pressure}$ ($\text{g MPa}^{-1} \text{s}^{-1}$).

Tangential spread was measured by blocking axial flow. To this end, 180° of distal outflow surface was occluded with glue such that the red-stained cells denoting axial flow were completely blocked and centred in the middle of the 180° glued section. The 20 mM filtered KCl was again pushed through the proximal inlet, and conductance measured through the partially occluded distal outlet (Fig. 2, III). High levels of outflow around the blocked axial route ($K_{\text{tangential}}$) indicate that fluid can transfer out of glued vessels and into unoccluded ones, suggesting integrated wood able to spread material throughout the crown.

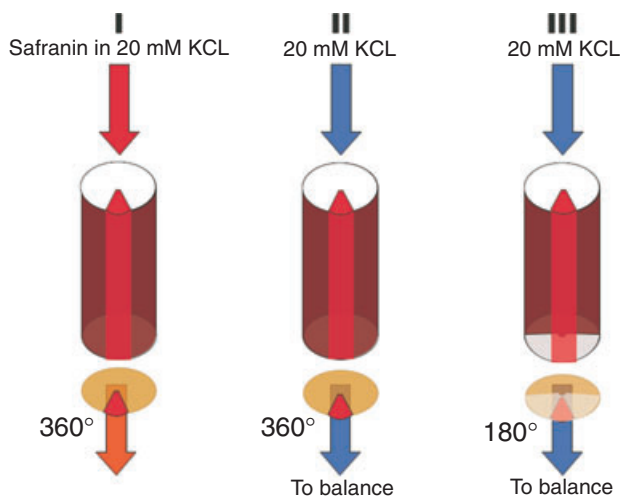


Figure 2. Method used to measure integration in wood. Step I. Safranin pushed through 60° inlet in proximal end of internode segment to visualize direct axial pathway. Step II. Axial conductance ($K_{\text{axial}} = \text{flow rate}/\text{pressure}$; $\text{g MPa}^{-1} \text{s}^{-1}$) measured by sending 20 mM KCl through inlet at 0.1 MPa, with flow at outlet weighed on a balance over time. Step III. Tangential conductance ($K_{\text{tangential}}$) measured by occluding axial outlet pathways and sending 20 mM KCl through the proximal inlet at 0.1 MPa, with flow rate again read on a balance.

Species vary greatly in K_{axial} and this variation could influence values of $K_{\text{tangential}}$ if rapid axial flow allows for more rapid tangential flow. Our Integration Index accounts for this variation, as each species served as its own internal control. Because $K_{\text{tangential}}$ was measured on a distal outlet with half the cross-sectional area of that for K_{axial} (180° of the distal end was unglued for $K_{\text{tangential}}$ vs. 360° for K_{axial}), we calculate the following:

$$\text{Integration Index} = 2(K_{\text{tangential}})/K_{\text{axial}}$$

Species with high values (approaching 1.0) are integrated, and those with low values (approaching 0.0) are sectored.

ANATOMY OF XYLEM

Traits related to intervacular pitting, vessel distribution and vessel diameter were measured because they are most likely to influence the pathway of water movement. Intervacular pitting was measured from wood macerates of 3–4 individuals per species using a modified Jeffrey's method (Ruzin, 1999). Wood segments

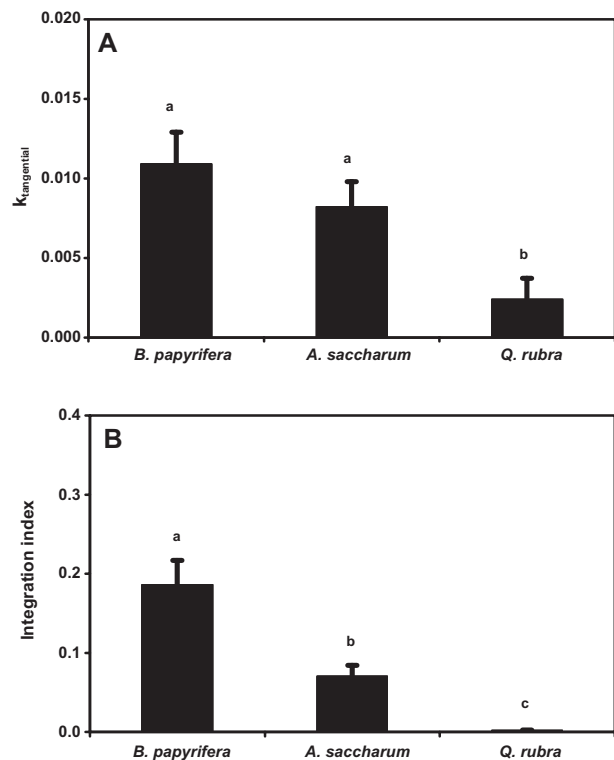


Figure 3. Hydraulic characteristics of 5-cm segments from 3-year-old branches. A, mean tangential conductance ($\text{g MPa}^{-1} \text{s}^{-1}$) in *Betula papyrifera*, *Acer saccharinum* and *Quercus rubra*. B, Integration Index: $2(K_{\text{tangential}})/K_{\text{axial}}$. $N = 4$ individuals per species. Bars with different lower case letters indicate significant differences based on Student's t -test at $P < 0.05$. Bars = +1 SE.

