Title: Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture

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Abstract

Across plant species, leaves vary enormously in their size and their venation architecture, of which one major function is to replace water lost to transpiration. The leaf hydraulic conductance ($K_{\text{leaf}}$) represents the capacity of the transport system to deliver water, allowing stomata to remain open for photosynthesis. Previous studies showed that $K_{\text{leaf}}$ relates to the vein density (= vein length per area). Additionally, venation architecture determines the sensitivity of $K_{\text{leaf}}$ to damage; severing the midrib caused $K_{\text{leaf}}$ and gas exchange to decline, with lesser impacts in leaves with higher major vein density that provided more numerous water flow pathways around the damaged vein. Because xylem embolism during dehydration also reduces $K_{\text{leaf}}$, we hypothesized that higher major vein density would also reduce hydraulic vulnerability. Smaller leaves, which generally have higher major vein density, would thus have lower hydraulic vulnerability. Tests using simulations with a spatially explicit model confirmed that smaller leaves with higher major vein density were more tolerant of major vein embolism. Additionally, for ten species ranging strongly in drought tolerance, hydraulic vulnerability determined as the leaf water potential at 50% and 80% loss of $K_{\text{leaf}}$ was lower with greater major vein density and smaller leaf size ($|r| = 0.80-0.86; P < 0.01$). These relationships were independent of other aspects of physiological and morphological drought tolerance. These findings point to a new functional role of venation architecture and small leaf size in drought tolerance, potentially contributing to well-known biogeographic trends in leaf size.

Key words: biological networks, cavitation, cuticular conductance, hydraulic architecture, leaf traits, safety margins, turgor loss point, venation architecture, vulnerability curves
Introduction

The leaf venation architecture has common functions across plant species, serving for mechanical support (Niklas, 1999), sugar and hormone transport (Kehr and Buhtz, 2008), and the replacement of water lost to transpiration during photosynthesis (Sack and Holbrook, 2006). However, venation architecture is highly diverse across species (Uhl and Mosbrugger, 1999; Roth-Nebelsick et al., 2001; Sack and Frole, 2006; Ellis et al., 2009; Brodribb et al., 2010). In dicotyledons, the leaf venation system typically consists of three orders of major veins and up to five higher orders of minor veins embedded in the mesophyll, with the vein orders arranged in a hierarchy; lower order veins are larger in diameter, with greater xylem conduit numbers and sizes, whereas higher order veins have greater length per area (= vein density; Sack and Holbrook, 2006; McKown et al., 2010). Species vary strongly in the density of given vein orders and their conductivities (Cochard et al., 2004b; Sack and Frole, 2006). The aim of this study was to test for novel functional consequences of variation in leaf venation architecture and leaf size, and particularly a role in drought tolerance.

The leaf is a critical component in the plant water transport system, accounting for 30% or more of whole-plant hydraulic resistance (Sack and Holbrook, 2006). The leaf hydraulic conductance ($K_{\text{leaf}}$, flow rate / water potential driving force, i.e., the inverse of hydraulic resistance) quantifies a complex microhydrological system, including the conductances in series of the vein xylem ($K_x$) and the mesophyll pathways outside the xylem ($K_{\text{ox}}$). The venation architecture is thus an important determinant of $K_{\text{leaf}}$ and its dynamics. Total vein density is a determinant of both $K_x$ and $K_{\text{ox}}$, because, all else being equal, higher densities represent more numerous xylem flow pathways in parallel per leaf area, and shorter pathways for water movement outside the xylem (Sack and Frole, 2006; Brodribb et al., 2007; McKown et al., 2010). Additionally, venation structure may contribute to the ability of $K_{\text{leaf}}$ to withstand vein damage (Sack et al., 2008). Minor veins had been classically hypothesized to provide "conductive overload", consisting of many parallel pathways for water flow such that a leaf could tolerate hydraulic disruption of major veins (Wylie, 1938). However, detailed studies found that $K_{\text{leaf}}$, stomatal conductance and photosynthesis were very sensitive to damage of the large major veins, which supply water to the downstream vein hierarchy (Nardini et al., 2001; Huve et al., 2002; Nardini and Salleo, 2003; Sack et al., 2003a; Salleo et al., 2003; Delaney and Higley, 2006). Additionally, the impact of severing the midrib near its base differed among species. The decline of $K_{\text{leaf}}$ was lower in palmately-veined species with greater major vein density providing flow pathways around the disrupted vein (Sack et al., 2008). The impact of midrib damage on $K_{\text{leaf}}$ also varied among pinnately
veined species. Smaller leaves, with their major veins spaced more closely and thus greater major vein densities, had greater tolerance of midrib damage (Sack et al., 2008).

Just as for leaves with damaged veins, $K_{\text{leaf}}$ declines in dehydrating leaves, resulting in reductions of leaf gas exchange and whole plant growth (Salleo et al., 2000; Brodribb and Holbrook, 2003; Sack and Holbrook, 2006; Johnson et al., 2009b). The $K_{\text{leaf}}$ decline with dehydration arises at least in part from embolism in the vein xylem (Kikuta et al., 1997; Salleo et al., 2000; Salleo et al., 2001; Nardini et al., 2003; Trifilo et al., 2003a; Trifilo et al., 2003b; Cochard et al., 2004a; Brodribb and Holbrook, 2005; Choat et al., 2005; Brodribb et al., 2009; Johnson et al., 2009a). Cavitation in turn will depend on the intrinsic vulnerability of each vein, with major veins likely to be more vulnerable because of their long and wide conduits (Choat et al., 2005). We hypothesized that higher major vein density, by providing transport pathways around embolised major veins, would confer tolerance of $K_{\text{leaf}}$ to dehydration, i.e., more negative $\Psi_{\text{leaf}}$ values at 50% and 80% loss of $K_{\text{leaf}}$ ($P_{50}$ and $P_{80}$ respectively). Such a role for leaf venation could be important in the optimization of leaf size.

Leaf size is highly variable across environments, with smaller leaves more frequent in dry habitats, both within and among species (Givnish, 1987; Sultan and Bazzaz, 1993; Gibson, 1998; Cunningham et al., 1999; Ackerly, 2003, 2004), as well as among community assemblages (Dolph and Dilcher, 1980; Fonseca et al., 2000). One advantage for small leaves is their thinner boundary layer enabling more rapid convective cooling (Vogel, 1968, 1970; Parkhurst and Loucks, 1972; Gibson, 1998; Vogel, 2009; Nobel, 2010). There may additionally be a direct hydraulic benefit of small leaves, if their greater major vein redundancy protects $K_{\text{leaf}}$ from decline and thus contributes to drought tolerance. To test these hypotheses, we conducted computer simulations of the impact of vein cavitation on $K_{\text{leaf}}$. We compared theoretical results with experimentally-measured relationships among leaf hydraulic vulnerability, leaf size, venation architecture and other aspects of leaf drought tolerance for species diverse in leaf form and drought sensitivity.

