

RESEARCH PAPER

Ionic control of the lateral exchange of water between vascular bundles in tomato

Maciej A. Zwieniecki^{1,4}, Colin M. Orians^{1,2}, Peter J. Melcher^{1,3} and N. Michele Holbrook¹

¹ Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA

² Department of Biology, Tufts University, Medford, MA 02155, USA

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Abstract

Ions can enhance water flow through the xylem via changes in the hydraulic resistance at border pit membranes. Because flow between adjacent xylem vessels occurs primarily via bordered pit fields, it is hypothesized that xylem sap ion concentrations would affect lateral movement of water more than longitudinal flow. Using tomato as a model system, evidence is presented for ion-mediated changes in xylem hydraulic resistance and the lateral transport of water. Water flow between adjacent xylem bundles increased by approximately 50% in the presence of ions while longitudinal flow only increased by approximately 20%. However, the enhancement of lateral exchange due to ions was magnified by the presence of a pressure difference between vascular bundles. These results indicate that the degree of nutrient-sharing among sectors of a plant may depend on both nutrient concentration and the availability of water in the root zone.

Key words: Ion-mediated lateral flow, *Lycopersicon esculentum*, tomato, vascular architecture.

Introduction

The distribution of resources within a plant may be constrained by the development of the vascular system. Plants in which lateral transport is restricted by vascular architecture are called sectoral (Watson and Casper, 1984; Sprugel *et al.*, 1991; Marshall, 1996; Vuorisalo and Hutchings, 1996; Orians and Jones, 2001). In the extreme case, one can think of a plant as consisting of numerous

autonomous subunits (Sprugel *et al.*, 1991). For example, Preston (1998) showed that the transport of carbohydrates between leaves with direct vascular connections is high, while transport between leaves in different sectors was minimal or even absent. Others have shown that sectoral transport extends from roots to shoots and vice versa (Rinne and Langston, 1960; Hay and Sackville-Hamilton, 1996; Murphy and Watson, 1996). The complete isolation of individual sectors, however, is probably quite rare. The fact that plants can survive and grow when water is applied to isolated lateral roots (Hansen and Dickson, 1979; Fort *et al.*, 1998; CM Orians, unpublished results) demonstrates that lateral transport can occur. However, Orians *et al.* (2002) found that nutrient fertilization to isolated lateral roots of tomato resulted in greater growth of those leaves and branches with direct vascular connections, indicating that nutrients are not easily shared among sectors.

Although the effect of vascular architecture on resource distribution is well established, whether the exchange among sectors can be subsequently modified has not been examined. Ionic mediation of flow within the xylem (Zimmermann, 1978; Van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001) suggests that xylem solutes might mediate the extent of resource sharing among sectors. Zwieniecki *et al.* (2001) showed that variation in ionic strength within the reported range of xylem sap concentration (Marschner, 1995; Schurr and Schulze, 1995) can induce significant changes in xylem axial resistance. Response to nutrient concentration was fast (seconds to minutes) and was localized at the vessel-to-vessel junctions (bordered pit fields). They concluded that contraction of hydrogels in the border pit membranes allowed for enhanced flow in the presence of increasing ionic concentration. Because transport among separate sectors (vascular bundles) requires the

³ Present address: Department of Biology, Ithaca College, Ithaca, NY 14850, USA.

⁴ To whom correspondence should be addressed. Fax: +1 617 496 5854. E-mail: mzwienie@oeb.harvard.edu

movement of xylem sap across walls or bordered pit fields, it is hypothesized that modifications in xylem sap ion concentration could result in marked changes in resistance restricting sector-to-sector resource exchange. Evidence is presented here for ion-mediated changes in xylem hydraulic resistance and the lateral transport of water in tomato. Tomato was used as a model system since leaves are linked by a specific pattern of vascular traces (Dimond,

1966; Orians *et al.*, 2000; Fig. 1) that allow the simultaneous quantification of the effects of ions on radial and longitudinal xylem hydraulic resistance.