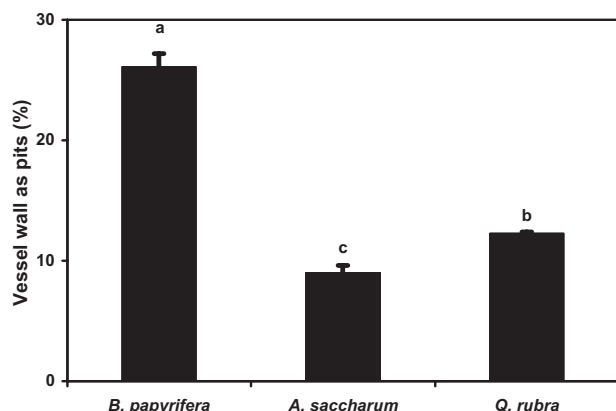


Figure 4. Mean percentage of pitted vessel wall area occupied by pit apertures. Based on ten samples from macerates of three individuals per species. Bars with different lower case letters indicate significant differences based on Student's *t*-test at $P < 0.05$. Bars = +1 SE. (Data for *Betula papyrifera* and *Acer saccharum* redrawn from Orians *et al.*, 2004.)

(3 cm long) were vacuum infiltrated in 1 : 1 10% HNO_3 and 10% CrO_3 , and macerated for 40 h at 60 °C. After washing in deionized water, macerates were stained in filtered safranin-O (1% in 100% EtOH) for 3 days at 60 °C. Samples were de-stained in EtOH, mounted on slides and photographed at 400 \times with a digital camera (Olympus BX40-F) attached to a microscope using Magnafire SP software (Olympus Optical Co. Ltd). Intervascular pitting in wide pit fields of vessel side-walls was measured from micrographs. From each wood macerate we measured pitting of ten vessel elements. These measurements were used to compare mean percentage pitfield area occupied by pits for each individual plant ($N = 3\text{--}4$ replicates per species). Images were printed and two parallel lines 25 μm apart were drawn along the transverse axis of each vessel element in broad pit fields where pits were clearly visible. In the segment between the lines, pits were counted. The lengths and widths of five pits down the centre of the segment were averaged and the formula for the area of an ellipse was used to estimate average pit chamber area. Total pit area of the segment was estimated by multiplying average pit area by the total number of pits. From these measurements, we present the percentage of pitfield wall area occupied by pits.

Differences in vessel distributions and diameters were determined from micrographs of wood cross-sections ($\sim 40\ \mu\text{m}$) stained in safranin (0.1% aq.), mounted in glycerol and photographed as above. To measure distance to nearest tangential neighbour, prints were made of wood cross-sections. Ten focal vessels were chosen randomly such that five vessels were measured each in springwood and summerwood of the 2003

growth ring. For each focal vessel, edge-to-edge distance to the nearest tangential neighbour was measured and average nearest-neighbour distance was calculated.

Statistical analyses were performed with JMP statistical software (Version 5.0.1.2, SAS Institute Inc.). A one-way analysis of variance was used to test for differences among species in the different traits. Significance of differences between species was determined using Least Squares Means Differences Student's *t*-test.

RESULTS

DYE DISTRIBUTION AND INTEGRATION INDEX

Saplings showed striking differences in their ability to distribute dye ($F = 162.32$, $P < 0.001$). In this study, *Q. rubra* was least capable of spreading point-source safranin throughout the crown. In a sample of four *B. papyrifera* saplings, stain spread to 17 out of 18 branches (94%) regardless of branch position relative to the root entry point of stain (Orians *et al.*, 2004). By contrast, safranin spread to 53% of branches of *A. saccharum* and only 38% in *Q. rubra*. Leaf biomass per branch was a poor predictor of branch staining in these species, because stain spread to branches with small leaf biomass as often as it did to branches with large leaf biomass. In *A. saccharum*, which has opposite phyllotaxis, stained branches tended to oppose unstained branches (data not shown).