Results

Impacts of vein cavitation depend on venation architecture: computer simulations

Simulations implemented in the program $K_{\text{leaf}}$ showed that the impacts of vein cavitation depended on vein density and leaf size (Fig. 1A, B and C; Table 1). Leaves were simulated of different sizes but with the same number of second-order ($2^o$) veins, and thus larger leaves had their $2^o$ veins spaced further apart, and major vein density declined geometrically with increasing leaf size (major vein density = 1.194 × leaf size$^{0.5}$; $r_p = 0.999; P < 0.001$). By
contrast, across the simulated leaves, minor vein density was varied independently of leaf
size \((r_p = 0.02; P = 0.89)\). \(K_x\) for uncavitated leaves correlated positively with both major vein
density and minor vein density \((r_p = 0.75; P < 0.001 \text{ and } r_p = 0.15; P = 0.01 \text{ respectively})\).

When major veins were reduced by 90% in cross-sectional conductivity to simulate
dysfunction of conduits due to embolism, the smaller leaves with greater major vein density
showed a lesser impact on total xylem and whole leaf hydraulic conductance per leaf area \((K_x\)
and \(K_{\text{leaf}}\), i.e., a lower percentage loss of conductance \((\text{PLC}; \text{Fig. } 1\text{A})\). Thus, across simulated
leaves, the PLC of \(K_x\) resulting from major vein cavitation correlated negatively with major
vein density \((r_p = -0.85, P < 0.001)\). For the simulated leaves with cavitated major veins, the
\(K_x\) itself correlated strongly with major vein density, due both to the increase of maximum \(K_x\)
by higher major vein density, and the protective role of higher major vein density \((r_p = 0.97; \newline P < 0.001)\). The importance of minor vein density was opposite from that of major vein
density. A higher minor vein density increased the impact of cavitation of the major veins on
the \(K_x\) (Fig. 1A). Thus, across the simulated leaves, the PLC of \(K_x\) resulting from major vein
cavitation correlated positively with minor vein density \((r_p = 0.42, P = 0.001)\). For these
simulated leaves with cavitated major veins, the \(K_x\) was not related to minor vein density \((r_p = 0.08; P = 0.57)\), because the greater \(K_x\) conferred by higher minor vein density was
counteracted by a greater sensitivity to major vein cavitation. Overall, because of the
contrasting effect of major and minor vein density, the PLC of \(K_x\) due to the cavitation of
major veins was least for leaves with highest major vein density and lowest minor vein
density, and correlated negatively with the ratio of major to minor vein density (Fig. 1C; \(r_p =\newline -0.97 P < 0.001\)).

When the minor veins rather than the major veins were cavitated, \(K_x\) and \(K_{\text{leaf}}\) had a
different dependency on venation architecture. When minor veins were reduced by 90% in
cross-sectional conductivity to simulate cavitation, the smaller leaves with greater major vein
density showed higher PLC of \(K_x\) (Fig. 1B). Thus, across simulated leaves, the PLC of \(K_x\)
resulting from minor vein cavitation was positively related to major vein density \((r_p = 0.77, P < \newline 0.001)\). By contrast, a higher minor vein density reduced the impact of cavitation of the
minor veins on the \(K_x\) (Fig. 1B). Thus, across the simulated leaves, the PLC of \(K_x\) resulting
from minor vein cavitation was negatively correlated with minor vein density \((r_p = -0.48, P = \newline 0.001)\). For the simulated leaves with cavitated minor veins, the \(K_x\) itself correlated positively
with minor vein density, due both to the increase of maximum \(K_x\) by higher minor vein
density, and the protective role of high minor vein density \((r_p = 0.66; P < 0.001)\). For these
simulated leaves with cavitated minor veins, the \(K_x\) also positively correlated with major vein
density, but more weakly than for uncavitated leaves, because the increase of maximum \( K_x \)
due to higher major vein density was counteracted by the greater PLC driven by minor vein
cavitation in leaves with higher major vein density \( (r_p = 0.35 \text{ rather than 0.75}; \ P < 0.001) \).
Overall, the PLC of \( K_x \) resulting from cavitation of the minor veins was strongly positively
correlated with the ratio of major to minor vein density \( (\text{Fig. 1C}; \ r_p = 0.92; \ P < 0.001) \).

Notably, in the model simulations, the PLC of \( K_x \) resulting from major vein cavitation
varied widely across the entire range of tested leaves with different major and minor vein
densities \( (22-87\%; \ \text{Fig. 1A and C}) \). By contrast, the PLC of \( K_x \) resulting from minor vein
cavitation was less variable and very strong across the entire range of tested leaves \( (62-90\%, \)
and > 80\% for most simulated leaves; \text{Fig. 1B and C}).

**Diversity in leaf venation and drought tolerance traits across species of moist and dry habitat**

Across the ten species diverse in drought tolerance there were strong differences in leaf
hydraulic conductance at full hydration, and in their vulnerability to dehydration. The species
varied in \( K_{max}, P_{50} \) and \( P_{80} \) by 12- to 32-fold. The \( K_{max} \) varied from 2.96 to 34.1 mmol m\(^{-2}\) s\(^{-1}\)
MPa\(^{-1}\) for *Comarostaphylos diversifolia* and *Platanus racemosa,* in \( P_{50} \) from \(-0.09 \) to \(-2.85 \)
MPa for *P. racemosa* and *C. diversifolia,* and in \( P_{80} \) from \(-0.35 \) to \(-5.25 \) MPa for *P. racemosa*
and *Cercocarpus betuloides* (Table S1).

The species differed strongly in leaf venation architecture and gross morphology, with
substantial variation between moist and dry habitat species (Table 2; Table S1). Species
varied fourfold in major vein density, threefold in minor vein density, and sevenfold in the
ratio of major to minor vein density. Species of moist and dry habitats did not differ
significantly in minor and total vein densities \( (P = 0.11-0.74), \) but dry habitat species had
18\% higher major vein density \( (\text{with 14-18}\% \text{ higher midrib, 2° and 3° vein densities}) \) and
50\% higher ratio of major to minor vein density. Moist habitat species had 24\% more free
ending veinlets per area, 13\% higher minor vein diameters and 14\% higher number of 2°
veins than dry habitat species \( (P < 0.001). \) Moist and dry habitat species did not differ in the
diameters of their major veins (Table S1). Species varied 18-fold in leaf area, with dry habitat
species having on average 30\% smaller leaves than moist habitat species. Leaf shape indices
(length: width and perimeter\(^2\): area) did not differ between habitats \( (P = 0.35-0.46). \)

Several venation traits correlated with leaf size. The densities of 1°, 2° and 3° veins
decreased with increasing leaf size \( (r_p \text{ and } r_s = -0.70 \text{ to } -0.92, \ P < 0.05), \) as did the total major
vein density \( (r_p =-0.95; \ r_s = -0.89, \ P < 0.001; \ \text{Figs 2A and 3}), \) and the ratio of major to minor
vein density \( (r_p = -0.67; \ r_s = -0.70; \ P = 0.03). \) Major vein density declined geometrically with
increasing leaf size (i.e., with an exponent of -0.5; Fig. 2A). By contrast, minor vein density
was independent of leaf size ($r_p$ and $r_s = -0.10$ to -0.20, $P > 0.05$; Figs 2B and 3), as were
total vein density (as minor vein density accounted for 73-95% of total vein density), vein
diameters and the number of free ending veinlets per area ($|r_p|$ and $|r_s| = 0.02-0.43$, $P > 0.05$;
Fig. 3; Table S2).