Materials and methods

Tomato seeds, *Lycopersicon esculentum* (Celebrity F₁ and Sun Gold F₁, Johnny's Selected Seeds, Albion, ME) were grown in a

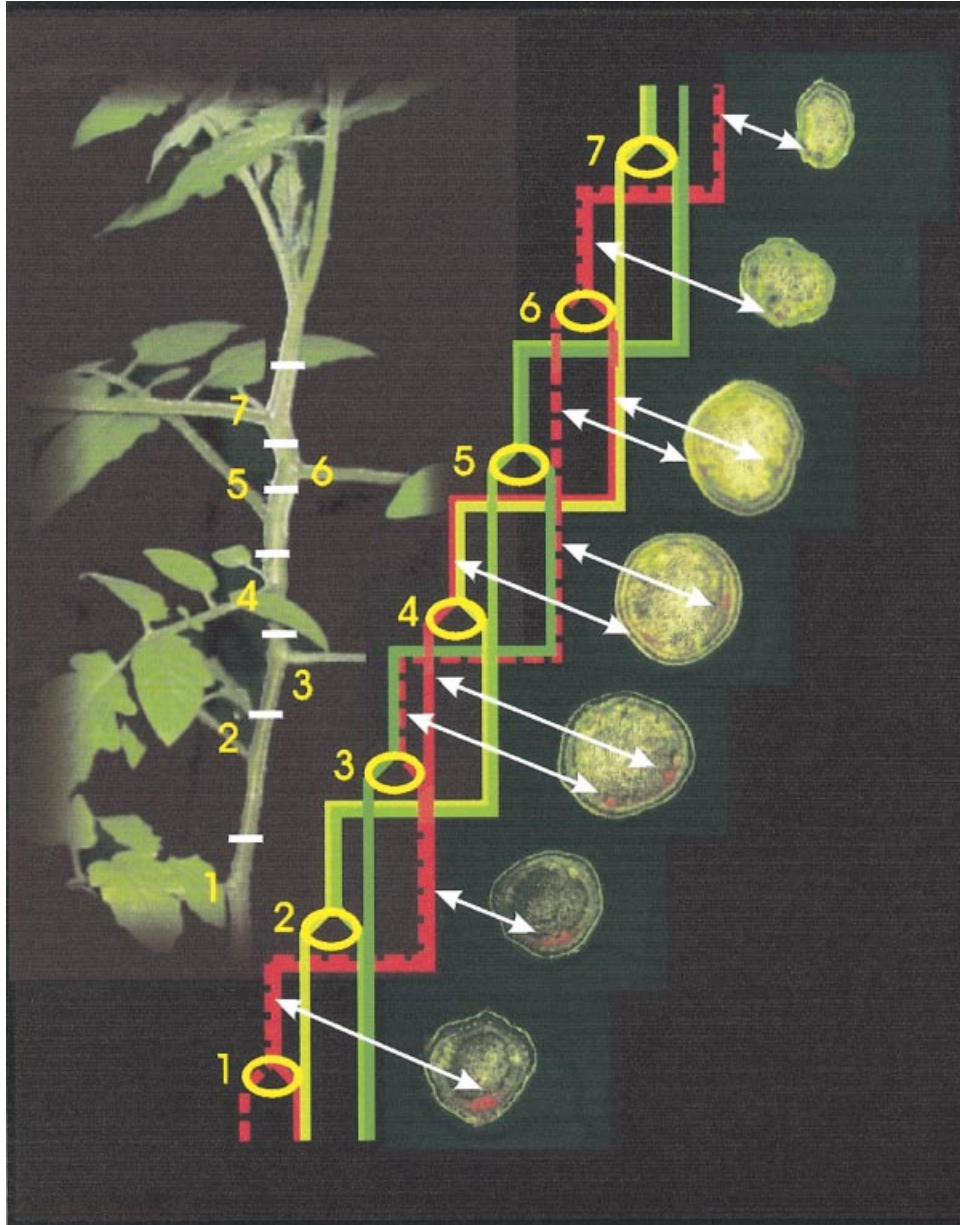


Fig. 1. Vascular bundle architecture in tomato. Each leaf is supplied by two bundles that coalesce above that leaf (leaf petioles are marked as ovals on the diagram). For example, two bundles (marked red, solid and dashed line) supply the first leaf. These two bundles coalesce in the stem just above leaf 1. Then both of them skip the second leaf and one (dashed line) supplies the third leaf while the other (solid line) supplies the fourth leaf. Above the third and the fourth leaves the two 'red' bundles coalesce with two other bundles (marked light and dark green). However, there is no dye exchange between adjacent bundles (note two separate red dots on the cross-sections). It is not until the sixth leaf that both 'red' bundles meet again. Cross-sections were taken from the locations marked along the stem with white bars.

