

Water flow through junctions in Douglas-fir roots

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ABSTRACT

Roots are important conduits for the redistribution of water within the rooting zone. Root systems are often highly branched, and water flow between regions undoubtedly involves passage through junctions between individual roots. This study considered junctions in the roots of Douglas-fir with regard to the resistances encountered by water flow through the xylem. Flow into the root branch distally along the main root encountered much greater resistance than flow into the branch and proximally along the main root (toward the plant stem). When the main root proximal to the junction was gradually shortened, the resistance to flow in the branch root and distally along the main root increased dramatically. Thus, flow in this manner appears to depend on lateral flow within the root over many centimetres proximal to the junction and not just within the direct connection at the junction. These results suggest that the hydraulic nature of junctions is an important aspect of hydraulic redistribution of water within the soil utilizing flow through roots.

Key-words: conductance; hydraulic redistribution; hydraulic resistance; tracheids; xylem.

INTRODUCTION

The root systems of terrestrial plants function in part for the uptake of water and nutrients from the soil and for their transport to the above-ground plant stem. More recently, it has become apparent that roots also play an important role in the distribution of water within the soil. For many deep-rooted desert species, roots in contact with the water table at great depths transport water upward and hydrate the soil adjacent to surface roots (Caldwell, Dawson & Richards 1998). It has also been suggested that redistribution of water laterally within the soil or even from wet upper to drier lower layers may be significant for species in other environments as well (Burgess *et al.* 2001; Hultine *et al.* 2004).

Junctions in above-ground branches have been studied extensively as potential constrictions to flow and as segmentation points in the overall hydraulic architecture of

tree crowns. Vessels or tracheids may be slightly narrower within junctions, or the occurrence of vessel endings may increase within the junction (Tyree & Ewers 1991). The segmentation concept (Zimmermann 1983) suggests that embolism within the junction may serve to sacrifice branches during periods of high water stress. In a related manner, junctions in root systems may serve to isolate the main root axis from branch roots during soil drying (North, Ewers & Nobel 1992).

Roots produce branches as do above-ground shoots, although the origin of these branches differs when considered from the developmental or anatomical perspective. The hydraulic significance of these junctions in root systems and their role in the redistribution of water within the soil is not well understood. Water taken up from the soil in one region and redistributed to another region undoubtedly passes through junctions in the root system. However, redistribution could also involve flow into a junction and distally out of the other component of the junction, leading to what others have referred to as reverse flow (Hultine *et al.* 2003). It is not clear if the resistances encountered within the junction during such reverse flows are the same as those encountered when water flows through roots and their junctions towards the main stem of the plant. Previous work on junctions in above-ground branches has suggested that junctions are not simple flow dividers, whereby the downstream branches would themselves be closely coupled (Schulte & Brooks 2003). Therefore, this study was designed to examine the nature of water flow through the xylem of root junctions in Douglas-fir trees [*Pseudotsuga menziesii* var. *menziesii* (Mirb) Franco]. Although perhaps an obvious assumption, the secondary purpose of this study is to demonstrate that flow through the xylem of a simple root segment shows no directional preference and that the hydraulic resistance will be the same regardless of flow direction.

MATERIALS AND METHODS

Plant material

Roots were collected by careful excavation at the base of Douglas-fir trees (15–20 m high, 24 years old) at the Wind River Experimental Forest of the Gifford Pinchot National Forest within the southern Washington Cascade Range (45°49'N, 121°59'W, 560 m elevation). Upon collection, the roots were wrapped with moist paper and sealed in plastic bags for processing that same day.