Lateral conductance in blocked vessels ($K_{\text{tangential}}$) measured in branch xylem of mature trees provided a hydraulic measure of transfer capacity, a wood's potential for allowing lateral spread driven by pressure gradients in the crown. The species differed in $K_{\text{tangential}}$ with *Q. rubra* exhibiting the lowest capacity for indirect transfer (Fig. 3A; $F = 8.18$, $P < 0.003$). Integration Index (Fig. 3B) provided a more precise gauge of relative integration (potential for lateral flow) because it has a defined theoretical maximum of 1.0 (completely integrated) and a minimum of 0.0 (completely sectorial). The highest Integration Index occurred in *B. papyrifera* followed by *A. saccharum* and finally by *Q. rubra* ($F = 19.61$, $P < 0.001$). Compared with *Q. rubra*, fluid was ten times more able to pass through lateral channels in *A. saccharum* and more than 50 times more able to take lateral channels in *B. papyrifera* (Fig. 3B), when supplied by the same pressure gradient.

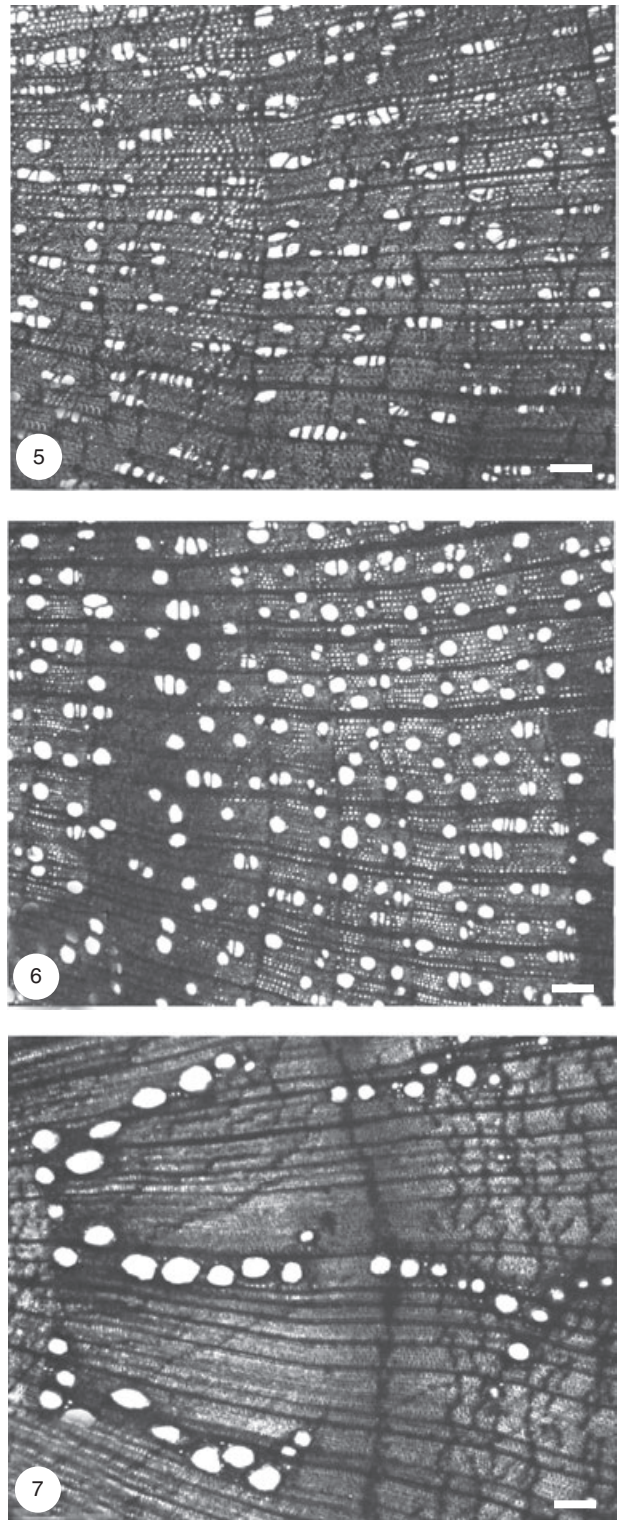
ANATOMY OF PATHWAYS

Wood macerates of branches from mature trees revealed the arrangement of intervacular pits in vessel walls (see Orians *et al.*, 2005a for images). *Betula*

papyrifera had the highest density of intervacular pits and its pits had small (2–4 µm) chamber size, features shared throughout the genus (Brown, Panshin & Forsaith, 1949; Orians *et al.*, 2004). The placement of pits produced alternate lateral wall pitting and a polygonal outline to the packed pits. Compared with *B. papyrifera*, intervacular pits in vessel walls of *A. saccharum* were more diffuse (less densely packed) and had larger pit chambers (6–10 µm diameter), in an alternate arrangement. In *Q. rubra*, pit chamber diameters again ranged from 6 to 10 µm, but were the most oval and widely scattered of the three woods tested, and had a vaguely alternate configuration. Based on ten samples of macerates made from each of three individuals for our test species, *B. papyrifera* had greater percentage of wall area in pits than did the two other species ($F = 162.3$, $P < 0.001$), with more than 24% of the cell wall area in pits. *Acer saccharum* and *Q. rubra* had 9 and 12%, respectively, of their wall space in pits.