greenhouse at the Tufts University. Seeds were placed into white cone containers (Steuwe and Sons, Corvallis, OR), and watered daily. Seedlings were fertilized at 2 weeks with 30 ml of 20-20-20 NPK fertilizer solution (1.1 g l^{-1}). At 8 weeks, each plant was transplanted into 10 cm round pots and refertilized with 60 ml of fertilizer solution. Plants were 10–12-weeks-old at the time of the experiments. Although two varieties were studied, no differences were expected between them. The varieties are morphologically similar and have yielded similar results in other experiments (CM Orians, unpublished data).

Prior to beginning hydraulic measurements, dye injections were used to map the connections between specific leaf traces and air injection was used to determine the maximum conduit length. Methods for dye injection are described below. To determine maximum conduit length, tomato plants were cut above the first leaf, submerged in water, and perfused with air at 0.1 MPa. Consecutive cuts were made, starting at the tip, until air bubbles were observed at the downstream end (Zimmermann and Jeje, 1981).

The effects of ion concentrations on lateral transport between vascular bundles were studied on well-watered tomato plants with at least nine leaves. The base of the petiole of leaves 5, 6, and 7 were wrapped in parafilm, leaves were severed with a razor blade, and the petiole stubs attached to tubes filled with distilled water (Fig. 2). The remaining leaves (2nd, 3rd, 4th, 8th, and shoot apex) were removed and their cut ends sealed with an acrylic-based glue (Super Bonder 409, Loctite Corporation). The main stem was then severed above the 1st leaf and attached to a hydraulic system that allowed switching between the solutions supplied to the stem. Xylem exudation occurred from all unsealed cuts, eliminating the possibility of air entry into the xylem.

Flow was generated by pressurizing the solution delivered to the base of the stem to 0.05 MPa and measuring the outflow from the 5th, 6th or 7th petiole using a balance ($\pm 0.01 \text{ mg}$ Sartorius 210). Each plant was flushed with distilled, deionized water until a steady-state flow was observed (usually more than 15 min), after which the flow rate through each petiole was recorded. Water was then replaced by 3 mM KCl solution, and the flow rates through each petiole were remeasured. Finally, the 3 mM KCl solution was replaced by a 20 mM KCl solution and the measurements repeated. Because all three flow paths were subject to the same driving gradient and the same perfusing solution, these data provide baseline information on the degree of longitudinal enhancement due to ions (control plants).

Two experimental approaches were used to examine the effects of ions on lateral transport. The first experiment followed the same procedure outlined above, apart from the fact that all vascular bundles were sealed (Super Bonder 409, Loctite Corporation), except for those that fed the first leaf. Glueing was performed under a stereo-microscope to prevent blockage of leaf 1 bundles. At the end of the experiment, each plant was perfused with safranin to determine whether the glue had completely sealed the open ends of the other bundles. If dye was observed in bundles other than the sealed bundles the data were not included in the analyses. In the second experiment, the cut end of the stem was split with a razor blade so that different perfusion solutions could be supplied simultaneously at the same pressure to leaf 1 bundles and to the rest of the stem. KCl solutions (3 and 20 mM) were supplied to leaf 1 bundles, and deionized distilled water was supplied to the other bundles. Experiment 1 mimics conditions where water and nutrients enter the plant through only a portion of the vascular system. By contrast, Experiment 2 tests for flow enhancement when water is supplied evenly, but nutrients are delivered to only a portion of the vascular system.