Species of dry habitats also had greater expression of leaf drought tolerance traits than
species from moist habitats, with 14% higher leaf mass per area (LMA), 11% thicker leaves,
18% higher modulus of elasticity, 15%-16% more negative values for osmotic potential at
full turgor and at turgor loss point, and 30% lower cuticular conductance. By contrast, species
of moist habitats had on average two- to threefold higher saturated water content and
capacitances before and after turgor loss point (Table S1; $P < 0.001$).

Relationships among hydraulic vulnerability, venation, and other drought tolerance and
morphological traits

Across species, $P_{50}$ and $P_{80}$ were strongly correlated and more negative values occurred in
leaves with higher major vein density and smaller leaf size ($|r_p|$ and $|r_s| = 0.78-0.90$, $P < 0.01$;
Figs 3, 4A and 4B; Table S2). These relationships for the major vein system also held for
component vein orders; the densities of 1°, 2° and 3° veins, all inter-correlated, were greater
in leaves with more negative $P_{50}$ and $P_{80}$ ($|r_p|$ and $|r_s| = 0.64-0.90$ $P < 0.01$). Because leaf size
and major vein densities were themselves negatively correlated (Fig. 2A), no correlation
could be observed of leaf hydraulic vulnerability with leaf size or with major vein density
independently of the other. Thus, in a partial correlation analysis, the relationships of $P_{50}$ and
$P_{80}$ with leaf area were not significant after partialing out major vein density, and their
relationships with major vein density were not significant after partialing out leaf area ($|r_{partial}|$
= 0.08-0.29; $P > 0.05$). By contrast with major vein densities, other vein traits did not
correlate with $P_{50}$ or $P_{80}$, including the minor vein density, total vein density, number of
secondary veins, vein diameters, and number of free-ending veinlets per area ($|r_p|$ and $|r_s| =
0.01-0.57$, $P > 0.05$). The ratio of major: minor vein density was positively correlated with
$P_{50}$ and $P_{80}$ ($r_p$ and $r_s = 0.77-0.67$, $P < 0.05$; Table S2)

Leaf hydraulic vulnerability also correlated with several other drought tolerance traits.
The osmotic potentials at full and zero turgor, which were inter-correlated ($r_s$ and $r_p = 0.95-
0.98$, $P < 0.001$), both correlated with $P_{50}$ and $P_{80}$ ($r_p$ and $r_s = 0.85-0.71$, $P < 0.05$; Table S2).
Leaves with higher LMA values tended to have more negative $P_{80}$ ($r_p = 0.71$, $r_s = 0.73$, $P <$
0.05; Table S2) but LMA was not correlated with $P_{50}$ ($P > 0.05$). However, both $P_{50}$ and $P_{80}$
were independent of other aspects of venation architecture and leaf morphology and
physiology, including leaf shape indices (length: width and perimeter²: area), the modulus of
elasticity, capacitances at full and zero turgor, saturated water content, leaf thickness and
density, and cuticular conductance (P > 0.05; Table S2).

The linkage of hydraulic vulnerability (P₅₀ and P₈₀) with major vein density was
independent from the relationships of hydraulic vulnerability to other leaf drought tolerance
traits. The linkage of P₅₀ or P₈₀ with major vein density was apparently more fundamental.
Thus, partialing out LMA, or osmotic potentials at full and zero turgor did not remove the
correlation of P₅₀ or P₈₀ with major vein density (rₚartial = 0.68 – 0.78; P < 0.05). However,
when partialing out the effect of major vein density on P₅₀ or P₈₀, their correlations with LMA
and with osmotic potentials at full and zero turgor were lost ([rₚartial] = 0.04– 0.29; P > 0.05).
Notably, the maximum leaf hydraulic conductance at full hydration did not correlate with any
venation architecture or morphological trait including leaf size, or leaf drought tolerance, for
this set of diverse leaves (P > 0.05).

Discussion

The importance of major vein density and leaf size in resistance to drought
We found novel, strong correlations of P₅₀ and P₈₀ with major vein density and leaf size,
across ten species with diverse leaves, consistent with findings from the computer model
simulations. All else being equal, leaf and whole-plant drought resistance would be conferred
by a higher major vein density, which is generally associated with small leaf size (Dunbar-Co
et al., 2009; McKown et al., 2010).

Such a role for venation and leaf size in determining hydraulic vulnerability has
important potential ecological and biogeographic implications. A link between leaf size and
P₅₀ and P₈₀ provides a new additional mechanism for the ecological distribution of leaf sizes.
Leaf size evolves relatively quickly via several independent genetic pathways (Ackerly,
2009; Gonzalez et al., 2010). Small leaves are more common in dry and exposed habitats, and
larger leaves in moister and/or shaded habitats (Dolph and Dilcher, 1980; Givnish, 1987;
Fonseca et al., 2000), and fossil leaf size is thus used as an indicator of past climate (Wilf,
1997). A direct hydraulic benefit of small leaves in drought tolerance, and the greater risk
associated with large leaves under dry conditions, should thus be considered in addition to
other demonstrated roles for leaf size in determining drought tolerance. A very well-
established benefit of smaller leaves in warmer environments is their thinner boundary layer
and more rapid convective cooling (Nobel, 1976; Nicotra et al., 2008; Vogel, 2009; Yates et
Another advantage of small leaves in exposed conditions is that more leaves can be packed into a smaller space to capture irradiance, though this benefit carries a greater cost in support mass; more branch allocation is needed to support many small leaves than for fewer larger leaves, and this outweighs the lower requirement for petiole and midrib support of smaller leaves (Bragg and Westoby, 2002; Niinemets et al., 2006, 2007). Future work needs to tease apart the importance of the direct hydraulic mechanism in providing an advantage in drought tolerance for smaller leaves from these other benefits across different species sets.

Is the relationship of hydraulic tolerance of dehydration to leaf size and higher major vein density necessarily causal? We considered the possibility that these relationships could be coincidental, i.e., that small leaf size and high major vein density on one hand, and more negative $P_{50}$ and $P_{80}$ on the other might be independently selected in drought-tolerant species. In our study, two lines of evidence supported a causal relationship. First, the relationship was established by the computer simulations of leaves with altered sizes and venation architecture, all else being held fixed. In these simulations, cavitation of the major veins as often observed during dehydration (see below), was better tolerated by smaller leaves with higher major vein density. Second, the partial correlation analyses of our experimental data showed that the relationship of venation architecture to leaf hydraulic vulnerability was independent of other drought tolerance traits, including turgor loss point ($\pi_{TLP}$). Indeed, $\pi_{TLP}$ is the most reliable single predictor of species’ drought tolerance to our knowledge (Auge et al., 1998; Sack et al., 2003b; Baltzer et al., 2008), and thus, if selection for drought tolerance were to result in a coincidental correlation of vulnerability and venation architecture, both should show underlying correlations with $\pi_{TLP}$. For our ten species, $P_{50}$ and $P_{80}$ were strongly correlated with $\pi_{TLP}$ (see also Crombie et al., 1985; Blackman et al., 2010), but venation architecture was unrelated to $\pi_{TLP}$. Further, $P_{50}$ and $P_{80}$ lost their relationship with $\pi_{TLP}$ after partialing out major vein density, whereas they remained correlated with major vein density even after partialing out $\pi_{TLP}$, indicating that the relationships of $P_{50}$ and $P_{80}$ to major vein density are more directly causal than any relationships with $\pi_{TLP}$ (Shipley, 2000).

