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Leaf Hydraulics and Its Implications in Plant Structure and Function

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Each year more than 40 trillion tons of water move through plant leaves, about 10% of the water leaving the planet's surface, two thirds of that from land (Hordon, 1998; USGS, 2004). This component of water's passage through the hydrologic cycle, the microhydrological process inside the leaf, contains many outstanding mysteries. Water flow through leaves has important implications for understanding whole-plant hydraulics and plant growth, as well as leaf structure, function, and ecology. Major questions relating to leaf hydraulics were reviewed in the 1980s (e.g., Boyer, 1985; Davies, 1986). Since the late 1990s, research has increased, including new perspectives from physics, anatomy, modeling, and ecology. In this chapter we describe the importance of the leaf in determining whole-plant hydraulic conductance, which can strongly influence gas exchange and growth. Next, we examine the basis of leaf hydraulic conductance in the pathways of water movement in the leaf. In the following sections, we describe how leaf hydraulic conductance is linked with other aspects of leaf structure/function, including venation design, leaf shape, water storage, and structural features important in plant carbon economy and drought tolerance. We survey recent work on the dynamics of leaf hydraulic conductance across plant growth conditions, as well as diurnally, and across leaf ages. Finally, we propose topics for future research.

Leaf Hydraulic Conductance in the Whole-Plant System

The leaf hydraulic conductance (K_{leaf}) is the ratio of flow rate (F_{leaf})—through the petiole to the sites of evaporation (where the liquid phase ends and the vapor phase begins)—to the driving force for flow, which is the

water potential difference across the leaf ($\Delta\Psi_{\text{leaf}}$). The hydraulic resistance is the inverse of conductance, defined as $\Delta\Psi_{\text{leaf}}/F_{\text{leaf}}$. Either definition models bulk liquid flow through a whole leaf as though through a piece of tubing of fixed length, where the hydraulic conductance indicates the diameter of the equivalent tubing. K_{leaf} is typically normalized by leaf area (i.e., $F_{\text{leaf}}/\Delta\Psi_{\text{leaf}}$ is further divided by lamina area; units of kg [or mmol of water] $\text{s}^{-1} \text{MPa}^{-1} \text{m}^{-2}$), based on the assumption that the number of parallel pathways for water flux will scale linearly with leaf area for a given type of leaf. This idea has been supported for several species (Meinzer and Grantz, 1990; Martre *et al.*, 2001; Sack *et al.*, 2004). K_{leaf} is apparently a substantial bottleneck in the whole-plant hydraulic pathway, and a potentially major determinant of gas exchange. The leaf lamina hydraulic conductance [$K_{\text{lamina}} = (K_{\text{leaf}}^{-1} - \text{petiole hydraulic conductance}^{-1})^{-1}$] varies at least 30-fold across species according to published measurements (Fig. 5.1A), indicating potentially strong ecological importance.

How does K_{leaf} , as a component of the whole-plant hydraulic system, impact gas exchange? The resistance of the stomata in the path of vapor diffusion out of the leaf is typically greater than two orders of magnitude higher than the hydraulic resistance to bulk flow through the whole plant, including the leaf, and so the hydraulic properties of the liquid path do not *directly* determine transpiration rates. However, while the transpiration rate is dictated by the stomatal aperture, at a given soil water supply and transpiration rate, the leaf water potential is determined by the plant hydraulic conductance (K_{plant} ; Cowan, 1972; Tyree and Zimmermann, 2002) and stomata close at low leaf water potentials to prevent leaf desiccation. Consequently, stomatal conductance is frequently suboptimal at typical midday leaf-water potentials because of hydraulic constraints on gas exchange (Tyree, 2003a). K_{plant} often correlates with maximum stomatal conductance within and across species (e.g., Küppers, 1984; Meinzer and Grantz, 1990; Nardini and Salleo, 2000).

Published data show that K_{leaf} scales with K_{plant} (Fig. 5.1A). This relationship indicates that on average the leaf lamina constitutes $\approx 1/4$ of whole-plant resistance (Fig. 5.1A; Sack *et al.*, 2003b). Further, K_{leaf} itself is correlated with both stomatal conductance and maximum photosynthetic rate per unit area across species for temperate deciduous trees (Aasamaa *et al.*, 2001), apparently via a tight correlation with total stomatal pore area, a major determinant of stomatal conductance (Fig. 5.1B; Sack *et al.*, 2003b). The coordination of K_{leaf} with K_{plant} and with gas exchange indicates the importance of K_{leaf} in defining plant function and driving differences in species function.

As described previously, the measurement of K_{leaf} models the leaf as though it were a piece of tubing. However, water flows through a leaf in a multitude of pathways. Past the petiole insertion point, water moves