Vessel diameter and radial distribution were measured from wood cross-sections of 3-year-old branches (Figs 5–7). Diffuse-porous *B. papyrifera* and *A. saccharum* have growth rings marked by denser fibres at the outer margin. In our *B. papyrifera* samples, larger vessel diameters ranged from 40 to 50 µm. These vessels were most often clumped in radial groups of three or more (Fig. 5). Vessel diameter did not vary consistently across the growth ring in *B. papyrifera*. Instead, wide and narrow vessels intermingled across the growth ring. The range of vessel diameters in *B. papyrifera* was much smaller than in *Q. rubra*. In our *A. saccharum* samples, vessel diameter was fairly uniform (50–60 µm) and vessels tended to be solitary (ungrouped) and evenly distributed throughout the growth ring (Fig. 6). In contrast to the two diffuse-porous species, strong differences in vessel diameter size classes occurred in the ring-porous *Q. rubra* (Fig. 7). Growth ring boundaries were easily distinguished by gradually reduced vessel diameter in summerwood. Springwood vessels were approximately 100 µm wide whereas summerwood vessels were 30 µm or less. In *Q. rubra* vessels did not aggregate into radial groups. Cross-sections showed *B. papyrifera* with the most radial vessel grouping, *Q. rubra* the least, with *A. saccharum* being intermediate between the two (Figs 5–7).

Average distance between tangentially neighbouring vessels was used as an estimate of vessel isolation related to tangential flow. Nearest-neighbour distance between vessels in cross-sections should be a good indicator of the likelihood for lateral flow between vessels in our 5-cm segments, as close vessels are likely to come into contact (Kitin *et al.*, 2004). Nearest tangential neighbours were more than three times further in *Q. rubra* and almost two times further in



Figures 5–7. Cross-sections (~40 µm) of sapwood from 3-year-old branches stained in safranin. Scale bars = 100 µm. Fig. 5. *Betula papyrifera*. Fig. 6. *Acer saccharum*. Fig. 7. *Quercus rubra*. (From Orians *et al.*, 2005a.)

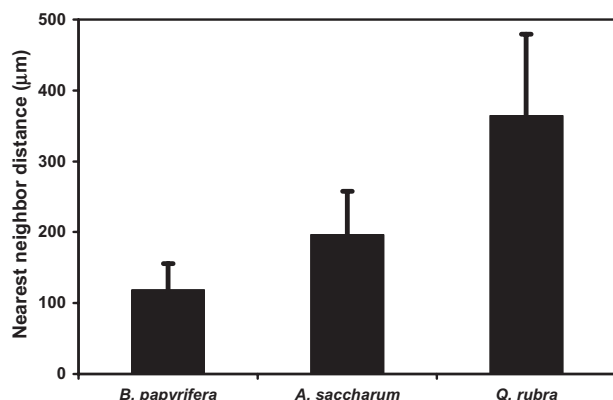


Figure 8. Mean distance from ten randomly selected focal vessels distributed throughout the growth ring to the nearest tangential neighbouring vessel in *Betula papyrifera*, *Acer saccharum* and *Quercus rubra*, measured from cross-sections. Bars = +1 SE.

A. saccharum than in *B. papyrifera* (Fig. 8). Thus, *B. papyrifera* vessels had the most extensive interconnections both radially (Fig. 5) and tangentially, probably facilitating spread of water around the stem. *Quercus rubra* had the least radial (Fig. 7) and tangential vessel interconnections, probably reducing spread of water around the stem.

DISCUSSION

Our results show that trees differ in their degree of sectoriality, and they help establish an integration–sectoriality continuum. Rather than describing species as integrated vs. sectorial, we use hydraulic and anatomical data to measure sectoriality as a comparative trait.

Movement of water and nutrients through constrained pathways from roots to shoots will in theory produce differences in tissue quality within the crown. Some portions of the crown will be better provided with water, nutrients and other resources than will others. Nitrate experimentally supplied through isolated lateral roots preferentially increased size and chlorophyll content of leaves directly above those roots (in the same orthostichy) in *Acer* sp., but not in *Betula* sp. (V. Gloser, K. Libera & C. Orians, unpubl. data). In the field, early leaf colour change in a subset of branches within an otherwise green crown may be an expression of tissue heterogeneity (Fig. 1). In *Betula* spp., leaf colour changes throughout the crown at the same time, leaving no indication of tissue quality differences among sets of branches. For those taxa (such as maple) that exhibit sectoriality, tissue quality differences within crowns may direct activity of herbivores and pathogens seeking nutrient-rich sectors (Orians & Jones, 2001).