To compare differences in flow enhancement at different leaf positions, a non-parametric test was used (Friedman's Method for Randomized Blocks; Sokal and Rohlf, 1981). In this test, each plant

is treated as a block and the degree of flow enhancement to leaves 5, 6 and 7 is ranked within each plant.

Results

Air injection indicated that the majority of vessels were shorter than four internodes, however a few are as long as five and extend into the leaf petioles. Dye injection confirmed that the vascular bundles link leaves within tomato plants in a systematic way (Fig. 1). Each leaf was supplied by traces branching from two vascular bundles. One of the bundles that supplies leaf 1 is directly connected to leaves 3 and 6 (red dashed line, Fig. 1), while the other bundle that supplies leaf 1 has direct connections to leaves 4 and 6 (solid red line, Fig. 1). By contrast, the 5th and 7th leaves had no direct links with the

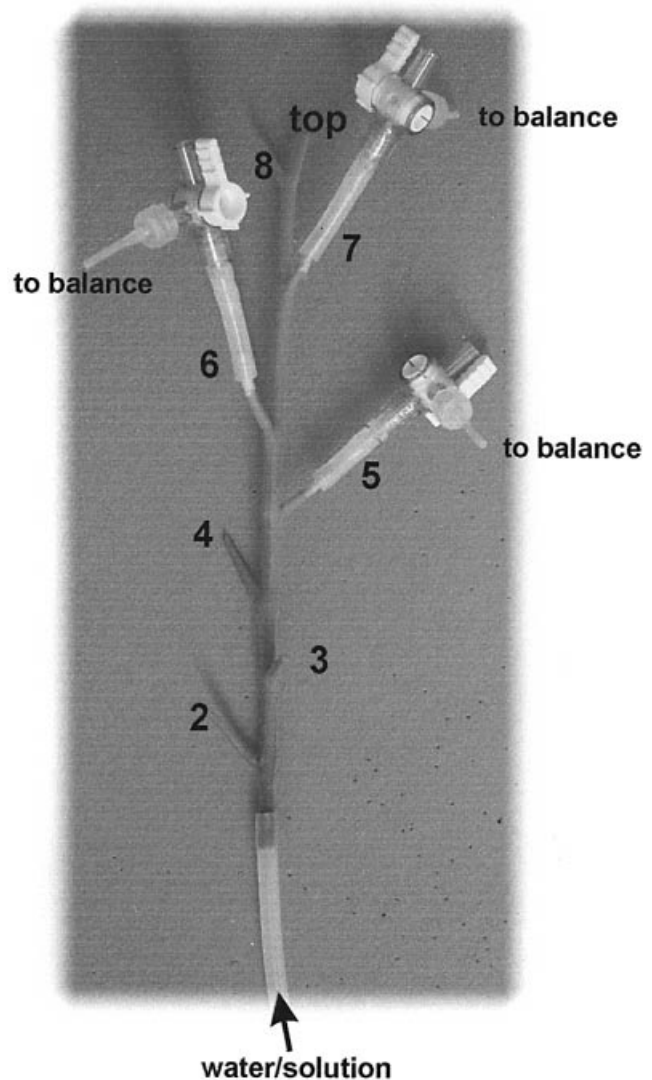


Fig. 2. Experimental set-up to determine flow enhancement through each petiole. All plants were cut just above the first leaf.

bundles that supply the 1st leaf (green solid lines; no red dye was observed in these bundles; Fig. 1). However, the vascular bundles that supply leaves 5 and 7 coalesced with bundles feeding leaf 6 (red lines), providing an opportunity for lateral exchange between these bundles. There was no movement of dye between them, indicating that vessels did not criss-cross between adjacent vascular bundles.

The addition of ions enhanced flow rate in all experimental manipulations. When the entire vascular system was supplied with water (control plants), the flow rate to leaves 5, 6 and 7 was relatively similar (61.98, 51.22, and 79.46 g s⁻¹ MPa⁻¹, respectively) and the change from deionized distilled water to 20 mM KCl enhanced the flow

to leaves 5, 6 and 7 equally (Fig. 3a; Friedman's test: $\chi^2=0.00$; $P=1.00$, $n=3$, $df=2$). Changes in solute concentration from 3 mM to 20 mM KCl had a similar, but reduced effect, on flow enhancement compared with 0 mM to 20 mM KCl, and there was no difference in flow enhancement among leaves 5, 6 and 7 (Fig. 3b; Friedman's test: $\chi^2=0.667$; $P=0.717$; $n=3$, $df=2$).