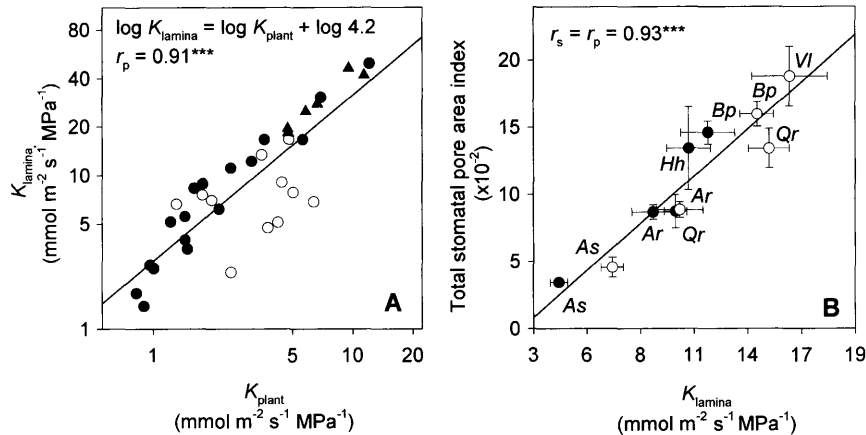


Figure 5.1 (A) The scaling of leaf lamina and whole plant hydraulic conductance, for 34 species; $K_{\text{lamina}} = 4.2 \times K_{\text{plant}}$. [$K_{\text{lamina}} = (K_{\text{leaf}}^{-1} - \text{petiole hydraulic conductance}^{-1})^{-1}$]. Filled triangles represent herbs, filled circles woody seedlings and saplings, and open circles mature trees and shrubs (data of Becker *et al.*, 1999; Nardini and Tyree, 1999; Nardini *et al.*, 2000; Nardini and Salleo, 2000; Tsuda and Tyree, 2000). (B) Coordination of total stomatal pore area index and K_{lamina} . Total stomatal pore area index = stomatal guard cell length² × stomatal density. Open/filled symbols represent sun/shade leaves. Species: *Ar*, *Acer rubrum*; *As*, *Acer saccharum*; *Bp*, *Betula papyrifera*; *Hh*, *Hedera helix*; *Qr*, *Quercus rubra*; *Vl*, *Vitis labrusca*. Error bars = 1 SE; ***: $P < 0.001$. From Sack *et al.* (2003b). Copyright Blackwell Publishing; adapted with permission.

through leaky xylem conduits within a typically reticulate venation wrapped in membrane-bound cells, and water leaving the veins flows potentially through multiple cell walls and perhaps membranes throughout the lamina before evaporating at many loci. Thus, K_{leaf} is an aggregate measure for a complex hydrologic system. To understand the basis of measuring K_{leaf} , it is necessary to understand the flow pathways through the leaf. There are several methods in common use for quantifying K_{leaf} (Table 5.1), and in applying these methods there remain outstanding issues. The most important unknown is the degree to which the different methods measure the conductance of the same flow pathways through the leaf, and how similar these flow pathways are to those of natural transpiration.

How Does Water Flow from the Petiole to the Sites of Evaporation?

Water enters the petiole through branch xylem bundles, which reorganize in a complex junction at the leaf insertion (Esau, 1965) and enter the

Table 5.1 K_{leaf} measurement methods for leaves transpiring *in vivo*, and for excised leaves

Method			Sample references
(a) <i>In vivo</i> methods	<i>Measurement of flow rate</i>	<i>Measurement of driving force</i>	
	Transpiration; measured as pot water loss rate <i>or</i> as porometer or gas exchange system measured 'transpiration' ^a <i>or</i> as sapflow proximally to the leaf	$\Delta\Psi_{\text{leaf}}$ measured as difference in pressure bomb water potential between transpiring leaf and bagged nontranspiring leaf ^b <i>or</i> as difference in water potential between a leaf sampled for psychrometry while transpiring, and a leaf measured for vein water potential ^c using <i>in situ</i> psychrometry ^d <i>or</i> by measuring within leaf gradients in water potential using psychrometry for vein water potential ^c and direct measurement of epidermal cell turgor and osmotic potential	Tsuda and Tyree, 2000; Brodribb and Holbrook, 2003a Matzner and Comstock, 2001 Shack et al and Brinckmann, 1985; Shack <i>et al.</i> , 1987
(b) Excised leaf methods			
i. Evaporative flux methods	F_{leaf} is estimated for excised leaves transpiring under natural or artificial conditions	$\Delta\Psi_{\text{leaf}}$ as for (a) above	Sack <i>et al.</i> , 2002 and references therein
ii. High pressure flow meter	Water driven through the leaf under positive pressure ^e , and flow rate and pressure driving force measured simultaneously proximally to the leaf		Tyree <i>et al.</i> , 1993; Yang and Tyree, 1994; Tyree <i>et al.</i> , 1999; Sack <i>et al.</i> , 2002
iii. Vacuum pump method	Water is pulled out of the leaf under several levels of partial vacuum ^e , and K_{leaf} is estimated as slope of F_{leaf} vs levels of vacuum		Kolb <i>et al.</i> , 1996; Nardini <i>et al.</i> , 2001; Sack <i>et al.</i> , 2002

Table 5.1—cont'd

Method		Sample references
iv. Rehydration kinetics method	K_{leaf} estimated analytically from rehydration kinetics of leaf water potential for partially desiccated leaves imbibing water	Tyree <i>et al.</i> , 1975; Tyree and Cheung, 1977; Brodribb and Holbrook, 2003b

For all methods but (b) iv, (the rehydration kinetics method), K_{leaf} determined as measured flow rate (F_{leaf})/driving force. There are theoretical grounds to speculate that the different methods might produce slightly different values of K_{leaf} . Tests for methods (b)i, (b)ii, and (b)iii have shown that when applied under specific conditions, similar values are produced (Tsuda and Tyree, 2000; Nardini *et al.*, 2001; Sack *et al.*, 2002).

^aThe potential overestimation of transpiration using gas exchange systems (due to removal of boundary layer resistance, and potential misestimation of leaf temperature; Tyree and Wilmot, 1990), need to be considered in the study design when using this method.

