

Phloem networks in leaves

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The survival of all vascular plants depends on phloem and xylem, which comprise a hydraulically coupled tissue system that transports photosynthates, water, and a variety of other molecules and ions. Although xylem hydraulics has been extensively studied, until recently, comparatively little is known quantitatively about the phloem hydraulic network and how it is functionally coupled to the xylem network, particularly in photosynthetic leaves. Here, we summarize recent advances in quantifying phloem hydraulics in fully expanded mature leaves with different vascular architectures and show that (1) the size of phloem conducting cells across phylogenetically different taxa scales isometrically with respect to xylem conducting cell size, (2) cell transport areas and lengths increase along phloem transport pathways in a manner that can be used to model Münch's pressure-flow hypothesis, and (3) report observations that invalidate da Vinci's and Murray's hydraulic models as plausible constructs for understanding photosynthate transport in the leaf lamina.

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Introduction

The xylem and phloem are hydraulically coupled and vital for the survival, growth, and reproduction of the vascular plants. Although xylem hydraulics has been extensively studied [1,2,3^{*},4^{**}], comparatively little is known about phloem hydraulics [5,6,7^{*}]. This bias is understandable because of the difficulties of directly measuring transport in living, thin walled cells obscured in vascular bundles experiencing physiologically positive pressures [8,9,10^{**},11]. Nevertheless, phloem transport is physically

directly dependent on water transport such that any attempt to model plant hydraulics must be informed about the architecture of both tissue systems [12,13^{*},14,15,16^{*}]. This requirement is illustrated by Münch's pressure-flow hypothesis [17,18,19^{*}], which posits that the movement of solutes in the phloem (loading from the source and unloading in the sink) results in an influx of water at the source that is equivalent (or nearly so) to the efflux at the sink [20,21,22^{*}]. This process generates the osmotically-mediated pressure gradient required to sustain the flow of sap within the phloem, i.e., the movement of water from the xylem into the phloem and the movement of sap in the phloem are hydraulically inextricably linked.

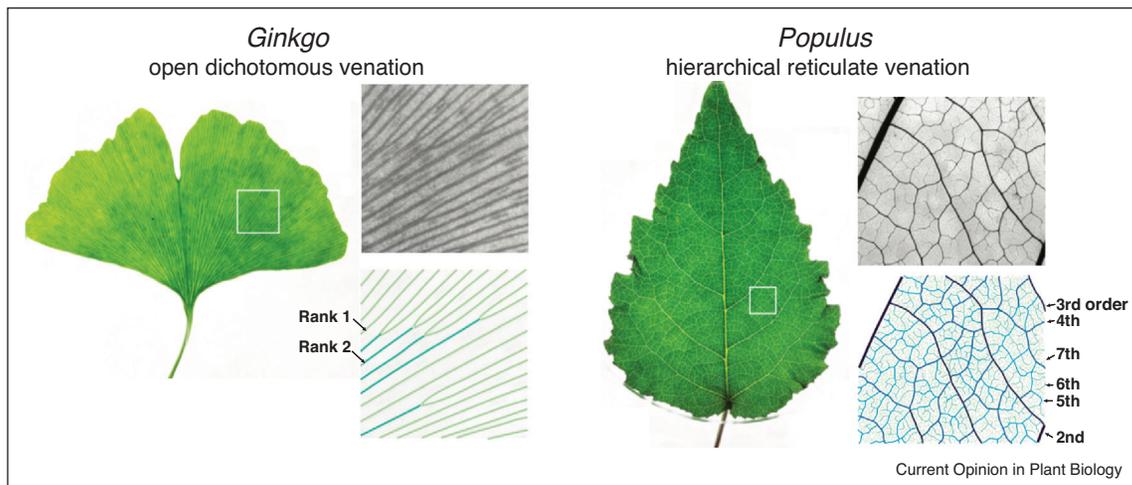
The quantification of phloem architecture is equally important to assessing whether hydraulic models proposed for the xylem are plausible when applied to the phloem. For example, da Vinci's model posits a conservation of cross sectional area whenever two transport conduits (e.g., sieve tubes) merge into one; Murray's model posits that the work expended on transport is minimized and that the mass or volume of the transported fluid is conserved whenever two transport conduits merge into one [23–25]. Both of these models, as well as others, can make rigorous quantitative predictions, but only if the dimensions of transport cells are known [26,27]. Without this information, the predictions of hydraulic models remain conjectural.