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Hydraulic conductance and flow measurements

Root junctions were excised and recut under water before being attached to a pressure-flow system for the measurement of flow rate under an applied pressure. Tissues external to the xylem were removed because of the presence of large resin ducts, which could release materials that would obstruct water flow through the xylem tracheids. The basal main section of the excised junction (component C, Fig. 1) was attached with tubing to a source of partial vacuum and a pressure meter. Pipettes were attached to the two branches for measurement of flow rate by timing the passage of the meniscus through the pipette. The tubing connections to the roots were sealed with a clear acrylic copolymer (Wet 'n Wild Markwins Beauty Products, City of Industry, California). Pressure (10–20 kPa) was applied and flow rate in the two branches was measured. For the first measurement sequence, the vacuum line was moved to component B and flow was measured in A and C. Flow was stopped in C, and changes in flow rate in A were noted. The initial arrangement was restored (vacuum applied to C) for flow measurements in A and B. Flow was then stopped in B, and flow rate changes in A were noted. Lastly, the junction was cut apart and segments were removed from all three components. These segments were sealed in a similar pressure-flow system for the measurement of hydraulic conductance per unit length (K_i , $\text{m}^4 \text{MPa}^{-1} \text{s}^{-1}$) (Schulte, Gibson & Nobel 1987, 1989). An estimate of the total flow resistance of each component of the junction was calculated by multiplying the inverse conductance per unit length by

the total length of that component of the junction. Solutions for flow studies were made from degassed water and 20 mM KCl (Zimmermann 1978). Hydraulic conductance is assumed to be inversely proportional to the viscosity of water, which depends in turn on temperature. Therefore, conductances as measured in the field were corrected to a reference temperature of 20 °C based on a standard relationship between water viscosity and temperature (Sengers & Watson 1986). Where the above description of methods refers to flow being stopped at a particular branch component, acrylic copolymer was applied to the cut xylem surface. However, pressures within the tracheids were slightly below atmospheric but not low enough to draw air through the pits of tracheids; thus, such sealing was probably not necessary. When flow needed to be re-established into a component of the branch that had been sealed, several millimetres of the root were excised so as to remove any air bubbles that might block the flow.

For the second measurement sequence, a partial vacuum was applied to component B (see Fig. 1) and flow rate was measured in component A, with the cut surface sealed at C. Next, a short segment of C was removed from the base (approximately 5–10 mm) and the flow rate was again measured in A. This process was repeated until component C was reduced from an initial length of 150 to 200 mm to less than 10 mm. Stain (safranin) was also forced through several junctions in the same manner in order to observe qualitatively the process of flow.

Several segments were obtained from the collected roots for the purpose of demonstrating that hydraulic conductance did not depend on flow direction. Tissues external to the xylem were removed and the root segments were sealed into the pressure-flow system for measuring hydraulic conductance. After the pressure was applied, several measurements of flow rate were obtained, the root segment was reversed in the tubing system and another series of conductance measurements followed. Several directional changes were applied in this manner to each root segment.

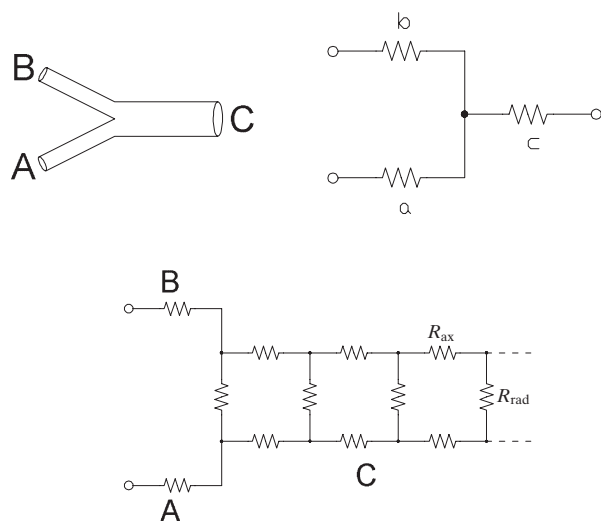


Figure 1. Conceptual diagram of a root junction showing a common basal segment (C) and the two adjoining segments (A and B). Component C in this diagram is facing towards the base of the plant, while the other components face the tips of the root system. The diagram on the right shows a preliminary electrical circuit model (the 'three-resistor model') of such a junction. Lower diagram shows a ladder network electrical circuit analogue for the root junction. For this model, component C of the junction has been expanded from the three-resistor model to incorporate axial (R_{ax}) and radial (R_{rad}) xylem resistances.