We propose that the relationship between leaf size and hydraulic vulnerability in both simulated and real leaves supports a general mechanism, to be tested in other species sets, including closely-related species within lineages in a phylogenetic context, because smaller leaves have evolved reliably in drier habitats (Ackerly et al., 2002; McDonald et al., 2003; Dunbar-Co et al., 2009; Santiago and Kim, 2009). In our modeled leaves and experimental species set, as across species in general, leaf size and major vein density were linked.
The potential roles of venation in determining \( K_{\text{leaf}} \) decline

The linkage of hydraulic vulnerability with venation architecture shown here would be expected because of the reduction of xylem conductivity, due to cavitation or collapse of conduits in the vein xylem, as previously shown by dye experiments, cryo scanning electron microscopy (cryoSEM), and acoustic methods (e.g., Kikuta et al., 1997; Salleo et al., 2000; Nardini and Salleo, 2003; Cochard et al., 2004a; Brodribb and Holbrook, 2005; Johnson et al., 2009a). Notably, other factors besides vein density can determine leaf hydraulic vulnerability, which is a higher-level trait influenced by multiple lower-level traits (cf. Marks and Lechowicz, 2006; McKown et al., 2010). Thus, species may additionally differ in the air-seeding thresholds of xylem conduits, and in the responses of extra-vascular tissues to dehydration. Some small-leaved species in moist habitats might not have the low vulnerability suggested by their leaf size, if other factors were to over-ride the benefit of high major vein density. However, our findings from simulated leaves and from our ten species diverse in drought tolerance indicated a strong potential role of major vein density and leaf size in determining \( P_{50} \) and \( P_{80} \) across diverse species.
the major veins would cause a greater decrease in $K_x$ and $K_{\text{leaf}}$ (Table 1). The model indicated that a higher major vein density is more effective for reducing hydraulic vulnerability due to major vein cavitation than a lower minor vein density. The model showed that reducing minor vein density only led to a strong reduction in PLC (i.e., to a strong gain in tolerance) at high major vein density. Further, a higher major vein density also increases maximum leaf hydraulic conductance for well-hydrated leaves ($K_{\text{max}}$), both in absolute terms, and relative to vein construction cost, all else being equal, whereas a lower minor vein density leads to losses of $K_{\text{max}}$ (McKown et al., 2010). Thus, for leaves with high major vein density, a low minor vein density might be a mechanism to achieve additional drought tolerance all else being equal, but at the cost of maximum hydraulic capacity and providing no gain in absolute conductance when the major veins are cavitated. The mechanism of achieving higher major vein density with small leaf sizes was found in the model simulations and experimental study to provide a strong benefit in reducing vulnerability and thus for drought tolerance.

Notably, the model findings indicated that leaves with higher major vein density, though less sensitive to cavitation of the major veins, were more sensitive to cavitation of the minor vein system. Thus, our empirical findings, of reduced vulnerability in leaves with higher major vein density suggested that major vein cavitation was more important than minor vein cavitation in driving loss of $K_x$ and $K_{\text{leaf}}$. There are four lines of evidence that support the greater probability of cavitation in the major than minor veins. First, the major veins have wide and long xylem conduits especially vulnerable to air seeding (Choat et al., 2005). Second, in naturally dehydrated leaves, embolism is readily observed by cryo-scanning electron microscopy (cryoSEM) of major veins (Ball et al., 2004, 2006; Marenco et al., 2006; Johnson et al., 2009a), and acoustic studies have indicated cavitation in the major veins at $\Psi_{\text{leaf}}$ values as high as -0.3 MPa (Crombie et al., 1985; Kikuta et al., 1997; Salleo et al., 2000; Johnson et al., 2009a). By contrast, the cryoSEM studies published thus far have not shown cavitation of minor vein conduits (Canny, 2001). Studies of dye uptake into transpiring leaves did show less staining of minor veins in dehydrated leaves (Salleo et al., 2001; Nardini et al., 2003; Trifilo et al., 2003b); these findings are consistent with cavitation occurring principally in major veins, blocking uptake to the minor veins. Third, leaves that did not render their minor vein system resistant to cavitation would tend to be extremely sensitive to decline during drought, regardless of their venation architecture. The model simulations showed that cavitation of minor veins led to drastic decline in $K_x$ across leaves of all venation architectures. As previously argued by Brodribb & Holbrook (2006), it seems improbable that leaves would invest in a fine vein network that becomes embolized at high
water potentials and thus decline strongly in function. Fourth, a study of diverse angiosperms found that the leaf \( P_{50} \) was more negative in species with thicker-walled conduits in their minor veins (Blackman et al., 2010). That finding suggested that conduits are built to resist collapse at the tensions experienced during strong dehydration. In wood, such investment to avoid collapse signifies that the xylem can withstand cavitation to close to that degree of tension, as cavitation precedes collapse (Hacke and Sperry, 2001; Hacke et al., 2001; Blackman et al., 2010). Thus, minor vein conduits too should resist both cavitation and collapse at high levels of dehydration.

The collapse of xylem conduits during leaf dehydration cannot be entirely excluded. Collapse of conduits in the major or minor veins has never yet been shown for angiosperms but has been found in conifer needles dehydrated to water potentials ranging -1.5 to -3.5 MPa (Cochard et al., 2004a; Brodribb and Holbrook, 2005). Future studies are needed to analyze in detail the progression of cavitation and collapse in veins of different orders during leaf dehydration. Such work will also need to consider other aspects of the structure of the vein system, e.g., vessel widths, lengths and the degree that conduits span across vein orders, as these factors have been found to have great importance in stem vulnerability (Sperry, 2003; Sperry et al., 2005), and vary greatly across species (Sack and Frole, 2006). These aspects may contribute to the correlation of vulnerability with low major vein density, because the major veins have especially long and wide vessels that span multiple orders. Other aspects of leaf vein arrangement, in addition to vein density, such as looping in the major veins might also influence resistance to hydraulic decline (Corson, 2010; Katifori et al., 2010).

While this discussion has focused on the decline of \( K_{\text{leaf}} \) with dehydration that is driven by declines in the xylem pathway conductivity, i.e., in \( K_s \), there is also a potential role for declines in \( K_{ox} \) in the correlation of \( P_{50} \) with major vein density and leaf size. The \( K_{ox} \) may decline in dehydrating leaves due to changes in the permeability of membranes (Sack and Holbrook, 2006). Notably, given that a high major vein density would entail a large bundle sheath area by which water exits the major veins, if the lamina near the major veins accounts for a large part of transpiration (Fricke, 2002; Sack et al., 2002; Nardini et al., 2010) then leaves with high major vein density would likely maintain a greater bundle sheath area, and a greater \( K_{ox} \) when cells lose turgor in dehydrating leaves (Kim and Steudle, 2007). Additionally, we note that as leaves dehydrate, it is possible that \( K_{\text{leaf}} \), the bulk leaf parameter, may not well describe the water transport pathways, if sectors or “patches” of lamina become isolated, each with their own hydraulic supply (cf. Barbour and Farquhar, 2004). Leaves with high major vein density may better provide for access of isolated leaf
sectors to the lower-order vein distribution system. Future studies are needed of the impact of dehydration on the bundle sheath and mesophyll tissues, and on the potential heterogeneity of water supply in dehydrating leaves. Detailed characterization of the causes of $K_{\text{leaf}}$ decline and its dependence on xylem and mesophyll characters will contribute to an ability to predict variation in species’ drought responses from cell and tissue-level properties.

