Journal of Experimental Botany, Vol. 68, No. 16 pp. 4479–4496, 2017
doi:10.1093/jxb/erx252 Advance Access publication 10 August 2017

DARWIN REVIEW

The causes and consequences of leaf hydraulic decline with dehydration

Christine Scoffoni* and Lawren Sack

Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095, USA

* Correspondence: cscoffoni@ucla.edu

Received 28 February 2017; Editorial decision 26 June 2017; Accepted 17 July 2017

Editor: Donald Ort, University of Illinois

Abstract

Resolving the drivers of hydraulic decline during drought is crucial for understanding drought tolerance in crops and natural ecosystems. In the past 15 years, studies of the decline of leaf hydraulic conductance (K_{leaf}) have supported a major role in controlling plant drought responses. We analyzed the variation in K_{leaf} decline with dehydration in a global database of 310 species, providing novel insights into its underlying mechanisms, its co-ordination with stem hydraulics, its influence on gas exchange and drought tolerance, and its linkage with species ecological distributions. K_{leaf} vulnerability varied strongly within and across lineages, growth forms, and biomes. A critical literature review indicates that changes in hydraulic conductance outside the xylem with dehydration drive the overall decline of K_{leaf}. We demonstrate a significant leaf hydraulic safety–efficiency trade-off across angiosperm species and discuss the importance of the large variation around this trend. Leaves tend to be more vulnerable than stems, with their vulnerabilities co-ordinated across species, and importantly linked with adaptation across biomes. We hypothesize a novel framework to explain diversity across species in the co-ordination of K_{leaf} and gas exchange during dehydration. These findings reflect considerable recent progress, yet new tools for measurement, visualization, and modeling will result in ongoing discoveries important across fields in plant biology.

Key words: Aquaporins, hydraulic segmentation, stem hydraulics, turgor loss point, vein density, water relations.

Introduction

Decline in hydraulic conductance with water stress occurs in all plant organs: dehydrating roots, stems, and leaves all experience to varying degrees a drop in their efficiency to conduct water. The 'vulnerability' of hydraulic conductance in each organ to water stress has important consequences as plant growth depends on the maintenance of an adequate leaf water supply. When stomata open to allow CO_2 capture for photosynthesis, water evaporates, generating high surface tensions in the mesophyll cell walls, pulling water from the soil through the roots and branches to the sites of evaporation to replace the water lost. As the soil dries, tensions in the soil and throughout the plant increase considerably, and the capacity for water movement (i.e. the hydraulic conductance, defined as the ratio of flow rate to water potential gradient driving force) will decline, reducing photosynthesis and plant growth, and eventually leading to mortality (Choat *et al.*, 2012; Brodribb *et al.*, 2015; Moshelion *et al.*, 2015). The bulk of early studies of plant water transport investigated how much tension plants could experience in their stem xylem before hydraulic failure (e.g. Peirce, 1936; Crafts, 1939; Temperley, 1947) and focused mainly on the physics of water transport. As the field of plant hydraulics shifted towards eco-physiological questions, leaf-level hydraulics gained considerable attention. Early studies on leaf hydraulic conductance (K_{leaf}) focused especially on drought-induced xylem embolism in leaf petioles (Milburn and Johnson, 1966; Crombie *et al.*, 1985). More recently, the development of laboratory techniques reproducing and measuring the flow of water through the leaf transpirational pathways, including pathways in the xylem and outside the xylem, provided insight into determinants of K_{leaf} and its responses to environmental factors (Sack and Holbrook, 2006). The last 15 years have seen a dramatic growth in the number of studies of K_{leaf} decline with dehydration, with an increasing recognition that leaves are an important bottleneck in the whole-plant hydraulic continuum, making up on average at least 30% of the whole-plant hydraulic resistance for hydrated plants (Sack and Holbrook, 2006), and that K_{leaf} decline has an important influence on plant productivity, water movement through the whole plant, and drought responses. At least five methods are in current use for constructing K_{leaf} vulnerability curves, and several studies have shown that these methods produce comparable results when applied rigorously to the same species (Hernandez-Santana *et al.*, 2016; Trifilò *et al.*, 2016).

We review five questions at the forefront of the field, and future directions. (i) What is the variation across species and lineages in K_{leaf} vulnerability? (ii) What are the mechanisms of K_{leaf} decline with dehydration and its recovery with rehydration? (iii) How do leaves and stems compare in their hydraulic vulnerability? (iv) What is the impact of decline in K_{leaf} on gas exchange and drought tolerance across species? (v) What are the ecological consequences of leaf hydraulic vulnerability to dehydration?