Ladder resistance model

A ladder network model for the common segment C (Fig. 1) was developed to produce predictions on the effect of shortening C on cross flow between A and B (the second measurement sequence as outlined previously). The axial xylem resistors of the model were estimated from measurements of the hydraulic resistance (inverse conductance) of root segments applied to the pressure flow system. The individual cross (radial) resistors were estimated from the initial (C at full length) measured resistance to flow between junction components A and B. The network model's radial resistance was computed given the measured total resistance and axial resistances. This model solution was obtained by using an iterative method analogous to Newton's method for finding the roots of a function and implemented with a short computer program (Microsoft Visual C ++). Essentially, the process starts with an initial guess for the radial resistances (see Fig. 1). The rightmost

three resistors are summed in series and the resulting resistance combined in parallel with the next radial resistor. This process continues all along the ladder forming component C until a final total resistance is obtained by summing the equivalent of C with A and B. The initial guess for the radial resistance is then refined based on a comparison between the measured and predicted total resistances. Newton's method is the iterative procedure employed for this refinement process (see any numerical methods text such as Gerald & Wheatley 1999). Predictions of total junction conductance (inverse resistance) were then made for a gradually shortened model analogous to the shortening of component C during the actual measurement process. A comparison of such predicted conductances with those measured was used to test the validity of the ladder network as a description of the nature of the root branch junction with respect to water flow in the xylem. As will be noted from the model results, this approach for estimating the radial resistance to flow from each junction starting with its full length means that the predicted and measured values will correspond perfectly for the junction at its full length. As the component C is shortened, the predicted values may deviate from the measured values depending on the model's ability to address the interplay between axial and radial resistances.

RESULTS

The first measurement sequence involved comparing the water flow rate in different directions through the junction and the effects of stopping the flow into one of the components of the junction. For this sequence (see Fig. 2), the vacuum was initially applied to the basal component C. Typically, flow into A and B was not identical but dependent on the relative sizes of the two branch components. Note that the flow out of the component where vacuum was applied was not measured but was calculated as the sum of the flows into components A and B based on mass conser-

vation and the steady-state nature of the established flow (i.e., no changes in water storage). When the vacuum was transferred from C to B, the flow rate into component A dropped dramatically despite maintaining the same applied pressure difference (Fig. 2, second panel from left). Stopping the flow at C partially restored flow into A (Fig. 2, middle panel). Following the re-establishment of flow into A and B with the vacuum on component C, the original flow rates into A and B were restored (Fig. 2, second panel from right as compared to first panel on left), indicating that no permanent disruptions to xylem conduits had occurred. Lastly, with the vacuum still attached to component C, halting the flow at branch B caused a slight increase in flow in A (Fig. 2, right panel).

The branch junctions considered in this study varied in size and in the relative balance between the branch components. In subsequent comparisons of six different root junctions, flow rates were normalized by dividing all flow rates by the initial measured flow in A. This procedure allows for the expression of the effects of the various junction manipulations by a relative change in the flow measured initially. Assuming the simple, three-resistor electrical circuit analogue of the junction (Fig. 1), the effects of the various manipulations of the junction on flow can be predicted based on Ohm's Law and by knowing the applied pressure and values for the resistance of each junction component. The method of normalizing for both measured and predicted flow rates results in an inherent match between these quantities for the initial flow set-up with the vacuum applied to component C and flows measured at A and B (Fig. 3, left panel). Flow rates following subsequent junction manipulations are then compared with this initial flow regime. Although applying the vacuum to B instead of C typically produced a large measured drop in flow into A, the simple resistor model predicts about the same flow or a slight increase in flow into A (Fig. 3, second panel from left). When flow at C was halted, both the predicted and measured flow rates in A increased (Fig. 3, middle panel).