^bThe non-transpiring leaf is thus assumed to have equilibrated with the branch water potential, at the leaf insertion.

^cIn methods using vein water potential and an estimate of transpiring leaf water potential, the conductance estimated is, of course, that between vein and evaporation sites, rather than that of the whole-leaf.

^dWe note that the water potential measured for 'transpiring' leaves taken for the pressure bomb, or sampled for psychrometry, are for leaves equilibrated and non-transpiring at the time of water potential determination. Hence the total water potential difference between 'transpiring' and non-transpiring leaves may not give the total driving force between base of the petiole and the 'average loci' of evaporation.

^eWhether a pressure gradient is equivalent to $\Delta\Psi_{\text{leaf}}$ to describe the driving force of water movement depends on the precise pathway of water movement in leaves—i.e., whether osmosis is a driving force to be considered—and should really be dealt with in terms of irreversible thermodynamics. The general assumption is that during steady-state flow through the leaf, osmosis is a negligible contribution to the driving force (but see Tyree *et al.*, 1999; Tyree, 2003b).

major veins. From this point onward, the exact water flow paths are uncertain. In most angiosperm leaves, water flows through several orders of major veins before entering the minor vein network (i.e., the network of small veins throughout the lamina embedded in the mesophyll, which in cross-section typically contain one or two xylem conduits). The reticulation of the venation means that water can exit major veins of any order into the minor veins, or, potentially, water might exit the major veins directly into the surrounding tissue. However, the density of minor veins accounts for 93% to 96% of the total vein density in temperate tree species (e.g., Armacost, 1944; Plymale and Wylie, 1944; Wylie, 1951; Dengler and Mackay, 1975; Russin and Evert, 1984). Thus, the bulk of water flow to the mesophyll and epidermis is probably supplied principally through the minor vein network.

The minor veins are typically wrapped in a bundle sheath of parenchymatous cells along most of their length (Esau, 1965). Classical anatomy and histology on a range of species suggest that a large part of the water leaving the minor veins necessarily passes through the bundle sheath cells; the

perpendicular cell walls may be suberized and might thus constitute a barrier analogous to the root Casparian strip, which forces water to move from apoplast to symplast (Van Fleet, 1950; Lersten, 1997). For a range of species, dye experiments have also suggested that the bulk of transpired water tends to exit the vein xylem into the bundle sheath cells—in leaves transpiring a solution of sulphorodamine G, an apoplastic dye, crystals form in the minor veins, indicating that water is diffusing out across the membrane (Canny, 1990a, 1990b). Other evidence comes from the temperature response of measured K_{leaf} . When water moves through leaves or leafy shoots, the conductance increases or declines as temperature is respectively increased or decreased, and the slope of the response is stronger than that expected to arise simply from changes in the viscosity of water (Tyree *et al.*, 1973; Boyer, 1974; Tyree and Cheung, 1977; Fredeen and Sage, 1999; Cochard *et al.*, 2000; Matzner and Comstock, 2001; Sack *et al.*, 2004). This extra-viscosity response suggests at least some water passes through membranes; indeed, such temperature sensitivity established the fact of water moving through membranes in root water transport (Kramer and Boyer, 1995). It is unclear whether *all* water leaves the xylem through membranes, and more research is needed to establish the details of this crucial stage.

What happens to water once out of the xylem and in the bundle sheath? According to early anatomical studies, water movement through the mesophyll would mostly occur between spongy mesophyll cells, which are in contact to a far greater degree than are palisade cells (Wylie, 1946). Water might move through the mesophyll to the sites of evaporation apoplastically (i.e., never crossing a membrane) or pass cell-to-cell, whether symplastically (i.e., via plasmodesmata, though water must pass one membrane to enter the symplasm and another membrane to exit to the evaporative surface), or transcellularly (i.e., crossing cell walls and membranes twice for every cell in the path). Theoretical considerations and early experiments on leaves of trees and herbs suggested that water movement from veins to evaporation sites was primarily via the apoplast, in cell wall nanochannels (Weatherley, 1963; Boyer, 1977), but other studies on one of the same species, sunflower, reported that symplastic or cell-to-cell movement cannot be excluded (Cruiziat *et al.*, 1980; Tyree *et al.*, 1981). Furthermore, in addition to water flow across the mesophyll, in many leaves an important potential pathway for water movement to the epidermis is through bundle sheath extensions, cells that bridge the epidermis and minor veins and that are commonly found in temperate tree species (Armacost, 1944; Wylie, 1952; McClendon, 1992). Evidence for the importance of a direct flow path to the epidermis includes the fact that in several species, areas of the epidermis remain hydrated even when having little vertical contact with the underlying mesophyll (LaRue, 1931; Warrit *et al.*, 1980). Additionally, sud-

den hydraulic pressure changes can be transferred quickly from the petiole to epidermis in *Tradescantia* leaves, which possess bundle sheath extensions (Sheriff and Meidner, 1974).