PATTERNS OF TANGENTIAL SPREAD

In a strongly sectorial plant, flow of material between roots and crown will be channelled narrowly. Our results show that the most narrowly channelled taxon was *Q. rubra*, which spread dye to one-third of its branches, whereas in *B. papyrifera* dye travelled to almost all branches in the same period (Orians *et al.*, 2004). By quantifying dye spread in terms of branches reached after 24 h, we have a measure of sectoriality that suggests *B. papyrifera* (94% of its branches stained) to be 2.5 times more integrated than is *Q. rubra* (38% stain coverage). Our dye-spread measurements in whole saplings are useful in comparing sectoriality between taxa rather than as absolute values. Compared with saplings, tall trees have longer root-to-branch pathways that probably increase the opportunity for lateral flow given the right pressure gradient. Double saw-cut experiments on *Betula*, *Acer* and *Quercus* sp. (Tyree & Zimmermann, 2002) provide evidence of differences in tangential conductance in older trees. Lateral flow pathways can bypass a xylem wound within 1 cm of overlapping cuts in *Betula*, and 10 cm in *Acer*, but require 8 m to do so in *Quercus*. Thus, we expect the pattern of sectoriality between species to remain similar between saplings and mature trees. This could be tested further by injecting dye into their trunk (Phair & Ellmore, 1984; Tyree & Zimmermann, 2002) and noting its distribution throughout the crown. Based on dye spread from roots to branches in saplings, the taxa we tested fall along an integration–sectoriality spectrum in order from most integrated to most sectorial: *B. papyrifera*, *A. saccharum*, *Q. rubra*.

In trees, the trunk and branches serve as links between roots and leaves. Having demonstrated a range of sectoriality in entire plants, we compared the hydraulics in 5-cm segments of their branches (Fig. 3). We expected branch hydraulics to indicate the similar patterns of sectoriality as did dye distribution in whole plants. This was indeed the case. Species varied greatly in $K_{\text{tangential}}$. When the pathway of axial flow was blocked $K_{\text{tangential}}$ was high in *B. papyrifera*, moderate in *A. saccharum* and low in *Q. rubra* (Fig. 3A). Similar relationships were found with our Integration Index (Fig. 3B). We recognize that this index does not account for known differences in vessel length among species: species with shorter vessels than the sample length should encounter greater axial resistance due to end walls and thus may appear more integrated. However $K_{\text{tangential}}$ is not compromised by this limitation because axial resistance is raised experimentally to 100% for all species. Overall, our method describes potential pathways along which resources can move but the likelihood of these pathways being used in intact trees depends on pressure gradients along the pathways.

In terms of hydraulics, differences in $K_{\text{tangential}}$ indicate that the wood of *B. papyrifera* was 1.4 times more integrated than that of *A. saccharum* and 5 times more integrated than that of *Q. rubra*, while the index of integration suggests that *B. papyrifera* is 2 and 50 times more integrated than *A. saccharum* and *Q. rubra*, respectively. Compared with dye distribution percentages, hydraulic conductance was measured on a constant 5-cm length of branch internode and revealed slightly greater differences in sectoriality than did dye distribution. Dye uptake data rated *B. papyrifera* saplings 2.5 times more integrated than saplings of *Q. rubra*.

We recognize that hydraulic conduction measurements taken from 5-cm segments overestimate axial flow, and hence overestimate sectoriality in intact trees. Species, such as *Quercus* spp., with average vessel lengths in excess of 5 cm are likely to have many vessels run entirely through the segment without supplying a vessel endwall. This should underestimate resistance to axial flow and explain why $K_{\text{tangential}}$ and the Index of Integration result in different estimates of relative sectoriality even though the relative ranking remained the same. Artefacts from vessel length disparities can be reduced by measuring hydraulics in branch segments that exceed average vessel length for the wood being tested (Zimmermann & Jeje, 1981).

ANATOMICAL IMPLICATIONS FOR SECTORIALITY

Patterns of sectoriality are mediated by xylem structure. Several anatomical features could allow flow to tangential pathways, driven by lateral pressure differences in the crown. Sectoriality can be seen as a consequence of a sharp difference between low axial resistance and high lateral resistance. As the difference diminishes, lateral flow becomes more significant and wood becomes more integrated, able to respond to pressure differences caused by wind currents, differential shading and other influences on transpiration that act asymmetrically on the crown. Resistance to axial flow increases with narrow vessels, short vessels and scalariform perforation plates (Schulte & Castle, 1993; Baas, Wheeler & Chase, 2004), all of which occur to a greater degree in *B. papyrifera* than in our other two test species. Vessel width and length are usually coupled (Zimmermann & Jeje, 1981), with narrow vessels also being shorter and hydraulically less efficient (more resistant to axial flow) because of Hagen–Poiseuille flow. In addition, shorter vessels have more endwalls along the axial pathway, where axial flow will be resisted by pits (Zwieniecki, Melcher, & Holbrook, 2001a). With increased pit resistance in the axial pathway, axial resistance approaches lateral resistance, making lateral flow more likely and favouring integration, as seen in *B. papyrifera* (Fig. 3B).