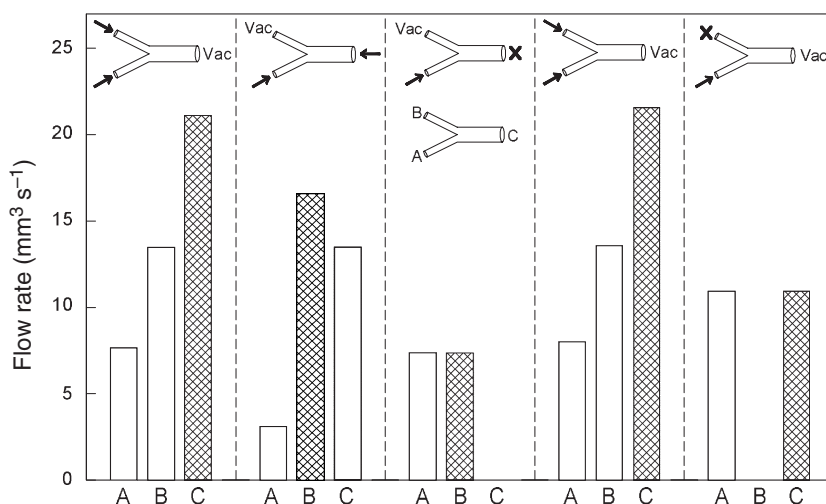


Figure 2. Flows at components A, B and C for the first measurement sequence for one typical root junction, with flows expressed as positive values regardless of direction (into or out of the component). Each of the five panels shows flow rates in the individual components of the junction for one portion of the experiment, as conducted from left to right in the figure. The y-shaped junction drawings show the arrangement with respect to where the vacuum was applied (Vac) and whether flow was allowed and measured (arrow) or blocked (x). Flow rates were measured at the locations shown by arrows (clear bars) and the flow out of the segment where the vacuum was attached (hatched bars) was calculated by summing the inflows.

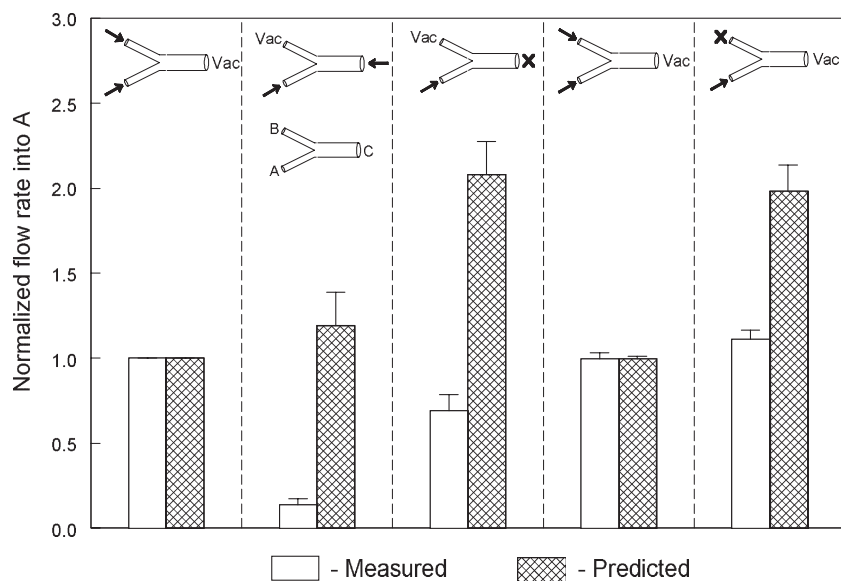


Figure 3. Measured and predicted flows into junction component A for six different root junctions. All flow rates were normalized by dividing the flow rates by that initially measured from the first pressure application. Error bars show the standard error of the mean ($n = 6$). The y-shaped junction drawings show the arrangement with respect to where the vacuum was applied (Vac) and whether flow was allowed and measured (arrow) or blocked (x).

Re-establishing the initial flow set-up restores the flows to the original rates (Fig. 3, comparing the left panel with the second from right panel). Lastly, with the vacuum still applied to C and the flow halted at B, the flow into A increased only slightly (12%), whereas the simple three-resistor model predicted a doubling of flow (99% increase; Fig. 3, right panel).