Finally, there is the question of where in the leaf water evaporates. If water evaporates preferentially from a certain group of cells, then pathways to those cells will be most important during transpiration. Some have presented arguments for water evaporating principally from the cells adjacent to the stomata—from the epidermal cells around the stomata, and/or from the layer of mesophyll (usually spongy) directly above the stomata, and/or from the guard cells themselves (Byott and Sheriff, 1976; Tyree and Yianoulis, 1980). In the archetypal dicotyledonous leaf, with only abaxial stomata and layers of spongy mesophyll above, this would mean that the cells most active photosynthetically, the palisade cells, which run beneath the adaxial surface, would not lie in the primary pathways of transpiration, and would be kept more turgid than the spongy mesophyll in normal transpiration, and thus buffered to a degree from potential water limitation. On the other hand, several have proposed an opposite scenario: that water tends to evaporate throughout the lamina, making the diffusion pathway of water vapor similar to that of CO₂ (though opposite in direction). Indeed the computation of intercellular CO₂ concentration using typical photosynthesis systems relies on this assumption (Field *et al.*, 2000). Further circumstantial evidence for this alternative scenario is the sheer amount of exposed cell wall throughout the leaf relative to that simply around the stomata (Davies, 1986), and the fact that, in at least several species, there is suberization inside the leaf around the stomata and adjacent mesophyll, which would reduce evaporation (Pallardy and Kozłowski, 1979; Nonami and Schulze, 1989). In studies using a cell pressure probe on transpiring leaves of *Tradescantia*, the water potential of the bulk mesophyll was reported to be lower than that in the epidermis, which might indicate that there is greater evaporation from the bulk mesophyll (Nonami and Schulze, 1989). However, the same finding might arise even if most water evaporated from cells close to the epidermis, if water is supplied to these cells with relatively high conductance (e.g., via the bundle-sheath extensions in this species; Sheriff and Meidner, 1974). The question of where water principally evaporates within the leaf is still open.

Such a linear description of flow paths, while important for mechanistic reduction, of course oversimplifies the reality. Water flux occurs simultaneously through many pathways, with local flow rates and pressure drops determined by resistances of each component in the context of the whole system. For full elucidation, the system could be modeled as a complex electronic circuit, looped in multiple places, but at present the component resistances are largely unknown. For instance, we do not know the resistance to flow through minor vein conduits, through vein xylem pit

membranes, bundle sheath membranes, mesophyll cell membranes, plasmodesmata, or cell wall microchannels. Further, because of uncertainty about where water evaporates, we cannot precisely locate the driving forces. However, even when all the resistances and driving forces are determined, a model will not be completely satisfactory in itself. Confirmation will be delayed until technology allows pressure probing of cells throughout the leaf during transpiration, or, most ideally, the direct visualization of the pressures within cells throughout the leaf.

Water flow pathways through the leaf have important bearing on the measurement and interpretation of K_{leaf} . Several methods for determining K_{leaf} (e.g., the evaporative flux method; Table 5.1) rely on estimating the overall driving force using the pressure bomb water potential for the transpiring leaf. It is unclear, however, how well the pressure bomb can estimate the overall driving force. If water evaporates preferentially from a population of cells near the stomata, a possibility described previously, then the pressure bomb water potential, as a volume-averaged water potential for all the leaf's cells, will underestimate the driving force, and thus overestimate K_{leaf} , though it is uncertain by how much. For instance, let us suppose that water evaporates principally from spongy mesophyll and epidermides, and negligibly from the palisade cells, located further from the stomata. During transpiration, the water potential of palisade cells would be nearly equilibrated with that of the adjacent minor venation, and the water potential in the spongy mesophyll and epidermides would be lower by some amount. The pressure bomb water potential will underestimate the driving force, because it integrates the water potential of the palisade, which is not in the transpiration path. The pressure bomb would estimate the driving force *well* only if the difference in water potential between palisade cells and sites of evaporation is not substantial. Such would be the case if the hydraulic conductance from the minor venation to the sites of evaporation is very high relative to the conductance of the xylem and bundle sheath; in this case, the water potential would not differ substantially among leaf cells.

Notably, the evaporative flux method produces K_{leaf} values similar to those measured using other methods that do not rely on the pressure bomb to determine driving force (Table 5.1; Sack *et al.*, 2002), the high pressure flowmeter (HPFM) and the vacuum chamber method (Sack *et al.*, 2002). These methods also have been hypothesized to overestimate K_{leaf} , for a different reason; these methods establish new flow paths through the leaf; once out of the veins, water may short-circuit transpirational paths, flowing through lacunae of the mesophyll airspaces. It is unlikely, however, that these three methods would all overestimate K_{leaf} to the same extent. The similarity among methods suggests that the flow paths are similar or that the differences in the flow paths are in components that contain minimal resistance (Sack *et al.*, 2002, 2004). Such would be the case if most of

the hydraulic resistance in the leaf (i.e., $1/K_{\text{leaf}}$) were in the venation system, rather than in the paths of water flow distal to the veins.

Coordination of K_{leaf} , Venation System Design, and Leaf Shape

How much does whole-leaf hydraulic function depend on properties of the venation? Leaves vary tremendously in venation architecture, from the gridlike system of grasses, to the radial venation of *Ginkgo*, to the radiator-like system of trees of the tropical genus *Calophyllum* (possessing midrib with close-set parallel secondaries that branch off at right angles), to the diverse dendritic architectures common in dicotyledonous trees. If the leaf venation is an important component of the leaf hydraulic resistance (i.e., an important bottleneck in K_{leaf}) then variation in venation architecture might significantly impact leaf water use. The importance of the venation in water transport would be coordinated with its role in nutrient supply, sugar distribution (see Chapter 6), as well as in support and defense against mechanical damage and herbivory (e.g., Niklas, 1999; Choong *et al.*, 1992). Additionally, as found for tropical tree species, major veins protruding from the lamina can play a significant role in breaking up the boundary layer of still air around the leaf, facilitating gas exchange (Grace *et al.*, 1980). Through selection, these roles together may be coordinated and co-optimized with respect to construction costs (Rosen, 1967; Givnish, 1987), though such optimization is contingent on the genes and developmental sequences available during evolution, and constrained by potential trade-offs arising among structures and functions.