To fill this gap, we review what is currently known about the phloem transport network in fully mature (source) leaves with three very different vascular topologies — needle-like leaves with a single vein (e.g., *Abies* and *Pinus*), leaves with open dichotomous venation (e.g., *Ginkgo*), and leaves with hierarchical reticulate venation (e.g., *Illicium* and *Populus*) (Figure 1). We detail the structural similarities and dissimilarities among these different topologies, discuss the insights they provide with regard to modeling phloem hydraulics according to Münch's pressure-flow hypothesis, and propose that da Vinci's and Murray's models are inappropriate for the leaf lamina hydraulic network. We also suggest that the hierarchical reticulate leaf topology is an evolutionary 'innovation' by virtue of amplifying the surface area in contact with photosynthetic cells, thereby enhancing the collection and export of photosynthates from the leaf. We also draw attention to the paucity of information regarding petiole hydraulics, which is the gateway to stem hydraulics.

Phloem hydraulic similarities and dissimilarities

The hydraulic structure of phloem in the leaf lamina consists of a collection of transport conduits formed by

Figure 1



The venation patterns of *Ginkgo* (with an open dichotomous pattern) and *Populus* (with a hierarchical reticulate pattern) and an illustration of how the different levels of veins within these venation patterns are ranked. In the case of *Ginkgo*, vein ranks increase toward the petiole (basipetal ranking); in the case of *Populus*, vein orders decrease basipetally.

individual cells called sieve elements [28,29,30^{*}]. In the leaf lamina, this structure can be viewed from two perspectives: (1) how the size of sieve elements changes in individual transport conduits (e.g., sieve tubes) along the phloem transport pathway, and (2) how the sum of the transport areas in vascular veins changes toward the petiole. The first approach structurally describes the transport pathway along individual veins in terms of the size, number, and connectivity among individual conduits, whereas the latter approach quantifies the sugar and water transport pathway at the whole leaf level.

Regardless of perspective, it is important to note that vein orders in the hierarchical reticulate topology of leaves like *Illicium* and *Populus* are categorized into ‘minor veins’ that mature as a leaf transitions into a source leaf and function primarily in phloem loading (sensu [31,32]), and ‘major veins’ that function to bring water and nutrients into an expanding leaf and subsequently function to export photosynthates from mature leaves. This division of function does not exist in leaves with an open dichotomous phloem topology like *Ginkgo* and obviously not in leaves with a single vein like those of *Abies* and *Pinus*.