Vessel diameter and perforation plate structure may be two wood characters that affect integration–sectoriality. Another is pitting. Resistance to flow through vessel walls, be they endwalls of the axial pathway or the tangential walls associated with lateral flow, is imposed by intervacular pits in the secondary cell wall (Chiu & Ewers, 1993). We predicted that the degree of hydraulic integration in our species would be proportional to the extent of intervacular pitting in each wood, but that was not the case. As predicted, vessel pit density was 2.5 times higher in *B. papyrifera* than in *A. saccharum*, and our hydraulics put *B. papyrifera* as 5 times more integrated than *A. saccharum*. In *Q. rubra*, however, the percentage of wall as pits was slightly higher than that of *A. saccharum* (Fig. 4), despite an extremely low Integration Index (Fig. 3B). Pitting alone appears to be an incomplete predictor of intervacular transfer capacity. Two factors may contribute to this result. First, pit resistance is determined by pit membrane porosity rather than by pit chamber diameter (Jarbeau, Ewers & Davis, 1995; Choat *et al.*, 2003; Sperry, 2003). Recently, Sperry & Hacke (2004) modelled the variation in pore sizes found in pit membranes, and Sano (2004, 2005) used scanning electron microscopy to find openings between microfibrils in pit membranes of *Betula platyphylla* and *Acer mono*. Porosity in those species was a function of pit membrane quality rather than size of the pit chamber. Large openings (high porosity) in the pit membrane will favour integration, as water can move more freely through walls bridged by porous pits. It is also possible that trees regulate their degree of integration, as pit membrane porosity changes in response to ion concentration (Zwieniecki, Melcher & Holbrook, 2001b). This was not a variable in our study because all species tested were perfused with the same solute concentration. Second, close vessels are likely to be connected within 2 cm or less of the plane of section, even in straight grained ring-porous wood such as that of *Fraxinus* (Kitin *et al.*, 2004). We found vessels in *B. papyrifera* and *A. saccharum* to be much closer to their nearest tangential neighbour than were vessels of *Q. rubra* (Fig. 8), thus better predicting hydraulic differences in sectoriality between *Q. rubra* and *A. saccharum* than did pitting. It may be that measuring the number of pit fields per unit vessel length, or the percentage of total wall that was pitted, would more accurately predict sectoriality than does our measure of pit percentage in pitted areas. Such a measure would account for portions of vessel wall that do not contact other vessels.

In summary, several anatomical traits seem to work together to determine degree of sectoriality. For the three species measured, we found sectoriedness to relate positively with increasing axial conductance, wide isolated vessels and low pitting density within

pit fields. That ring-porous species are more sectorized agrees with recent measures of sectoriality along leaf-to-leaf pathways (Orians, Smith & Sack, 2005b).

COMPARATIVE INTEGRATION–SECTORIALITY

In documenting differences in sectoriality we open the way for co- and multivariate linkage between extent of sectoriality and xylem structure (Baas, Wheeler & Chase, 2000; Orians *et al.*, 2005a), evolution (Sperry, 2003; Baas *et al.*, 2004; Maherali, Pockman & Jackson, 2004), life history suites (Reich *et al.*, 2003; Ackerly, 2004), climate (Wiemann *et al.*, 1998), shoot allometry and plant architecture (Preston & Ackerly, 2003). We are extending our methods to other species to understand better the range of lateral flow expressed in wood.

Different environments may select for species cohorts that concentrate near one end of an integration–sectorized axis. The most integrated species in our study (*B. papyrifera*) is diffuse-porous and occupies spatially heterogeneous (rocky soils), often disturbed habitats (Fralish & Franklin, 2002). It has high potential for sharing patchily acquired resources throughout the fast-growing crown. The price to be paid in temperate zones may be a short life because, along with water, integrated wood can spread embolisms and vascular pathogens, and is vulnerable to air seeding, all of which may restrict short-lived *B. papyrifera* in the north-east USA (Marchand, 1987; Wargo & Auclair, 2000). Integrated wood may be most limiting in seasonally arid areas, such as chaparral, known to have many ring-porous species (Davis *et al.*, 2002; Baas *et al.*, 2004). It remains to be seen if similar limitations are experienced by trees growing in disturbed spatially heterogeneous areas less prone to embolism, such as riparian sites in gravel or rocky riverbeds of tropical lowlands.