The importance of the leaf venation to the hydraulics of the whole-leaf depends on how much of the total resistance to transpirational water flow is in the vein xylem, and how much is outside the xylem. Recent work on venation pressure distribution, using the xylem pressure probe, indicated a large resistance within the leaf vasculature in *Laurus nobilis* (Zwieniecki *et al.*, 2002). Vein cutting experiments also have indicated a large proportion of the leaf's resistance in the vasculature, 64% in *Acer saccharum* and 74% in *Quercus rubra* (Fig. 5.2; see also Color Plate section; Sack *et al.*, 2004). In these experiments, leaves were submitted to K_{leaf} determination by the HPFM, and subsequently veins were cut, beginning with the minor veins, and then the higher orders of major veins, such that water leaked out of the cuts, shorting out downstream resistance. A major location of resistance is between the major and minor veins, or in the minor veins themselves. The lower order major veins typically contain little of the leaf resistance (Yang and Tyree, 1994; Tyree *et al.*, 2001; Sack *et al.*, 2004). The proportion of the leaf resistance that is vascular depends on the xylem conduit number and dimensions, and on the

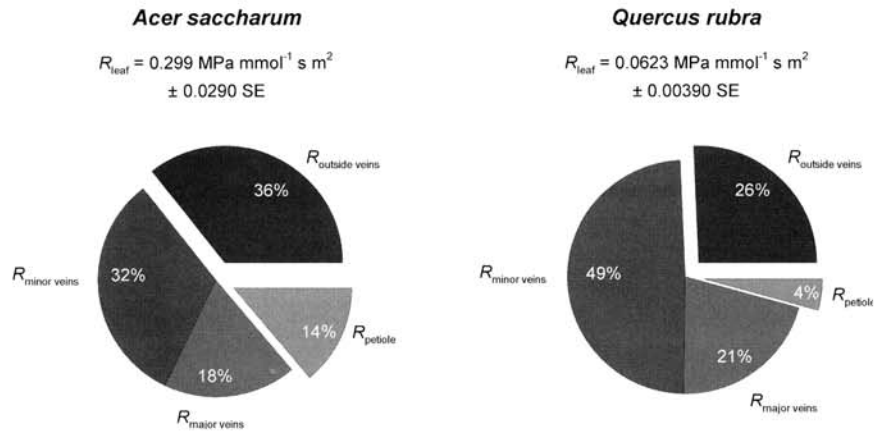


Figure 5.2 The distribution of hydraulic resistance ($=1/\text{hydraulic conductance}$) in the leaves of sugar maple (*Acer saccharum*) and red oak (*Quercus rubra*). (See also Color Plate section.) From Sack *et al.* (2004). Copyright American Society of Plant Biologists; adapted with permission.

extravascular pathway (i.e., the permeability of the membranes, the plasmodesmata, or the nanochannels formed by the cell wall microfibrils), as well as the transfer areas of the cell-to-cell pathways. The possible coordination of K_{leaf} with membrane properties such as aquaporin populations is a current field for pioneering research (Martre *et al.*, 2002; Siefritz *et al.*, 2002).

The leaf lamina is supplied by the petiole. This single-point supply to the lamina raises the problem of supplying water equitably, so that gas exchange rates and cell water deficits are distributed uniformly (i.e., bringing the water potential of each square centimeter of the leaf within a narrow range). Data from porometer studies (Sack *et al.*, 2003a) and thermal photography (Jones, 1999) on temperate tree species and grapevine suggest that for an evenly lit leaf under well-watered conditions, stomatal conductance is relatively even, from petiole insertion to leaf tip (though transpiration can be locally highly patchy, especially from stomate to stomate throughout the leaf (Terashima, 1992; Mott and Buckley, 1998). If transpiration is indeed relatively homogeneous across the lamina from leaf base to tip (at say square centimeter resolution), for water potential to show a similarly narrow spatial variation from leaf base to tip, water must be distributed relatively equitably. Several models of leaf hydraulic design proposed in the literature might account for equitable distribution, based on the relative hydraulic conductances of vein orders, and the conductance of the vasculature relative to the pathways from veins to evaporation sites (Tyree and Cheung, 1977; Canny, 1993; Nardini *et al.*, 2001; Roth-Nebelsick *et al.*, 2001; Zwieniecki *et al.*, 2002; Sack *et al.*, 2004).

Another key feature of the leaf venation is its damage tolerance. The lower order major veins are essential in water supply. Such a conclusion was at odds with the classical demonstration that leaves of many temperate woody species survive the cutting of major veins, including primaries, in apparently perfect health (Plymale and Wylie, 1944). More recent work has shown that the apparent health in *Quercus rubra* leaves with severed midribs belies a strong loss of function; K_{leaf} , transpiration, and photosynthetic quantum yield were reduced dramatically (Fig. 5.3; Sack *et al.*, 2003a). However, leaves with different venation architectures might be able to sustain different amounts of major vein severing while maintaining function at different amounts. Redundancy in the vasculature—including the multiple conduits in parallel within veins, multiple veins of a given order, and the reticulation of the minor vein network—may provide alternative pathways for water flow, buffering to some extent the effects of vascular damage, as well as of drought-induced embolism (see discussion below).