The second measurement sequence considered cross flows between junction components A and B in more detail. The flow between A and B was strongly dependent on the length of the common segment C (Fig. 4, symbols). Although the three-resistor model would predict that shortening C has no effect on flow between A and B, the hydraulic conductance for this pathway declined to near

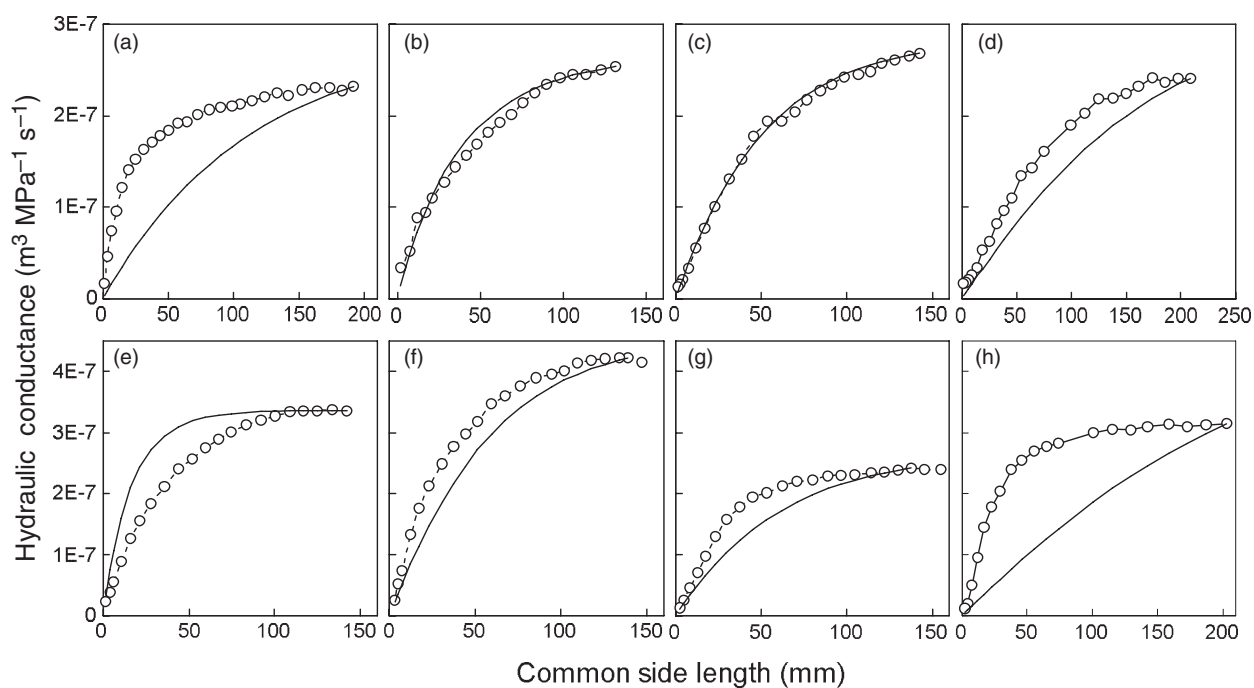


Figure 4. Effect of shortening component C on the measured hydraulic conductance to flow between A and B (circles). Model predictions from the ladder network (solid lines) were based on estimates of the radial and axial resistances of the roots. Note that the measurement process started with a long common side length (C) and proceeded to shorter lengths (right to left in each graph).

Table 1. Xylem outer diameters for each component of the root junctions tested in the cross flow measurements of hydraulic conductance to flow between branch components A and B

Junction	Diameter (mm)		
	A	B	C
A	3.19	8.10	8.74
B	3.70	4.62	5.94
C	2.30	4.30	5.30
D	3.90	5.40	6.10
E	2.79	3.05	4.44
F	3.35	4.28	5.69
G	1.93	5.21	5.55
H	3.60	7.70	8.40

Junction designations correspond to the data sets presented in Fig. 5. Diameters were measured after removal of bark and phloem tissues.

zero as the component C was cut shorter. Stain applied to similar junctions did not flow directly from component A to B across the junction, but along component C away from the junction even though the partial vacuum was applied only to component B. The stain gradually circled around and across the stem of component C before beginning to flow towards component B.