**Relationship of leaf vulnerability to leaf and whole-plant drought tolerance**

Our results also highlighted the importance of cell properties and leaf morphology in drought tolerance. Species from dry habitats had higher leaf mass per area (LMA) and modulus of elasticity, more negative osmotic potentials at full and zero turgor ($\pi_{\text{TLP}}$) and lower cuticular conductance than species from moist habitats, which by contrast had higher saturated water content (SWC) and capacitance. Further, the $\pi_{\text{TLP}}$ correlated with $P_{50}$ and $P_{80}$ as shown in two previous studies (Crombie et al., 1985; Blackman et al., 2010). Given that stomata tend to close near the $\pi_{\text{TLP}}$ (Hao et al., 2010), this linkage points to a control of stomatal aperture during drought by hydraulic vulnerability. The linkage might arise mechanistically, if a low osmotic potential in leaf tissues, reflected by $\pi_{\text{TLP}}$, acted to reduce turgor loss and decline in membrane permeability (Canny and Huang, 2006). Alternatively, the $\pi_{\text{TLP}}$ might be co-selected with $P_{50}$ and $P_{80}$ in drought tolerant leaves such that stomatal closure precedes hydraulic dysfunction. Our results were most consistent with this second possibility. In the partial correlation analysis, $\pi_{\text{TLP}}$ had no impact on $P_{50}$ and $P_{80}$ when major vein density was partialed out. Thus, as found in the model simulations, leaf size and major vein density are putative causes of high $P_{50}$ and $P_{80}$, whereas $\pi_{\text{TLP}}$ is apparently a structurally independent but coordinated trait that modulates the leaf and plant response to drought.

The finding that major vein density and small leaf size reduce leaf hydraulic vulnerability points to potential roles in determining whole-plant drought tolerance. These traits may be especially important because the leaf is a key locus in whole-plant vulnerability, with typically greater hydraulic sensitivity than stems and roots (Hao et al., 2008; Brodribb and Cochard, 2009; domec et al., 2009). Thus, for three of the four chaparral species in this study, the leaf $P_{50}$ was 2.7 to 4.7 MPa lower than that previously reported for stems (Jacobsen et al., 2007), with only *Q. arbutifolia* having stems apparently similar to leaves in their vulnerability (-2 versus -2.4 MPa respectively). The importance of leaf hydraulic vulnerability in whole-plant drought tolerance was further supported in this study both by the more negative $P_{50}$ and $P_{80}$ values for species of dry than moist habitats, and by the general correlation of these traits with others related to leaf drought tolerance. It is important to
recognize, however, that there can be other possible routes to leaf drought tolerance than a low hydraulic vulnerability and thus being able to maintain hydraulic and photosynthetic function during drought. Thus, some species can achieve substantial drought tolerance via a low $g_{\text{min}}$ and water storage, with an extreme development of this mechanism in succulent plants (Ogburn and Edwards, 2009). In this study, *Hedera canariensis* showed these mechanisms (Sack et al., 2003c; Metcalfe, 2005) possibly explaining its relatively high $K_{\text{leaf}}$ vulnerability for a species that can tolerate drought. Other species can achieve drought tolerance via an ability to tolerate low tissue water potentials via dehydrin expression that prevents mechanical failure of the cell walls (e.g., the resurrection fern *Polypodium polypodioide*; Layton et al., 2010). Species with these alternative mechanisms to maintaining hydraulic function can achieve large leaf sizes even given dry conditions (Nobel and Jordan, 1983). Further, we note that drought tolerance achieved at the level of the whole plant may not always correspond to leaf-level drought tolerance. Some species with drought-sensitive leaves can tolerate dry soil by shedding leaves or achieving deep roots, as is the case for *Lantana camara* (Castillo et al., 2007), which had relatively high $K_{\text{leaf}}$ vulnerability. By contrast, some species with drought-tolerant leaves may be sensitive to drought in the field, due to relatively shallow roots (e.g., *Magnolia grandiflora*; Klos et al., 2009). Future work will establish the degree that even despite such complexity, key leaf traits such as major vein density, leaf size, and $P_{50}$ and $P_{80}$ contribute to drought tolerance. This work has strong potential to explain leaf function during drought from cell, tissue and organ-level physiological properties, and to predict the drought tolerance of diverse species and landscapes in current and extinct vegetation from their leaf traits.

### Materials and Methods

*Computer simulations of the importance of vein architecture in leaf hydraulic vulnerability*

Simulations of the impact of cavitation in leaves with varying venation architecture were generated using the program $K_{\text{leaf}}$, version 6 (written by H. Cochard, Institut National de la Recherche Agronomique, Clermont-Ferrand, France; Cochard et al., 2004b; McKown et al., 2010; available on request, Herve.Cochard@clermont.inra.fr). Based on specified parameters, $K_{\text{leaf}}$ creates a spatially explicit model of a leaf with up to six vein orders represented as a square grid of xylem resistors and outside-xylem resistors (“mesophyll” resistors) branching orthogonally from each junction of the vein grid. In modeled leaves, water exits through the mesophyll resistor located at each vein junction, and the bulk of the water thus exits from the
numerous minor veins. The model determines three parameters, $K_x$ (leaf xylem conductance per leaf area), $K_{ox}$ (outside-xylem conductance per leaf area), and $K_{leaf}$, where:

$$K_{leaf} = \left( \frac{1}{K_x} + \frac{1}{K_{ox}} \right)^{-1}$$

EQN 1

for leaves given specified size, densities and cross-sectional conductivities for each vein order, and mesophyll conductance. Simulations were modeled using an elliptical leaf with 12 pairs of second-order veins ($2^o$ veins) and with vein densities and conductivities based on those for a *Juglans regia* terminal leaflet (Cochard et al. 2004). Our findings should be applicable to other leaves with hierarchical, reticulate venation (McKown et al., 2010). Individual vein conductivities ($k_v$) were based on estimations from xylem conduit lumen dimensions in *Juglans* vein cross-sections using the formula:

$$k_v = \sum \left( \frac{\pi a b}{6 \eta L} \right)$$

EQN 2

where $a$ and $b$ are the major and minor axes of ellipses, and $\eta$ is the viscosity of water at 25°C (units are mmol m s$^{-1}$ MPa$^{-1}$; Lewis and Roose, 1995; Cochard et al., 2004b; Sack and Frole, 2006). The $K_x$ calculated by the model depends on the individual vein order conductivities and densities, and $K_{ox}$ depends on the specified mesophyll conductance and the total vein density, which determines the number of junctions and thus of mesophyll resistors in parallel.