Venation system design is tantalizingly linked with leaf shape, in *Arabidopsis* mutants and in leaf development (Dengler and Kang, 2001). The linkage of venation and shape may have a hydraulic basis (Thoday 1931; Sack *et al.*, 2002; Zwieniecki *et al.*, 2002). Leaves with higher outline complexity (i.e., lobing) have their mesophyll regions at the leaf edge closer to low-order “supply” veins (e.g., primaries and secondaries). By contrast, in more entire leaves, the relatively larger areas of mesophyll far from the lower-order supply veins are supplied via a greater length of low-conductance higher-order veins, and these areas may thus contribute to a lower overall K_{leaf} than for lobed leaves. Such a linkage between K_{leaf} and lobing may be termed structural—arising from common anatomy. Additionally, K_{leaf} and lobing might be functionally linked (i.e., without sharing a common structural basis), if exposed conditions lead to selection of a high K_{leaf} to maintain water supply for high transpiration rates, in parallel

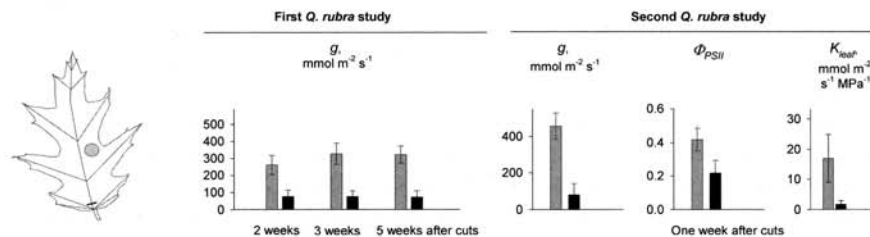


Figure 5.3 Two studies of the effects of severing the midrib on *in vivo* stomatal conductance, photosynthesis, and K_{leaf} in *Quercus rubra*. Stomatal conductance (g) and the quantum yield of photosystem II (Φ_{PSII}) were measured in the indicated area; black line represents the cut. Black bars represent treated leaves and gray bars paired control leaves (from Sack *et al.*, 2003a). Copyright Botanical Society of America; adapted with permission.

with selection for lobing. Lobing may be an advantage in exposed conditions, because the less well-supplied mesophyll regions in entire leaves are prone to desiccation under high evaporative demand or limited water supply (Thoday, 1931; Zwieniecki *et al.*, 2002, 2004). Further, lobed leaves have a thinner still-air boundary layer, which minimizes lamina overheating (Vogel, 1968, 1970). A few recent studies support a linkage of K_{leaf} and leaf outline complexity. *Quercus rubra* sun leaves have greater outline complexity than shade leaves, as well as greater K_{leaf} (Sack *et al.*, 2003b; Zwieniecki *et al.*, 2004). Across species of *Quercus*, leaves with deeper lobes had higher K_{leaf} (Sisó *et al.*, 2001). In a study of sun and shade leaves of six temperate deciduous species, leaf perimeter/area (an index of outline complexity) correlated with K_{leaf} , though one species, *Vitis labrusca*, was a strong outlier (Sack *et al.*, 2003b). One hypothesis is that leaf shape and vascular architecture may be structurally coordinated within species, and across species that have similar vascular design (i.e., similar conduit numbers and diameters in each vein order). Across sets of species that differ strongly in vascular design, however, the leaf shape and venation architecture might well be structurally independent, though functionally coordinated for their advantages in exposed conditions.

Coordination of K_{leaf} and Leaf Water Storage

Leaf water storage occurs in the vacuoles of mesophyll or epidermal cells, and/or in specialized thin-walled water storage cells, which in some species are achlorophyllous, and/or in apoplastic mucilage (Roberts, 1979; Nobel and Jordan, 1983; Morse, 1990; Sack *et al.*, 2003b). The leaf-area specific capacitance—the water released per change in leaf water potential, per leaf area—is the product of tissue-specific capacitance, leaf dry mass per area, and leaf water content per dry mass. These characters vary strongly, though smoothly, across species. For instance, in a set of semidesert species ranging from nonsucculents, to semileaf succulents, to full leaf succulents, leaf water content per dry mass varied smoothly by nearly 20-fold along a continuum (Von Willert *et al.*, 1990).

Commonly, water storage in the leaf has been associated with desiccation avoidance (or desiccation delay)—i.e., for sustaining cuticular transpiration when the stomata have closed (Levitt, 1980; Lamont and Lamont, 2000). Could stored water play a role in normal transpiration? Certainly stored leaf water in most cases cannot supply typical mid-day rates of transpiration under high illumination if water were not supplied from the soil. Leaf water storage often accounts for less than 1% of daily transpiration needs in tropical trees to up to 16% in *Thuja* trees (Tyree *et al.*, 1991; Machado and Tyree, 1994). On the other hand, stored water may play an

important role in sustaining a relatively stable leaf water potential as transpiration rate and root water supply fluctuate. In principle, the lower the water storage, the greater impacts these fluctuations will have in driving transient changes in leaf water potential, desiccating leaf tissue, and causing stomatal closure. Leaves with high K_{leaf} and high maximum transpiration rates would benefit most from stored water as a buffer of fluctuations in water potential. Consistent with this principle, in leaves of temperate woody species, leaf area-specific capacitance was correlated with K_{leaf} (Fig. 5.4; Sack *et al.*, 2003b). A large capacitance might be especially useful in semidesert leaves, which would benefit by maintaining stably high stomatal conductance, when moisture is available. A linkage between high K_{leaf} and water storage might contribute to the finding for temperate tree species that at a given transpiration rate excised leaves with high K_{leaf} close their stomata relatively slowly (Aasamaa *et al.*, 2001). The two potential roles of leaf capacitance, drought survival and buffering of water potential fluctuations, will need to be teased apart—perhaps most profitably in a phylogenetic context, especially in relation to the frequent evolution of succulence.