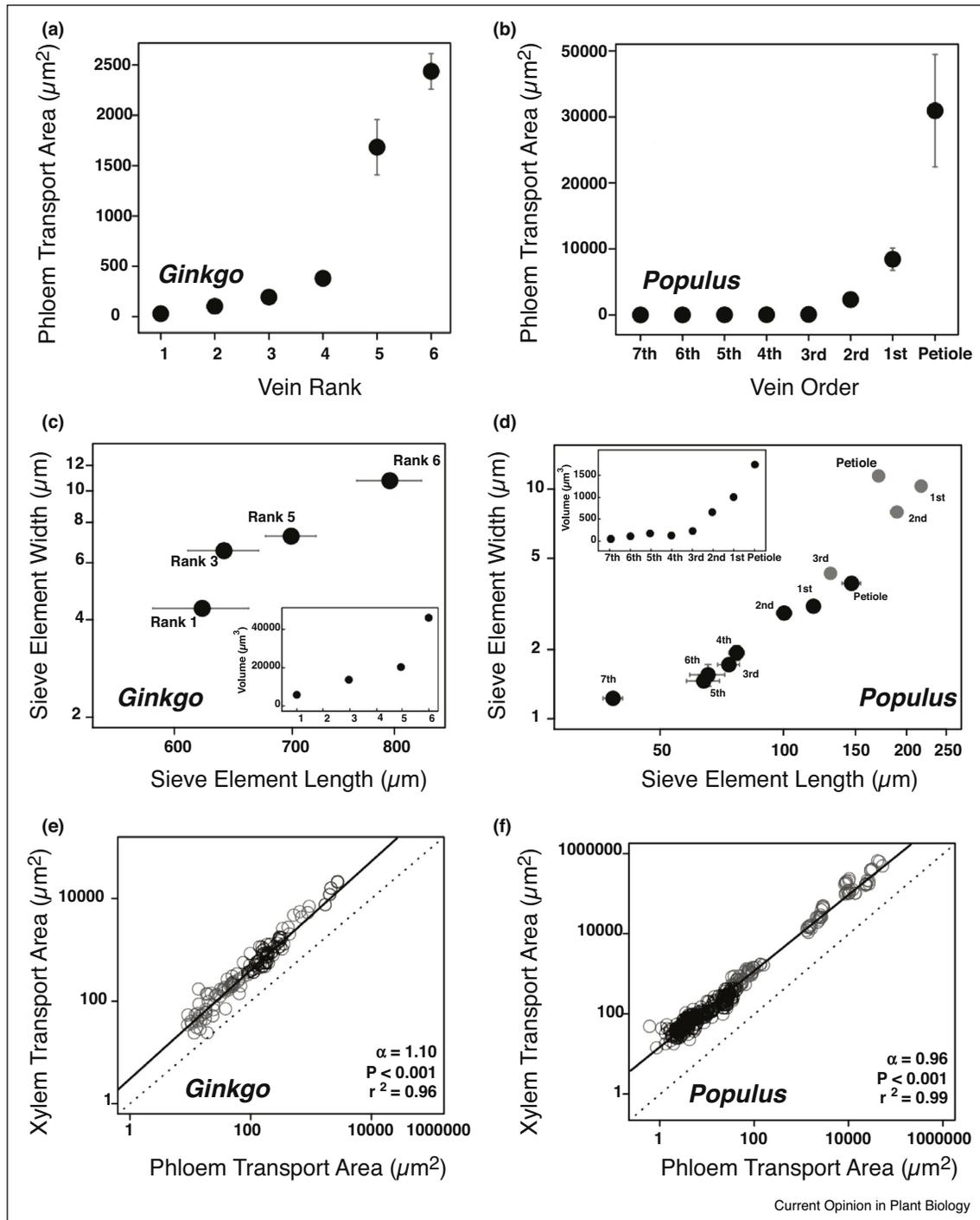
Nevertheless, for all three topologies, the total transport areas of phloem and xylem in individual veins increase from sugar loading sites along the phloem transport pathway toward the petiole (Figure 2a–c). In the case of single-veined leaves, this trend is reported to result from an increase in the number of sieve elements toward the base of leaves and not the result of increasing cell diameters [33]. In the case of *Illicium*, *Populus*, and *Ginkgo*, the increase in total transport areas is the result of

an increase in cell size (transverse area and length; Figure 2c,d) as well as an increase in the number of transport conduits in individual veins [30^{*},34^{*},35^{*}]. For all the topologies thus far quantified, the transport areas of phloem and xylem are isometrically correlated across the entire vasculature of the leaf lamina, although the area of the xylem significantly exceeds that of the phloem. This trend also holds true for individual veins as well as across the entire leaf lamina (Figure 2c,f).