Values of $K_x$, $K_{ox}$, and $K_{leaf}$ were determined in typical units, normalized by leaf area (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$). The relative responses of $K_x$, $K_{ox}$ and $K_{leaf}$ to alteration of venation features in our simulations are expected to accurately indicate relative trends and principles of leaf venation design; however, the empirical values are not to be taken as meaningful, and units are not presented in our simulation results. For instance, the simulations based on the *Juglans* leaflet anatomical data set produced a $K_x$ of 462 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$, which is very high relative to experimentally measured $K_x$ and many times greater than measured $K_{ox}$ (Cochard et al., 2004b). Cochard et al. (2004b) introduced the “xylem hydraulic efficiency” parameter in $K_{leaf}$ (XHE; modeled $K_x$ divided by measured $K_x$) to calibrate the modeled $K_x$ with measured values and thus to account for other factors than xylem conduit numbers and diameters that cannot currently be modeled, such as pit membrane resistance (Sperry et al., 2005) or conduit blockage by embolism or tyloses (Salleo et al., 2002; Choat et al., 2005). In our simulations, XHE was set to 1. While not significant for this study, future work should better reconcile modeled $K_x$ with experimentally measured values (McKown et al., 2010).

For this study, we focused on the impacts of simulated cavitation on $K_x$ values, which would result in a reduction of $K_{leaf}$ by a degree that depends on the value of $K_x$ relative to $K_{ox}$. The ratio of $K_{ox}$ and $K_x$ depends on species and on environmental variables that affect
these compartments differently, but the available data suggest that $K_{ox}$ and $K_x$ are of similar magnitudes on average (Sack and Holbrook, 2006), and in that case, a given decline of $K_x$ would reduce $K_{leaf}$ by approximately half that amount. We also note that dehydration could also impact on the extra-xylem pathways, e.g., due to cell shrinkage and/or aquaporin deactivation (Kim and Steudle, 2007), which would lead to stronger overall impacts on $K_{leaf}$.

The program generates leaves of a specified size and number and arrangement of 2° veins, from which it determines the 2° vein density. Thus, designating leaves of given sizes leads to the 2° veins being spaced further apart, just as observed in real leaves (Sack et al., 2008; see Results). The density of the minor veins (in this model, 3° and higher) depends on the areole size, which is specified independently, and thus is manipulated independently of major vein density. We altered leaf size (and thus the density of 1° and 2° veins) and also minor vein densities, simulating a total of 42 leaves of six different sizes (4.8 to 65.4 cm²), associated with a 3.5-fold range of major vein densities (0.15-0.53 mm mm⁻²) × seven different minor vein densities (spanning a 3.5-fold range; 2.8-8.7 mm mm⁻²). For each leaf, we additionally applied cavitation “treatments” to determine the impact on $K_x$: (1) To test the impact of cavitating the major veins, we reduced the cross-sectional conductivities of the 1° and 2° veins by 90%, to simulate the great majority of vessels being cavitated; and (2) To test the impact of cavitating the minor veins, we reduced the cross-sectional conductivities of the 3°, 4°, 5° and 6° veins by 90%. For the simulated leaves of contrasting venation architecture, we present the percent loss of conductance of $K_x$ that resulted from these treatments, i.e., the decline relative to a control, uncavitated leaf.

Plant material and leaf hydraulic vulnerability

Leaf hydraulic vulnerability was determined for ten species sampled in and around the campus of University of California, Los Angeles and Will Rogers State Park, Los Angeles, California in May-September 2008 (Table 1). Leaves were collected from mature trees and shrubs of nine species. Leaves from sunflowers (Helianthus annuus, var. Sunspot; Botanical Interests, Colorado, USA) were collected from greenhouse plants grown from seeds in 3.6 L pots (average minimum, mean and maximum values for temperature: 21.1, 23.2 and 26.0°C; for humidity: 44, 51 and 59%). Sunflowers were irrigated every two days, with 200-250 ppm of 20:20:20 N:P:K; the light availability measured at mid-day on a sunny day was up to 550 µmol photon · m⁻² · s⁻¹, and on average 300 µmol photon · m⁻² · s⁻¹ (LI-250 light meter; LI-COR Biosciences, Lincoln, Nebraska, USA). Species spanning a wide range of drought
sensitivity were selected across nine families to include phylogenetic diversity. Five species were native to dry habitats and five species to moist habitats (Table 2).

Mature, healthy leaves were excised from sun-exposed branches rehydrated overnight. We used the evaporative flux method (EFM) to determine leaf vulnerability curves. We measured $K_{\text{leaf}}$ as the light-acclimated steady-state transpirational flow rate for excised leaves ($E$, mmol·m$^{-2}·$s$^{-1}$) divided by the water potential driving force ($\Delta \Psi_{\text{leaf}}$, MPa; Sack et al., 2002; Scoffoni et al., 2008). The EFM was modified to allow determination of $K_{\text{leaf}}$ at low $\Psi_{\text{leaf}}$. Shoots were cut into segments of three or more leaves under ultrapure water, and dehydrated with a fan for different periods of time to achieve a range of $\Psi_{\text{leaf}}$ values. Shoots were allowed to equilibrate for at least 30 min and then two leaves were excised and measured for initial $\Psi_{\text{leaf}}$ ($\Psi_0$) using a pressure chamber (Plant Moisture Stress Model 1000; PMS Instrument Co, Albany, OR, USA). The third leaf (typically the middle leaf) was used to determine $K_{\text{leaf}}$ with the EFM. The vulnerability curve was obtained by plotting $K_{\text{leaf}}$ against whichever was lowest, $\Psi_0$ or $\Psi_{\text{final}}$ (“$\Psi_{\text{lowest}}$”), assuming this to be the $\Psi_{\text{leaf}}$ associated with the strongest dehydration experienced during the experiment (bench-drying and $K_{\text{leaf}}$ measurement). Species show different shapes in their vulnerability curve trajectories, as expected given variation in the importance of multiple mechanisms for the decline of $K_{\text{leaf}}$ with dehydration (Brodribb and Holbrook, 2006). Thus, we determined each species’ vulnerability curve, selecting among four functions used in the previous literature using maximum likelihood (Burnham and Anderson, 2002), using the optim function in R 2.9.2 (http://www.r-project.org; Burnham and Anderson, 2004; Sack et al., 2006; our scripts are available on request): linear ($K_{\text{leaf}} = a \Psi_{\text{leaf}} + \gamma_0$), sigmoidal ($K_{\text{leaf}} = \frac{a}{1+e^{-(\Psi_{\text{leaf}}-b)}}$), logistic ($K_{\text{leaf}} = a/(1 + (\Psi_{\text{leaf}}/x_0)^b)$) and exponential ($K_{\text{leaf}} = \gamma_0 + ae^{-b\Psi_{\text{leaf}}}$). We used the best fit function for each species to estimate the maximum $K_{\text{leaf}}$ for the hydrated leaf ($K_{\text{max}}$), and the $\Psi_{\text{leaf}}$ at 50% and 80% loss of $K_{\text{leaf}}$ ($P_{50}$ and $P_{80}$ respectively).