Coordination of K_{leaf} with Other Aspects of Leaf Structure, Carbon Economy, and Drought Tolerance

How is K_{leaf} integrated in the complex of characters involved in leaf structure and function? It is clear that within and across biomes leaves vary

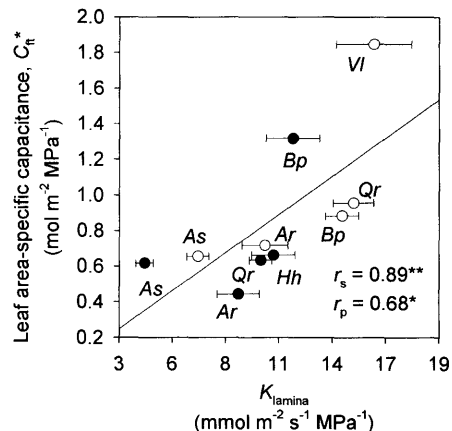


Figure 5.4 Coordination of leaf area-specific capacitance with K_{leaf} in northern temperate tree and climber species. Symbols as in Fig. 5.1(B) * $P < 0.05$; ** $0.01 > P > 0.001$ (from Sack *et al.*, 2003b). Copyright Blackwell Publishing; adapted with permission.

tremendously in venation, shape, and water storage, as discussed previously, and also in area, thickness, gas exchange, and desiccation tolerance. The underlying basis for variation in leaf traits related to carbon economy has been extensively investigated, and several of these traits are correlated across diverse leaves. One example is the correlation of high nitrogen per unit mass, high light-saturated photosynthetic rate per unit mass, low leaf mass per area (LMA), high leaf water content, and short leaf lifespan (e.g., Field and Mooney, 1986; Reich *et al.*, 1999; Wright and Westoby, 2002).

There appears to be similar coordination of leaf characters associated with water flux through the leaf, which also bear on carbon economy (Grubb, 1984; Sack *et al.*, 2003b). Indeed, K_{leaf} may constrain gas exchange per area (see earlier discussion), and thus play a direct role in carbon economy. Some work suggests that K_{leaf} may be strongly coordinated with leaf thickness (Sack *et al.*, 2003b; Fig. 5.5). Thicker leaves tend to have higher mesophyll surface area per leaf area for CO_2 fixation, and thus, higher rates of drawdown of intercellular CO_2 . Thus, thickness is expected to be positively coordinated with stomatal pore area, which in turn should be coordinated with K_{leaf} , so that water supply matches demand. Stomatal pore area would turn in constrain maximum photosynthetic rate per unit area. Photosynthetic rate per unit leaf mass, probably the more appropriate measure of gain in leaf-level carbon economy, depends additionally on leaf mass per area, which seems to be independent of K_{leaf} (Tyree *et al.*, 1999; Salleo and Nardini, 2000; Sack *et al.*, 2003b), despite the relationship of K_{leaf} to leaf thickness, a component of LMA (K_{leaf} is apparently independent of leaf density, the other component of LMA). Further, K_{leaf} appears to be unrelated to leaf lifespan (Tyree *et al.*, 1999; Nardini, 2001; but see Sobrado, 1998). Thus, K_{leaf} represents a potential constraint on carbon economy independent of other inter-related characters that influence carbon economy (LMA, nitrogen concentration, leaf lifespan). The overall importance of K_{leaf} and associated traits in defining species' ecology, and potential crop performance requires further investigation.

Is K_{leaf} important in determining drought tolerance? A high K_{leaf} in itself may contribute a measure of drought tolerance (i.e., because a lower drop in water potential occurs across the leaf at a given transpiration rate), and, as seen previously, K_{leaf} is positively coordinated in temperate woody species with capacitance leaf area-specific, an index of water storage. For leaves of temperate woody species growing in moist soil, however, K_{leaf} is unrelated to other leaf features that contribute to the ability to function at low water potential—a low turgor loss point, high modulus of elasticity, and low cuticular transpiration rate (Sack *et al.*, 2003b). The independence of K_{leaf} and drought tolerance is consistent with the fact that both drought tolerant and intolerant species can have high maximum stomatal conductances and transpiration rates when moisture supply is high (Maximov,

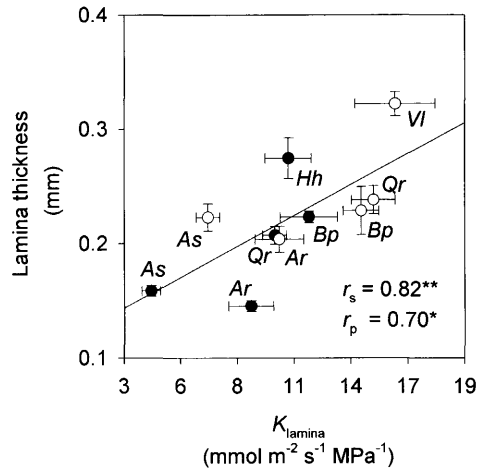


Figure 5.5 Coordination of K_{leaf} and leaf thickness. Symbols as in Fig. 5.1(B) and Fig. 5.4 (from Sack *et al.*, 2003b). Copyright Blackwell Publishing; adapted with permission.