Another similarity observed among *Ginkgo*, *Illicium*, and *Populus* leaves is the maintenance of a maximum distance between the bundle sheath of any given vein and any mesophyll cell. For example, in the open dichotomous topology of *Ginkgo*, the divergence angle between adjoining veins (i.e., $22^\circ \pm 2^\circ$) and the frequency with which it occurs over the lamina surface maintains a maximum number of five to six mesophyll cells between neighboring veins. In the hierarchical reticulate topology of the *Populus* leaf, the fractal like distribution of minor veins establishes ‘islets’ of cells in which an individual cell is also rarely more than five or six cells from a vein. In the case of single veined leaves, the distance between photosynthetic cells and the vein decreases toward the petiole (i.e., basipetally), but the number of cell layers between the bundle sheath and sieve elements is typically greater than one.

A major difference exists between single veined leaves and *Ginkgo* on the one hand and the leaves of *Illicium* and *Populus* on the other. The sum of all phloem transport areas increases toward the base of single veined leaves and *Ginkgo* (Figure 3a), but decreases dramatically toward

Figure 2

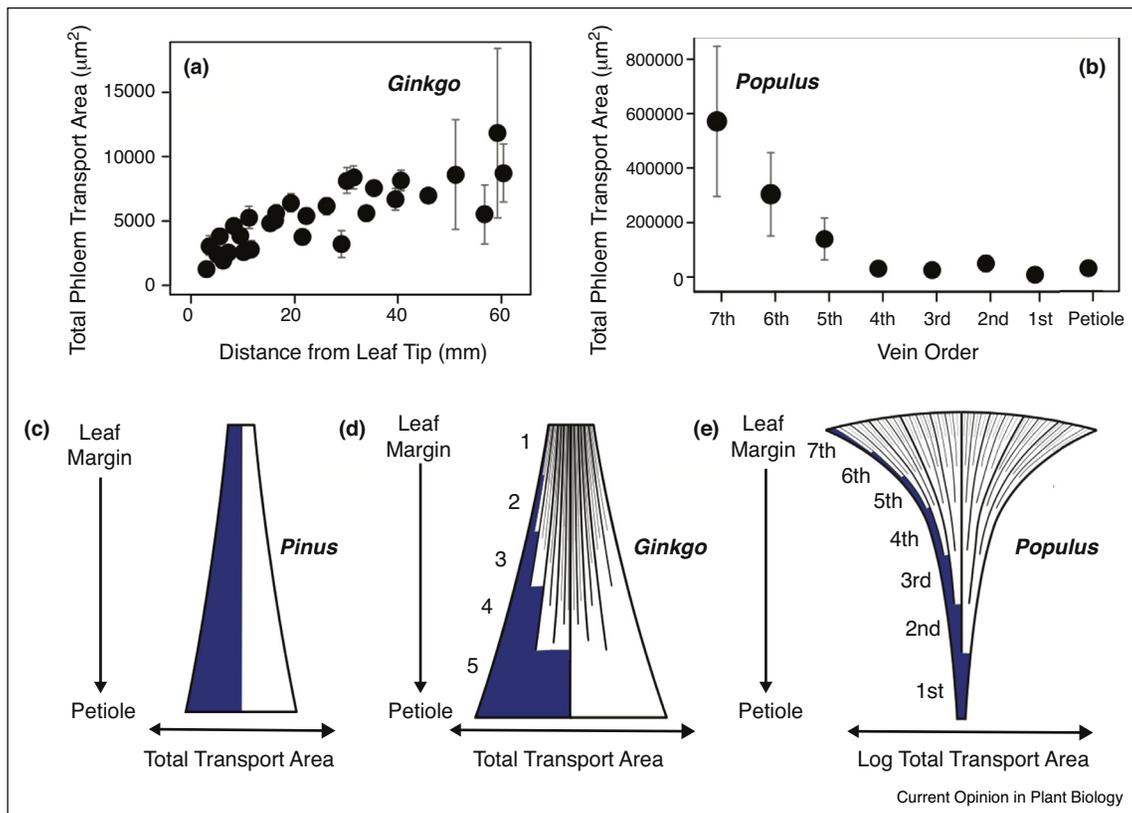


Comparisons between the hydraulic networks of *Ginkgo* and *Populus* leaves. (a,b) Total phloem areas of all comparable vein ranks and orders plotted against vein rank and order, respectively, for *Ginkgo* and *Populus*. (c,d) Sieve element diameter plotted against length for different vein ranks of *Ginkgo* and vein orders of *Populus* (black) and *Illicium* (gray). Inserts report cell volume relationships. (e,f) Isometric scaling between phloem and xylem transport areas in individual veins of *Ginkgo* and *Populus*, respectively. Dashed lines show isometric scaling relationship; solid lines are linear regression curves (regression data reported in inserts).