Quantification of leaf form and venation architecture

We determined venation traits for leaves from one leaf from an exposed branch for three individuals per species, from the same individuals measured for hydraulic vulnerability. Leaves were collected in May-September 2007 and fixed in formalin-acetic-acid solution (37% aqueous formaldehyde solution, 50% ethanol and 13% glacial acetic acid solution). Leaves were chemically cleared with 5% NaOH in ethanol, stained with safranin and...
counterstained with fast-green (Berlyn and Miksche, 1976). Leaves were mounted with water in transparency film (CG5000, 3M Visual Systems Division, Austin, TX, USA) and scanned (flatbed scanner; Canon Scan Lide 90; 1200 pixels/inch). The leaf area, length, width, perimeter, and numbers and lengths of 1° and 2° veins were measured using Image J software, v. 1.42q (U. S. National Institutes of Health, Bethesda, Maryland, USA). Two indices of leaf shape were calculated: the length: width ratio and the \( \text{perimeter}^2: \text{area} \) ratio (a size-independent index of edge relative to size; Sack et al., 2003b). The 3° vein lengths were measured for three rectangles per leaf (10 to 300 mm², depending on leaf size), located centrally in the top, middle and bottom thirds of the leaf. For each vein order, the vein density was calculated as length divided by leaf area; for 3° veins, the vein densities were averaged across the three subsampled rectangles. Vein diameters, excluding the bundle sheath, were measured for each vein order by averaging six measurements (two made centrally in veins in the top, middle and bottom thirds of the leaf).

Measurements of the minor vein system were made under a light microscope (DMRB Leica Microsystems, Germany) with a 5× or 10× objective and digital camera (14.2 Color Mosaic, DIAGNOSTIC Instruments Inc., ENG0950, USA). Three rectangles were imaged (areas of 1.5 mm² or 6 mm²) centrally in the top, middle and bottom thirds of the leaf, and the number of vein orders, density of minor veins (length per area) and the number of free vein endings per area, and vein diameters measured centrally in six segments were averaged across the rectangles. The major vein density was determined as the sum of 1°, 2° and 3° vein densities and the minor vein density as the sum for 4° and higher order veins. The ratio of major to minor vein density was calculated for each leaf for every species.

**Quantifying other key traits related to leaf drought tolerance**

Morphological and physiological traits related to leaf drought tolerance were measured for six leaves from each of three to six individuals per species. Measurements were made of leaf area and of dry mass after oven-drying at least 48h at > 70°C to allow calculation of leaf mass per area (LMA, g · m⁻²). Leaf thickness was determined using digital calipers (Fowler, Chicago, IL), and leaf density was calculated as LMA divided by leaf thickness (Witkowski and Lamont, 1991). Cuticular conductance (\( g_{\text{min}} \)), the minimum conductance to vapor diffusion across the epidermis when the stomata are closed, was measured by weighing leaves as they dehydrated (Sack et al., 2003b; Sack et al., 2010). Parameters were determined from pressure-volume curves constructed by measuring leaf water potential and relative water content as leaves dehydrated (Tyree and Hammel, 1972; Sack et al., 2003b), including
osmotic potential at full turgor ($\pi_o$, MPa) and at turgor loss point ($\pi_{TLP}$, MPa), saturated water content (SWC, g · g$^{-1}$), modulus of elasticity ($\varepsilon$, MPa), and capacitances at full turgor and at turgor loss point ($C_{FT}$ and $C_{TLP}$, MPa$^{-1}$; Sack and PrometheusWiki, 2010).

Statistical analysis of trait differences and correlations across species

To test trait differences between moist and dry habitat species (Table 2 and Table S1), we performed ANOVAs with species nested within habitat type (Minitab Release 15). Prior to tests, data were log-transformed to improve normality and heteroscedasticity (Sokal and Rohlf, 1995). We performed $t$-tests for leaf density, maximum $K_{leaf}$, $P_{50}$ and $P_{90}$ where only species mean values were available (Table S1).

A correlation matrix was determined to reveal the inter-correlative structure of hydraulic parameters, leaf size, venation architecture, and other traits putatively related to drought tolerance. For a conservative estimation, correlations were considered significant only if $P < 0.05$ for both Spearman and Pearson coefficients ($r_s$ and $r_p$ respectively). Because many relationships were non-linear, we determined Pearson correlations for both raw and log-transformed data.

When three variables of interest were inter-correlated across species, we performed partial correlation analysis (Sokal and Rohlf, 1995), testing the relationship between two variables holding the third variable constant (corpcor package; R; Schaefer et al., 2007).

Acknowledgments

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Table 1. Results of computer model simulations of the percentage loss of whole-leaf xylem hydraulic conductance (PLC of $K_x$) after reducing (a) major and (b) minor vein conductivities to simulate cavitation, in realistic leaves varying in major or minor vein density.

<table>
<thead>
<tr>
<th>Leaf simulation</th>
<th>(a) Cavitation in major veins</th>
<th>(b) Cavitation in minor veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher major vein density</td>
<td>Lower $K_x$ decline</td>
<td>Greater $K_x$ decline</td>
</tr>
<tr>
<td>Higher minor vein density</td>
<td>Greater $K_x$ decline</td>
<td>Lower $K_x$ decline</td>
</tr>
<tr>
<td>Higher major : minor vein density</td>
<td>Lower $K_x$ decline</td>
<td>Greater $K_x$ decline</td>
</tr>
</tbody>
</table>

*Note:* Contrasting impacts were found for the impacts of higher major and minor vein densities on the PLC of $K_x$ due to vein cavitation, and contrasting impacts were found when cavitating major or minor veins. These effects can be understood in terms of the relative leverage of major or minor veins on the overall vein system. When the major vein density is increased, its greater redundancy gives the minor vein system a greater leverage; thus, the leaf is less sensitive to cavitation in the major veins and more sensitive to cavitation in the minor veins. By contrast, when the minor vein density is increased, its greater redundancy gives the major vein system a greater leverage; thus, the leaf is more sensitive to cavitation in the major veins and less sensitive to cavitation in the minor veins.
Table 2. Species, family, native range, and mean values ± standard error for morphological and physiological traits, and results of analyses of variance for the difference between moist and dry habitat species and among species nested within those categories. ***$P < 0.001$; NS, $P > 0.05$