1931; Wright *et al.*, 2001). More work is needed to elucidate how leaf hydraulics, structure, and function may be coordinated, co-selected, and co-optimized across the diversity of leaf types.

Variability of K_{leaf} Across Environments, Diurnally, and with Leaf Age

The coordination of K_{leaf} and other leaf characters has typically been studied for plants grown under high-irradiance, in moist soil. However, K_{leaf} is a highly variable trait, even among leaves of the same species, or, indeed, of the same plant. First, K_{leaf} is environmentally plastic, higher for leaves of given species grown in higher resource conditions: sun leaves of temperate deciduous trees grown in higher resource conditions have been found to have K_{leaf} values up to 67% higher than shade leaves—consistent with the higher degree of exposure and associated water flux (Sack *et al.*, 2003b). Similarly, leaves of temperate deciduous tree seedlings grown in higher water or nutrient supplies may tend to have higher K_{leaf} (Aasamaa *et al.*, 2001), as do leaves of *Quercus ilex* seedlings associated with ectomycorrhizae, relative to those of nonsymbiotic plants (Nardini *et al.*, 2000). Additionally, K_{leaf} declines with leaf age, moving down the stem from young to old leaves in sunflowers, and seasonally in tree species (Salleo *et al.*, 2002; Brodribb and Holbrook, 2003a; Sack, L., unpublished data). More work is needed, but the age-related decline of

K_{leaf} might result from the accumulation of embolism in leaf veins, from the formation of tyloses (Salleo *et al.*, 2002), or from changes in the permeabilities of membranes and cell walls in the leaf (Van Fleet, 1950).

Evidence is accumulating that K_{leaf} is also reduced during diurnal water stress and during drought. One study reports diurnal changes in petiole hydraulic conductivity in two of three temperate tree species tested (Zwieniecki *et al.*, 2000). Acoustic and dye studies show that embolisms in minor veins arise during desiccation (Kikuta *et al.*, 1997; Salleo *et al.*, 2001, 2003; Lo Gullo *et al.*, 2003). An ideal method for measuring K_{leaf} in desiccated leaves is elusive. Methods that measure K_{leaf} on desiccated leaves by driving flow through the leaf at higher pressure than ambient may redissolve embolisms rather quickly. However, using such methods, considerable declines in K_{leaf} due to desiccation have been reported (Nardini *et al.*, 2001, 2003; Salleo *et al.*, 2001; Brodribb and Holbrook, 2003b; Lo Gullo *et al.*, 2003; Trifilo *et al.*, 2003a and b). The redundancy of conduits within veins and the reticulation of the minor veins may buffer K_{leaf} from the effects of cavitation-induced vein embolism to some degree, but at high levels of embolism, the decline of leaf water potential associated with decline in K_{leaf} may drive stomatal closure (Salleo *et al.*, 2001; Nardini *et al.*, 2003; Trifilo *et al.*, 2003b; Brodribb and Holbrook, 2003b). In excised sunflower leaves allowed to rehydrate, cavitated conduits in the leaf veins will apparently refill (Trifilo *et al.*, 2003a). The question of whether, *in vivo*, leaves may refill embolized conduits (i.e., redissolve embolism either when under high tension, or when tension is low) is currently entirely open.

Because the leaf represents a substantial component of the whole-plant resistance, reduction of K_{leaf} would have significant consequences at the whole-plant level (Meinzer, 2002). Declines of K_{leaf} may trigger stomatal closure and may play an important role in driving the well-recognized declines of gas exchange late in the day, late in the season, and in aging leaves (Jones, 1992; Kitajima *et al.*, 1997).

Summary of Directions for Future Research

The field of leaf hydraulics is still in discovery phase, and new work is needed to remove the many unknowns. More research is needed in examining the methods for measuring K_{leaf} , as well as in each of the areas described previously. Our understanding of pathways of water flow through the leaf remains rudimentary, and new approaches are needed, based either on probes or on visualization. The possible coordination of K_{leaf} with venation architecture and/or with particular aspects of membrane physiology (e.g., aquaporins and plasmodesmata) is an exciting field for future

research. Also, the coordination of K_{leaf} and other leaf features merits further study, as do the dynamics of K_{leaf} in changing environmental conditions, at time scales ranging from minutes, to hours, to seasons.

Finally, it will be important to examine the possibility of coordination of K_{leaf} with other organs and systems in the plant, within and across species. Do plants with high K_{leaf} have high root conductance? Is K_{leaf} coordinated with particular root morphologies and anatomies? Research is needed on the coordination of nutrient and water supply within the leaf, and, of course, the integration of the water transport system with phloem (see Chapters 10 and 11). Because leaf hydraulics is important on the global scale and intricate at the smallest scale, its clarification will illuminate many topics in leaf physiology, structure, evolution, and ecology.

Acknowledgments

Lawren Sack was supported by The Arnold Arboretum of Harvard University (Putnam Fellowship), and the Smithsonian Tropical Research Institute (Short Term Fellowship), and Mel Tyree by the USDA Forest Service and the Andrew Mellon Foundation. We thank our project collaborators for generous insights and discussion, and Kevin Boyce, Matthew Gilbert, Missy Holbrook, and Ken Shack et al for comments on the manuscript.

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