Starting with the full length of component C present, a plateau region was generally observed, initially with a gradual decrease in conductance, followed by a steep decline for shorter lengths (Fig. 4). Model predictions (Fig. 4, solid lines) always showed a decline in conductance with shortening of C, matching some root junctions very closely. For others, however, the predicted curve either did not have a long enough plateau or had a too extensive plateau region. Two junctions in particular showed a mismatch between model and measurement (Fig. 4a & h), whereby the modelled conductance declined much more rapidly than actually measured. However, for one junction (Fig. 4e), the modelled conductance did not decline as quickly as measured. The junctions where the model underpredicted the conductance as component C was shortened (Fig. 4a & h) were those with large B and C components relative to component A (Table 1). On the other hand, the junction with the greatest overprediction of conductance (Fig. 4e) had the narrowest B and C components, which were more similar to component A than for the other junctions.

The hydraulic conductance for flow does not appear to change when root segments are reversed in the pressure-flow system (Fig. 5). Although in some cases flow conductance appears to decline gradually through the extended measurement period, the trend is consistent regardless of the orientation of the root and hence the direction of flow.

DISCUSSION

Considering the initial flow set-up with the vacuum connected to the basal component C and flow occurring into

the two branches A and B, when the vacuum was applied, the pressure drop would have propagated along the stem and through the junction such that flow would occur because of the established pressure gradient. If flow were then halted at one of the branches, the pressure gradient within that branch would disappear. This would lead to a decrease in the pressure within the junction and hence an increase in the pressure gradient driving the flow into the remaining branch. Thus, stopping the flow in one branch of a junction would be expected to lead to an increase in flow in an adjacent branch. This expectation was supported in both cases during the first measurement sequence when vacuum was applied at C and flow was halted at B, and when vacuum was applied at B and flow was halted at C. Flow into component A increased in both cases, although to a lesser extent than predicted based on the three-resistor model.

The second aspect of the first measurement sequence (manipulating flow directions) allowed for a comparison of flow into a root branch and towards the base of the plant as opposed to flow into the branch and back out the adjacent branch. Although the three-resistor model predicted a slight increase in flow into the branch when the outflow direction was changed, results consistently showed a large decrease in flow. Therefore it appears that flow into one branch of a root junction and back out along the adjacent branch (a reverse flow as described in the hydraulic redistribution literature) encounters much greater resistance than flow towards the base of the plant. The nature of water flow through root junctions in Douglas-fir does not appear to match the preliminary notion of the junction as a simple three-resistor circuit, a finding that has also been observed for shoot branch junctions (Schulte & Brooks 2003). Such a model would have suggested a much greater intercon-

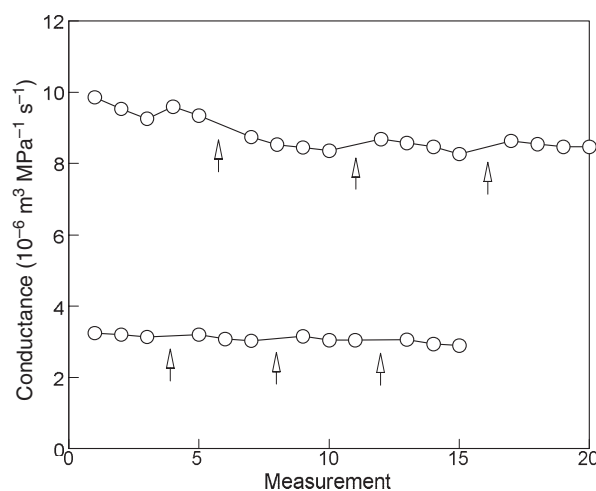


Figure 5. Effect of flow direction on hydraulic conductance as measured on simple root segments (not involving a junction). Conductance was measured repeatedly and the segment orientation was reversed three times during the measurement series. Arrows indicate the points at which the root segment was reversed within the pressure-flow system.

nectedness between the distal portions of the junction than was observed. Measurements indicate that flow occurs preferentially (lower resistance) between a distal branch and the main root than between the branches themselves. It is apparent from measurements with simple root segments that the root xylem is not inherently directional. Thus, the directional effects identified within the root junctions must be due to xylem tracheid orientations within the junction, which provide good connections from branch to main root rather than between root branches.