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Native range</th>
<th>Leaf area (cm²)</th>
<th>Leaf mass per area (g.m⁻²)</th>
<th>Major vein density (mm⁻¹)</th>
<th>Minor vein density (mm⁻¹)</th>
<th>Ratio major : minor vein density</th>
<th>Cuticular conductance (mmol · m⁻² · s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry habitat species :</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cercocarpus betuloides</em></td>
<td>Rosaceae</td>
<td>California. Mexico</td>
<td>7.04 ± 1.73</td>
<td>156 ± 19.9</td>
<td>1.40 ± 0.14</td>
<td>7.74 ± 0.76</td>
<td>0.19 ± 0.03</td>
<td>3.99 ± 0.41</td>
</tr>
<tr>
<td><em>Comarostaphylis diversifolia</em></td>
<td>Ericaceae</td>
<td>California. Mexico</td>
<td>7.93 ± 1.89</td>
<td>254 ± 7.73</td>
<td>1.57 ± 0.18</td>
<td>4.17 ± 0.18</td>
<td>0.38 ± 0.04</td>
<td>2.87 ± 0.35</td>
</tr>
<tr>
<td><em>Hedera canariensis</em></td>
<td>Araliaceae</td>
<td>Canary Islands</td>
<td>53.2 ± 14.4</td>
<td>78.1 ± 6.32</td>
<td>0.53 ± 0.06</td>
<td>3.00 ± 0.10</td>
<td>0.18 ± 0.01</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td><em>Heteromeles arbutifolia</em></td>
<td>Rosaceae</td>
<td>California. Mexico</td>
<td>14.6 ± 2.89</td>
<td>146 ± 13.2</td>
<td>0.88 ± 0.04</td>
<td>4.63 ± 0.11</td>
<td>0.19 ± 0.005</td>
<td>4.21 ± 1.22</td>
</tr>
<tr>
<td><em>Quercus agrifolia</em></td>
<td>Fagaceae</td>
<td>California. Mexico</td>
<td>13.5 ± 1.32</td>
<td>166 ± 7.64</td>
<td>1.07 ± 0.07</td>
<td>7.30 ± 0.23</td>
<td>0.15 ± 0.02</td>
<td>1.72 ± 0.23</td>
</tr>
<tr>
<td>Moist habitat species :</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Camellia sasanqua</em></td>
<td>Theaceae</td>
<td>Japan</td>
<td>11.1 ± 0.45</td>
<td>144 ± 13.4</td>
<td>0.78 ± 0.04</td>
<td>3.31 ± 0.26</td>
<td>0.24 ± 0.03</td>
<td>1.77 ± 0.13</td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>Asteraceae</td>
<td>Across N. America</td>
<td>44.3 ± 1.64</td>
<td>56.2 ± 6.98</td>
<td>0.48 ± 0.03</td>
<td>9.32 ± 0.44</td>
<td>0.05 ± 0.002</td>
<td>18.3 ± 1.92</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>Verbenaceae</td>
<td>Pantropical</td>
<td>12.8 ± 3.09</td>
<td>79.0 ± 4.48</td>
<td>0.97 ± 0.12</td>
<td>9.75 ± 0.40</td>
<td>0.11 ± 0.02</td>
<td>12.0 ± 0.85</td>
</tr>
<tr>
<td><em>Magnolia grandiflora</em></td>
<td>Magnoliaceae</td>
<td>Southern U.S.</td>
<td>69.5 ± 5.51</td>
<td>180 ± 17.3</td>
<td>0.48 ± 0.02</td>
<td>5.16 ± 0.29</td>
<td>0.09 ± 0.003</td>
<td>3.88 ± 0.41</td>
</tr>
<tr>
<td><em>Platanus racemosa</em></td>
<td>Platanaceae</td>
<td>California. Mexico</td>
<td>80.9 ± 2.79</td>
<td>109 ± 6.54</td>
<td>0.40 ± 0.06</td>
<td>4.97 ± 0.14</td>
<td>0.08 ± 0.01</td>
<td>6.61 ± 0.41</td>
</tr>
<tr>
<td>Average trait values</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dry area species</td>
<td></td>
<td></td>
<td>19.2 ± 4.44</td>
<td>162 ± 11</td>
<td>1.09 ± 0.09</td>
<td>5.18 ± 0.35</td>
<td>0.22 ± 0.04</td>
<td>2.65 ± 0.45</td>
</tr>
<tr>
<td>Moist area species</td>
<td></td>
<td></td>
<td>43.7 ± 14.3</td>
<td>113 ± 9.75</td>
<td>0.62 ± 0.05</td>
<td>6.23 ± 0.33</td>
<td>0.12 ± 0.03</td>
<td>8.44 ± 0.82</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dry / moist Species</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

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Supplemental Data

**Supplemental Table S1.** Species means ± standard errors for 24 morphological, anatomical and physiological traits and results of analyses of variance testing for species differences, and for differences between moist and dry habitat species. ***P < 0.001; **P < 0.01; *P < 0.05; NS, P > 0.05.

**Supplemental Table S2.** Correlation matrix for the relationship of leaf hydraulic vulnerability traits with venation architecture and other traits related to leaf morphology and drought tolerance. For each correlation the Spearman coefficient is presented, and the Pearson coefficient calculated with untransformed data and log-transformed data. Correlations are highlighted as significant only when Spearman and Pearson coefficients are both significant. ***P < 0.001; **P < 0.01; *P < 0.05.
**Figure 1.** Results of computer model simulations of the percentage loss of whole-leaf xylem hydraulic conductance (PLC of $K_x$) after reducing by 90% (A) major and (B) minor vein conductivities to simulate cavitation for realistic leaves varying in major or minor vein density ($n = 42$ simulated leaves; see Materials and Methods). (C) The dependence of PLC of $K_x$ due to cavitation of major (grey) and minor (black) veins on the ratio of major: minor vein density.

**Figure 2.** The scaling of vein density with leaf size for ten species varying strongly in drought tolerance. (A) Major vein density versus leaf area. (B) The independence of minor vein density with leaf size. Symbols: grey, dry habitat species; white, moist habitat species. Fitted regression in (A): Major vein density = $0.32 \times \text{Leaf area}^{0.53}$. ***$P < 0.001$; NS $P > 0.05$.

**Figure 3.** Relation of the vulnerability of leaf hydraulic conductance to major and minor vein densities for ten species varying strongly in drought tolerance. Vulnerability curves are plotted in the left column; each point represents a different measured leaf ($n = 26$-74 per curve). For each vulnerability curve the fitted line is the maximum likelihood function for given species (linear for *C. sasanqua*, *C. diversifolia*, *Q. agrifolia*, and *H. arbutifolia*, logistic for *M. grandiflora*, *P. racemosa*, *H. annuus*, *H. canariensis* and *L. camara* and sigmoidal for *C. betuloides*; $R^2 = 0.39$-0.89; $P < 0.001$; see Materials and Methods) and the vertical line represents the water potential at 80% loss of conductivity ($P_{80}$). Leaf schematics are drawn to scale, with major veins (first- and second-order veins). Micrographs of the minor vein architecture are represented on the right, indicating the independence of minor vein density from leaf size; in each image, the largest vein at the top is a second-order vein, with third-order veins branching off, and the minor veins make up the rest of the network.

**Figure 4.** Dependence of leaf hydraulic vulnerability, quantified as the water potential at 80% loss of conductivity ($P_{80}$) on (A) major vein density and (B) leaf area. Symbols: grey,
dry habitat species; white, moist habitat species. Fitted regressions: (A) $P_{80} = 4.2 \times \text{Major vein density} - 0.92$; (B) $P_{80} = 28.2 \times \text{Leaf area}^{-0.84}$. 
A  Cavitation in the major veins

B  Cavitation in the minor veins

C  Cavitation in the major veins
   Cavitation in the minor veins

Loss of whole leaf xylem conductance (%)

Major vein density (mm·mm⁻²)

Minor vein density (mm·mm⁻²)

Major/minor vein density

0.00 0.05 0.10 0.15 0.20 0.25
**A**

Major vein density (mm/mm²)

- \( r_p = -0.95^{***} \)
- \( r_s = -0.89^{***} \)

**B**

Minor vein density (mm/mm²)

- \( r_p = -0.2^{NS} \)

Leaf area (cm²)
A

$P_{80}$ (MPa)

$r_p = 0.85^{**}$

$r_s = 0.83^{**}$

Major vein density (mm·mm$^{-2}$)

B

$P_{80}$ (MPa)

$r_p = -0.85^{**}$

$r_s = -0.78^{**}$

Leaf area (cm$^2$)