When water was supplied only to the vascular bundles associated with leaf 1 and no flow was generated in the remaining bundles (Experiment 1), the flow rate to leaves 5 and 7 was significantly less than to the 6th leaf (10.81, 20.34 and 40.54 g s⁻¹ MPa⁻¹, respectively). Switching to a 20 mM KCl solution resulted in greater enhancement to leaves 5 and 7 than to leaf 6. The flow enhancement to the 5th and 7th leaves was twice that to the 6th leaf (Fig. 3a; Friedman's test: $\chi^2=6.33$; $P=0.04$; $n=6$, $df=2$). The same pattern of flow enhancement was observed when 3 mM solution was replaced by 20 mM solution, although the magnitude of enhancement was less. Nevertheless, enhancement to the 5th and 7th leaves was still twice that to the 6th leaf (Fig. 3b; Friedman's test: $\chi^2=4.667$; $P < 0.1$; $n=3$, $df=2$).

This pattern was reversed when the sequence of water/KCl solution was supplied to the vascular bundles feeding the 1st leaf and distilled deionized water was supplied to all other bundles (Experiment 2). In this case, the presence of the 20 mM KCl solution enhanced the flow rate to the 6th leaf more than to either the 5th or the 7th leaf. Enhancement of flow to leaf 6 was similar to that observed in both the control and Experiment 1 (approximately 20%), but was consistently lower to either the 5th or the 7th leaf (<10%, Fig. 3a; Friedman's test: $\chi^2=4.667$; $P < 0.1$; $n=3$, $df=2$). Changes in solute concentration from 3 mM to 20 mM produced a similar but reduced effect (Fig. 3b; Friedman's test: $\chi^2=4.667$; $P < 0.1$; $n=3$, $df=2$).