Among the three species in our study, ring-porous *Q. rubra* is the most strongly sectorized. This may allow this species to be long-lived by concentrating resources into growing or reproductive sectors, and by isolating sectors that are diseased or embolized (Castello, Leopold & Smallidge, 1995). The price to be paid may be slower growth, but if the association between sectorized ring-porous wood and seasonality holds up as we study more species, we predict that the prominence of sectorized species may increase as the pattern of wet and dry events intensifies with global climate change (Meehl & Tebaldi, 2004).

Having developed hydraulic and structural measures of integration–sectoriality as a functional trait, more species can be tested for their distribution along this continuum, grouped by architecture (monopodial vs. sympodial), ease of propagation, pest resistance, climate, occurrence along resource gradients, phylog-

eny, etc. In this way, we hope to use integration–sectoriality as an axis of ecophysiological variation to help explain tree diversity and forest dynamics in heterogeneous environments, including those associated with global climate change.

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REFERENCES

- Ackerly D. 2004.** Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* **74**: 25–44.
- Baas P, Ewers FW, Davis SD, Wheeler EA. 2004.** Evolution of xylem physiology. In: Hemsley AR, Poole I, eds. *The evolution of plant physiology. From whole plants to ecosystems*. London: Elsevier, 273–295.
- Baas P, Wheeler E, Chase M. 2000.** Dicotyledonous wood anatomy and the APG system of angiosperm classification. *Botanical Journal of the Linnean Society* **134**: 3–17.
- Brown HP, Panshin AJ, Forsaith CC. 1949.** *Textbook of wood technology, vol. I. Structure, identification, defects, and uses of the commercial woods of the United States*. New York: McGraw-Hill.
- Carlquist S. 2001.** *Comparative wood anatomy. Systematic, ecological, and evolutionary aspects of dicotyledon wood*. Berlin: Springer.
- Castello JD, Leopold DJ, Smallidge PJ. 1995.** Pathogens, patterns, and processes in forest ecosystems. *Bioscience* **45**: 16–24.
- Chiu ST, Ewers FW. 1993.** The effect of segment length on conductance measurements in *Lonicera fragrantissima*. *Journal of Experimental Botany* **44**: 175–181.
- Choat B, Ball M, Luly J, Holtum J. 2003.** Membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. *Plant Physiology* **131**: 41–48.
- Davis SD, Ewers FW, Sperry JS, Portwood KA, Crocker MC, Adams GC. 2002.** Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *American Journal of Botany* **89**: 820–828.
- Delcourt HR, Delcourt PA. 2000.** Eastern deciduous forests. In: Barbour MG, Billings WD, eds. *North American terrestrial vegetation*, 2nd edn. Cambridge: Cambridge University Press, 357–395.
- Fralish JS, Franklin SB. 2002.** *Taxonomy and ecology of woody plants in North American forests*. New York: John Wiley & Sons.
- Jarbeau JA, Ewers FW, Davis SD. 1995.** Mechanism of water-stress-induced embolism in two species of chaparral shrubs. *Plant Cell and Environment* **18**: 189–196.