Flow between two root branches appears to utilize lateral or radial flow within the xylem of the common segment on the proximal side of the junction, as evidenced by the observed stain movements and the quantitative reduction in flow conductance as the common segment was shortened. A ladder-like resistor network model of the junction produced cross flow conductance estimates that matched measurements to a varying extent depending on the relative sizes of the branches in the junction. The nature of the suggested ladder network is such that a relatively small radial resistance compared to the axial resistance would tend to confine the cross flow to short distances beyond the junction (the conductance curve would have a steep initial increase with a long plateau). Conversely, an increase in the radial resistance or a decrease in the axial resistance tends to produce a more gradual corner in the conductance curve such that the cross flow depends on a greater distance along the common junction segment. A comparison of the relative effectiveness of the model with the various junctions tested suggests that while the importance of lateral flow is supported, the ladder network as proposed does not fully account for the effects of relative size between the parts of a junction. In particular, when one side of the junction was considerably smaller than the other (component A compared to component B), the model underpredicted conductance changes as component C was shortened. A small root branching from a large root may be effectively connected to only a small portion of the main root xylem, in a sense requiring an uneven ladder for the model (the axial resistances on the side of the smaller branch would be greater than those on the opposite side of the ladder).

As noted, cross flow between root branches appears to depend on radial or lateral flow across the xylem. In a more general sense, the ability of the xylem to either allow flow readily or not in the lateral direction may be significant for long-lived tree species. The term 'sectorialized' has been used to describe xylem with apparently poor lateral connections (Larson, Doubt & Matthes-Sears 1994). Studies of lateral flow in the xylem of woody plants have demonstrated that considerable variation is found among different plant species. It has also been suggested that variation between species may be due to differences in the pitting characteristics of the xylem conduits (Orians *et al.* 2004). Therefore, the pitting characteristics of the sidewalls in xylem conducting elements, such as tracheids, for the Douglas-fir described herein may be important in determining the relative ease of cross flow between root branches.

The redistribution of water within the soil profile by flow through plant roots has been described for woody plants in many ecosystems (Meinzer *et al.* 2004). Depending on rainfall patterns, individual plants may facilitate the redistribution of water from deep wet regions to drier surface layers or, after rainfall wets the upper layers of a dry soil profile, from wet surface layers to drier deeper regions (Burgess *et al.* 1998). Although simulations (Ryel *et al.* 2002) suggest that the redistribution of soil water by unsaturated flow (as opposed to flow through roots) accounts for a portion of the water movement (depending on soil hydraulic conductivity), redistribution of water within the soil through the root system would depend on the reverse flow of water along roots and hence a flow across junctions between roots (referred to as cross flow in this paper). Such flows are likely to encounter greater resistance than flows directed towards the base of the plant stem. Such flows also depend on the relative magnitude of radial or lateral flow resistances within the xylem. Thus, the resistance encountered for a specific path between parts of a root system may depend on the direction of flow through junctions and the number of such junctions. Brooks *et al.* (2002) speculate that their observed patterns of redistribution of deuterium-labelled water applied to specific locations are affected by the degree of interconnectedness within the xylem. In addition, Burgess *et al.* (2000) present evidence for the isolation of lateral roots within the main stem (non-integrating nature of xylem). However, other studies with grape vines (Smart *et al.* 2005) suggest that for some species, despite the presence of fibre cells seemingly limiting lateral flow between xylem vessels, water can flow between opposite sides of the plant by lateral movement around the xylem within the stem. Further studies on the junctions in root systems with regard to how radial flow resistances in the xylem may determine coupling between the components of a junction would be useful to increase our understanding of their hydraulic nature and potential role in hydraulic redistribution through root systems.

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