the petioles of *Illicium* and *Populus* leaves (Figure 3b). This contrasting trend is attributable to the fact that hierarchical reticulate venation pattern has a functional

distinction between ‘fine veins’, which are numerous and provide a large collective surface area, and ‘major veins’, which are comparatively few in number but large in their

Figure 3



Comparison of the phloem hydraulic architecture of three different leaf vascular topologies. **(a,b)** Total phloem transport areas plotted against distance from the margin of a *Ginkgo* leaf and vein order for *Populus*. **(c)–(e)** Schematic of how the total phloem transport area changes along the export pathway (shown in white) and how the transport area changes along an individual phloem conduit changes (shown in blue) in a single veined leaf (c), an open dichotomous leaf (d), and a hierarchical reticulate leaf (e). Note that the transport area along an individual phloem conduit increases basipetally in all three topologies.

collective transport area, a distinction that is absent in single veined leaves and leaves with an open dichotomous venation pattern (see Figure 1).

Implications of anatomy to modeling phloem hydraulics

Münch's pressure-flow hypothesis

An important consideration when modeling phloem hydraulics is that there are two classes of loading mechanisms for photosynthates in mature leaves: (1) a symplastic route through plasmodesmata that relies on the passive diffusion of sugars down a concentration gradient [36^{••},37[•]], and (2) an apoplastic transport route through the cell wall that involves the active transport of photosynthates mediated by H⁺/sucrose antiporters in the CC/SE complex [31]. Symplastic entry can be either completely passive [38] or aided by active mechanisms that trap simple sugars by converting them into larger polymers in specialized companion cells (i.e., polymer trapping) [39,40]. Despite the controversy around the ability of symplastic passive loading mechanisms to generate the osmotic-mediated pressure gradient between

sources and sinks [41,42], the passive loading strategy is reported for some tree species, and it has been shown to drive flow and regulate photosynthesis under experimental conditions [43].

Regardless of the loading mechanism, the standing hypothesis for the export of photosynthates in the phloem is Münch's pressure-flow hypothesis. This hypothesis proposes that the bulk flow of photosynthates from sugar source (leaves) to sink tissues (roots, active meristems) is driven by an osmotically generated hydrostatic pressure gradient. Münch's hypothesis makes no direct predictions about the dimensions of phloem transport cells. However, the dimensions and numbers of sieve elements can inform attempts to model how (or if) osmotic-mediated pressure gradients can be generated and how the efflux of photosynthates from leaves can be expedited. For example, based on the diameters and lengths of sieve elements (see Figure 2a–d), it is clear that the hydraulic capacity of individual phloem conduits basipetally increases in each of the three vascular topologies, regardless of whether total transport area increases or decreases along the

phloem transport network (see Figure 3). The isometric scaling of phloem transport area with respect to xylem transport area (see Figure 2e–f) shows the extent to which the xylem provides water to the phloem for the efflux of photosynthates.

Likewise, attempts to model Münch's hypothesis are informed by the fact that leaves with a hierarchical reticulate topology appear to have a hydraulic advantage over those with either a single vein or an open dichotomous topology because of the division of labor between fine veins and major veins. The anatomy and geometry of the fine veins (ranks 1–4) show that they are specialized for the collection of photosynthates and water, whereas the major veins (ranks 5–7) are specialized for the rapid transport of materials out of the leaf lamina. This degree of specialization is not observed for the veins in leaves with a single vein or an open dichotomous topology, which likely collect photosynthates all along their lengths.