- Jones CS, Lord EM. 1982.** The development of split axes in *Ambrosia dumosa* (Gray) Payne (Asteraceae). *Botanical Gazette* **143**: 446–453.
- Kitin PB, Fujii T, Abe H, Funada R. 2004.** Anatomy of the vessel network within and between tree rings of *Fraxinus lanuginosa* (Oleaceae). *American Journal of Botany* **91**: 779–788.
- Kozlowski TT, Winget CH. 1963.** Patterns of water movement in forest trees. *Botanical Gazette* **124**: 301–311.
- Larson DW, Doubt J, Matthes-Sears U. 1994.** Radially sectorized hydraulic pathways in the xylem of *Thuja occidentalis* as revealed by the use of dyes. *International Journal of Plant Sciences* **155**: 569–582.
- Maherali H, Pockman WT, Jackson RB. 2004.** Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85**: 2184–2199.
- Marchand PJ. 1987.** *North woods*. Boston: Appalachian Mountain Club.
- Marshall C. 1996.** Sectoriality and physiological organization in herbaceous plants: an overview. *Vegetatio* **127**: 85–97.
- McWilliams WH, Heath LS, Reese GC, Schmidt TL. 2000.** Forest resources and conditions. In: Mickler RA, Birdsey RA, Hom J, eds. *Responses of northern US forests to environmental change*. New York: Springer, 3–26.
- Meehl GA, Tebaldi C. 2004.** More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* **305**: 994–997.
- Orians CM, Ardon M, Mohammad BA. 2002.** Vascular architecture and patchy nutrient availability generate within-plant heterogeneity in plant traits important to herbivores. *American Journal of Botany* **89**: 270–278.
- Orians CM, Babst B, Zanne AE. 2005a.** Vascular constraints and long-distance transport in dicots. In: Holbrook NM, Zwieniecki M, eds. *Vascular transport in plants*. Oxford: Elsevier, 355–371.
- Orians CM, Jones CG. 2001.** Plants as resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. *Oikos* **94**: 493–504.
- Orians CM, Pomerleau J, Ricco R. 2000.** Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *Journal of Chemical Ecology* **26**: 471–485.
- Orians CM, Smith SDP, Sack L. 2005b.** How are leaves plumbed inside a branch? Differences in leaf-to-leaf hydraulic sectoriality among six temperate tree species. *Journal of Experimental Botany* **56**: 2267–2273.
- Orians CM, van Vuuren MM, Harris NL, Babst BA, Ellmore GS. 2004.** Differential sectoriality in long-distance transport in temperate tree species: evidence from dye flow, ^{15}N transport, and vessel element pitting. *Trees* **18**: 501–509.
- Pearcy RW, Gross LJ, He D. 1997.** An improved dynamic model of photosynthesis for estimation of carbon gain in sunfleck light regimes. *Plant Cell and Environment* **20**: 411–424.
- Phair WE, Ellmore GS. 1984.** Improved trunk injection for control of Dutch elm disease. *Journal of Arboriculture* **10**: 273–278.
- Preston KA, Ackerly DA. 2003.** Hydraulic architecture and the evolution of shoot allometry in contrasting climates. *American Journal of Botany* **90**: 1502–1512.
- Price EAC, Hutchings MJ, Marshall C. 1996.** Causes and consequences of sectoriality in the clonal herb *Glechoma hederacea*. *Vegetatio* **127**: 41–54.
- Reich PB, Wright J, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB. 2003.** The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* **164**: S143–S164.
- Robinson D. 1994.** The response of plants to non-uniform supply of nutrients. *New Phytologist* **127**: 635–674.
- Ruzin SE. 1999.** *Plant microtechnique and microscopy*. New York: Oxford University Press.
- Samuelson LJ, Hogan ME. 2003.** *Forest trees: a guide to the southeastern and mid-Atlantic regions of the United States*. Upper Saddle River, NJ: Prentice Hall.
- Sano YZ. 2004.** Intervascular pitting across the annual ring boundary in *Betula platyphylla* var. *japonica* and *Fraxinus mandshurica* var. *japonica*. *International Association of Wood Anatomists Journal* **25**: 129–140.
- Sano YZ. 2005.** Inter- and intraspecific structural variations among intervacular pit membranes, as revealed by field-emission scanning electron microscopy. *American Journal of Botany* **92**: 1077–1084.
- Schulte PJ, Castle AL. 1993.** Water flow through vessel perforation plates – a fluid mechanical approach. *Journal of Experimental Botany* **44**: 1135–1142.
- Shigo AL. 1985.** How tree branches are attached to trunks. *Canadian Journal of Botany* **63**: 1391–1401.
- Sperry JS. 2003.** Evolution of water transport and xylem structure. *International Journal of Plant Science* **164**: S115–S127.
- Sperry JS, Hacke UG. 2004.** Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membranes. *American Journal of Botany* **91**: 369–385.
- Stegmann EW, Primack RB, Ellmore GS. 1988.** Absorption of nutrient exudates from terrapin eggs by roots of *Ammophila breviligulata* (Gramineae). *Canadian Journal of Botany* **66**: 714–718.
- Terashima I. 1992.** Anatomy of non-uniform leaf photosynthesis. *Photosynthesis Research* **31**: 195–212.
- Tichane R. 1987.** *Ash glazes*. Painted Post, NY: New York State Institute for Glaze Research.
- Tyree MT, Zimmermann MH. 2002.** *Xylem structure and the ascent of sap*. Berlin: Springer.
- Wargo PM, Auclair AND. 2000.** Forest declines in response to environmental change. In: Mickler RA, Birdsey RA, Hom J, eds. *Responses of northern US forests to environmental change*. New York: Springer, 117–145.
- Watson MA, Casper BB. 1984.** Morphogenetic constraints on patterns of carbon distribution in plants. *Annual Review of Ecology and Systematics* **15**: 233–258.
- Wiemann MC, Wheeler EA, Manchester SR, Portier KM. 1998.** Dicotyledonous wood anatomical characters as predictors of climate. *Palaeo* **139**: 83–100.

- Zimmermann MH, Jeje AA. 1981.** Vessel-length distribution in stems of some American woody plants. *Canadian Journal of Botany* **59**: 1882–1892.
- Zwieniecki MA, Melcher PJ, Holbrook MN. 2001a.** Hydraulic properties of individual xylem vessels of *Fraxinus americana*. *Journal of Experimental Botany* **52**: 257–264.
- Zwieniecki MA, Melcher PJ, Holbrook MN. 2001b.** Hydrogel control of xylem hydraulic resistance in plants. *Science* **291**: 1059–1062.