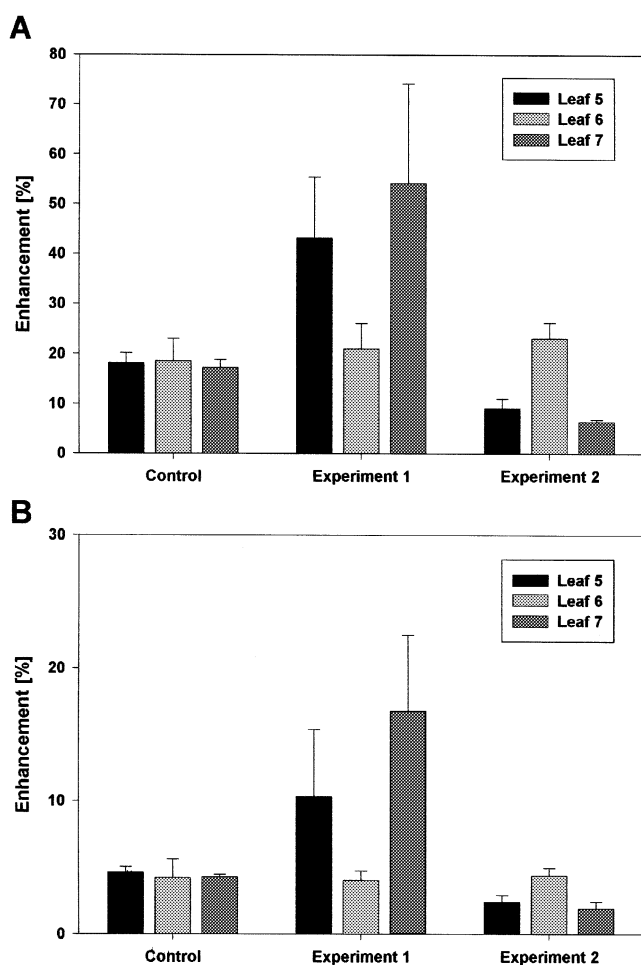


Fig. 3. Flow enhancement (%) resulting from switching between deionized water and 20 mM solution of KCl (A) and between 3 and 20 mM solutions of KCl (B). In the control set-up all bundles were perfused with the same sequence of water/KCL solutions. In Experiment 1, water/KCl solutions were perfused only through the bundles supplying the first leaf. In Experiment 2, water/KCl solutions were perfused through the bundles feeding the first leaf while water was perfused through all other bundles. Sample sizes were: three (control), six (0 to 20 mM) and three (3 to 20 mM) plants in Experiment 1, and three (control), three (0 to 20 mM) and three (3 to 20 mM) plants in Experiment 2. Bars represent SE.

Discussion

The presence of ions in the perfusing solution had a significant effect on the hydraulic properties of tomato stems. The effect observed here is similar to that reported for several woody and herbaceous species (Van Ieperen *et al.*, 2000; Zwieniecki *et al.*, 2001). Because some vessels extend over multiple internodes, one may expect that the enhancement effect of ions on flow within a bundle will be relatively minor when compared to flow between vascular bundles where the water is likely to have to traverse numerous pit membranes and/or travel through cell walls (Fig. 4). Indeed, it is shown that lateral transport is enhanced more by ions than axial flow through the xylem (~50% versus ~20%). Provided that water is not uniformly available to all portions of the vascular system (i.e. there is pressure difference across the lateral pathway), the presence of solutes in the xylem will facilitate lateral transport between bundles (Fig. 4A). Thus,

when water availability is patchy, ion-mediated flow enhancement between adjacent vascular bundles will lead to increased exchange of resources between sectors. However, when water flow is high to all sectors, the driving force for lateral flow is greatly reduced, limiting

the effect of ions on resource sharing between sectors (Fig. 4B). This explains why fertilizing an isolated tomato root results (when water is readily available to all roots) in larger leaves and greater side-shoot growth in sectors with direct connections to the fertilized root (Orians *et al.*,