The da Vinci and Murray hydraulic models

A number of hydraulic models have been proposed to describe the flow of water (and by inference, the flow of phloem sap) through the plant body. The two most prominent of these models are da Vinci's model [25] and Murray's model [23]. Each have been applied to understand leaf hydraulics [44,45,46**] and each predicts the size of conduits across different levels of branching when two or more independent conduits merge into one. The transport area-preserving scaling relationship described by da Vinci's model was originally based on empirical observations of branching in trees; Murray's model was derived from optimization principles that minimize flow energy loss in closed, branching hydraulic networks such as the cardiovascular system.

Importantly, the data available for phloem in the leaf lamina are inconsistent with either model because the veins in all three leaf vascular topologies are tapered basipetally along their lengths before veins branch owing to an increase in conduit number, or an increase in both conduit number and conduit cell size. Thus, measurements taken at different locations along unbranched veins show that the sum of transport cross sectional areas increases, which violates the assumption that the cross sectional areas of individual conduits are conserved until two or more conduits merge [33,47*]. In addition, both models assume closed hydraulic systems, i.e., there is no loss of the fluid being transported. Although this assumption holds (more or less) true for rachides, stems, and woody roots, it is violated in the leaf lamina because of evapotranspiration and the influx of water into the phloem. There are mathematical tools to deal with open hydraulic systems [48]. However, there is insufficient data to parameterize models relying on these tools [49*].

It is equally important to note that when measurements of transport area (or volume) are taken directly above where two conduits converge and directly below the point of convergence, total cross sectional area (or volume) is conserved in a manner that complies with both da Vinci's model and Murray's model [35*]. However, this agreement does not substantiate either model because the conservation of transport area (or volume) must occur provided that a large number of new conduits are not added directly at the point two or more conduits converge.

Future directions

Understanding phloem hydraulics is imperative to understanding how the transport system operates in vascular plants. Unfortunately, until recently, this aspect of plant hydraulics has not been sufficiently investigated, both among the flowering plants and across the different vascular plant lineages. At a macroscopic anatomical level, a broad species survey is required because the phylogenetic position of species does not necessarily correlate with their physiological complexity, e.g., *Amborella*, an early divergent angiosperm [50], uses symplastic rather than apoplastic loading [51]. Another poorly understood research area is how petiole anatomy influences the hydraulics of the leaf lamina. For example, the sieve elements in the petiole of *Illicium* are shorter than those entering the petiole and may play an important regulatory role in sugar export [34*,52*]. Similarly, little is known about how different phloem conduits interconnect as total transport cross sectional area decreases basipetally in leaves with a hierarchical reticulate topology [53,54].

At a microscopic anatomical level, improved and new techniques (e.g., cryoSEM and transgenic manipulation) are required to visualize the size and frequency of plasmodesmata connecting sieve elements to adjoining cells [55], and quantitative data for plasmodesmata cell wall permeability are needed to understand the hydraulic 'connectivity' of different tissues [56]. Technologies are also required to measure the velocity (e.g., [57]), viscosity (e.g., [10**]) and flow rates of sap within the phloem, since these variables are critical for calculating differential pressures within the phloem.

Conclusions

A review of data drawn from mature leaves manifesting three distinctively different vascular topologies (leaves with a single vein, open dichotomous venation, and with hierarchical reticulate venation) indicates that phloem and xylem transport areas scale isometrically with respect to one another and that the transport areas of individual phloem elements increase basipetally toward the petiole. The data also reveal significant differences among the three topologies, particularly with respect to how the total cross sectional area of the phloem changes along the phloem loading-unloading pathway toward the petiole.

Despite these differences, the data indicate that the hydraulic capacity of individual phloem conduits (e.g., sieve tubes) increases basally in all three vascular topologies, which informs attempts to model Münch's pressure-flow hypothesis. The data also show that the da Vinci and Murray models are inappropriate when dealing with the hydraulics of leaf laminae.

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