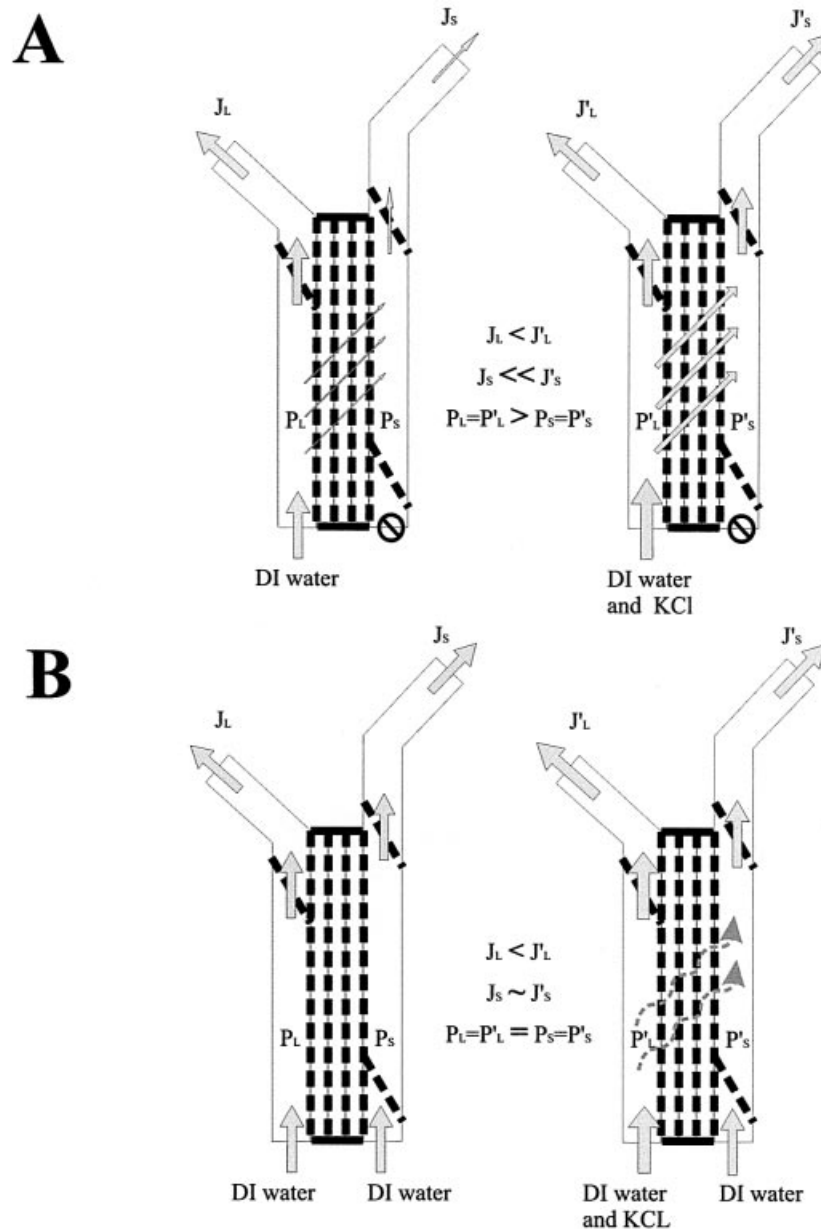


Fig. 4. Diagram of ion-mediated changes in hydraulic resistance within and between xylem sectors (vascular bundles). (A) When water is available only to one sector (as in Experiment 1) there is a pressure difference between adjacent sectors ($P_L > P_S$). In this case, flow within the left sector (J_L) would be greater than to the right sector (denoted J_S). Flow in the right sector occurs entirely through the lateral connection with the left sector and is limited by the high hydraulic resistance of the sector-to-sector connection. When ion concentration is higher in the left sector, longitudinal and lateral flow is up-regulated due to a drop in resistance across bordered pit fields. Lateral flow increases ($J_S \ll J'_S$) more than longitudinal flow ($J_L < J'_L$) due to the more restricted pathway (multiple bordered pit fields and walls). (B) When water is equally available to both sectors (as in Experiment 2), exchange between sectors is limited due to high lateral hydraulic resistance and the fact that there is no pressure difference between adjacent sectors ($P_L = P_S$). Despite higher ion concentration in the left sector, there will be little effect on lateral transport since there is little or no hydraulic pressure difference between sectors.

2002), but when the water supply is also asymmetrically applied (i.e. to isolated, fertilized roots), the plant produces similar-sized leaves in all sectors (CM Orians; unpublished data).

Sectoral transport is not unique to tomatoes. Many species of herbaceous and woody plants have compartmentalization of the transport pathways feeding adjacent leaves (Esau, 1965). When sectoral transport is coupled with patchy nutrient availability (Lechowicz and Bell, 1991; Jackson and Caldwell, 1993), there is the potential for a non-uniform distribution of resources within the plant (Orians *et al.*, 2002). This, however, depends on patterns of water uptake. These findings provide an insight into how sharing of limited resources among sectors might be enhanced. When roots encounter nutrient-rich patches they often increase their rate of nutrient uptake (Jackson *et al.*, 1990; Caldwell, 1994), which might result in an increase in xylem solute concentrations in that portion of the vascular system. Increased xylem sap concentrations would, in turn, result in a reduction in the hydraulic resistance of that root, and may ultimately lead to preferential water uptake from these roots. Higher water availability in one sector of the transport system would further promote lateral exchange by the creation of a pressure differential between vascular bundles.

In summary, it has been shown that ion concentration directly modifies the properties of lateral transport in the tomato stem. The process studied in this report is passive, i.e. changes were implemented and the resulting distribution of fluxes within the plant observed. An open question is whether there is an active, i.e. plant-controlled, modification of fluxes between leaves or branches. If living cells can exchange ions with the xylem sap it could lead to modifications in lateral transport characteristics and the distribution of resources among leaves and branches. These resources might include nutrients, plant growth hormones and perhaps signal molecules involved in the regulation of genes important to plant–pathogen and plant–herbivore interactions.

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