Species variation in leaf hydraulic vulnerability

The decline of K_{leaf} is steep on average, and strongly variable within and across plant lineages. We compiled data for 310 species for which K_{leaf} vulnerability curves were constructed (see Supplementary Table S1 at *JXB* online). On average, across species, K_{leaf} declined considerably at leaf water potentials of above –1 MPa, and above turgor loss point, regardless of the method used for constructing K_{leaf} vulnerability curves. At water potentials of –1 MPa, K_{leaf} had declined on average across methods by 19–54% across angiosperms, by 8–39% across gymnosperms, and by 8–59% across pteridophytes (Fig. 1). By turgor loss point, K_{leaf} had declined on average across methods by 57–80% across angiosperms and by 30–81% across gymnosperms (Fig. 1). The variation in P_{50} and P_{80} reflects species-level variation in the shape and steepness of K_{leaf} decline, with drought-sensitive species typically experiencing very steep and strong declines at mild water potentials (Scoffoni *et al.*, 2012).

Our data compilation revealed differences across major plant lineages in the leaf water potentials at which K_{leaf} declined by 50% and 80% (P_{50} and P_{80}) (Fig. 2). Gymnosperms were on average more resistant (mean ±SE for P_{50}= –2.9 ± 0.17 MPa; P_{80}= –4.0 ± 0.23 MPa; n=70) than angiosperms (P_{50}= –2.4 ± 0.09 MPa; P_{80}= –2.8 ± 0.12 MPa; n=234) and pteridophytes (P_{50}= –1.6 ± 0.31 MPa; P_{80}= –2.2 ± 0.40 MPa; one-way ANOVA, P=0.006 and <0.001 for P_{50} and P_{80}, respectively; n=6). Yet, the ranges of P_{50} and P_{80} overlapped across lineages as species with K_{leaf} strongly vulnerable or tolerant to decline with dehydration evolved within each major lineage (Fig. 2).

Mechanisms of K_{leaf} decline with dehydration and recovery with rehydration

What causes K_{leaf} to decline? There are two pathways in series through which liquid water moves before it evaporates and diffuses as water vapor through stomata (Rockwell *et al.*, 2014; Buckley, 2015; Buckley *et al.*, 2017). Water first enters the xylem, consisting mainly of dead, hollow, elongated cells, before moving through living tissue outside the xylem conduits. Leaf pathways outside the xylem involve liquid water movement through living cells and/or cell walls in the vascular parenchyma, bundle sheath, mesophyll, and epidermis, and, in addition, vapor diffusion through intercellular airspaces. Updating a previous analysis (Sack and Holbrook, 2006), we compiled the data from studies that partitioned the xylem versus outside-xylem resistances in well-hydrated leaves and confirmed that on average across the 45 dicotyledonous species, the resistances are similar in magnitude inside and outside the xylem, with the resistance outside the xylem (R_{ox}) slightly higher, 57.1 ± 3.5% of the total resistance (Supplementary Table S2; Sack *et al.*, 2003, 2005; Salleo *et al.*, 2003; Cochard *et al.*, 2004*a*; Taneda *et al.*, 2016; Trifilò *et al.*, 2016; Scoffoni *et al.*, 2017*a*). However, the partitioning varies strongly across species, with R_{ox} as low as 11% of total resistance in *Lindackeria laurina* and as high as 97% in *Viburnum wrightii* (Sack *et al.*, 2005; Scoffoni *et al.*, 2016).

Given that the pathways in the xylem and outside the xylem differ in hydraulic vulnerability, the partitioning of resistances would shift during leaf dehydration (Scoffoni *et al.*, 2014). Because both inside and outside xylem pathways are important in determining K_{leaf} in hydrated leaves, the decline of K_{leaf} with dehydration could potentially be caused by changes in either or both of these pathways.

Leaf xylem as a driver of K_{leaf} decline with dehydration

Leaf veins are organized in a hierarchy: after water flows through the petiole xylem, it enters that of the midvein and other major veins (secondary and tertiary), then the minor veins that typically constitute the bulk of the total leaf vein length (Sack and Scoffoni, 2013). Water moves under tension in a metastable state. As the leaf dehydrates, increasing tensions in the xylem can cause air seeding through xylem conduit pits, breaking the cohesion of water molecules, which evaporate and conduits become gas filled. When xylem conduits 'embolize' (i.e. fill with gas), they become non-functional for water movement and the hydraulic conductance of

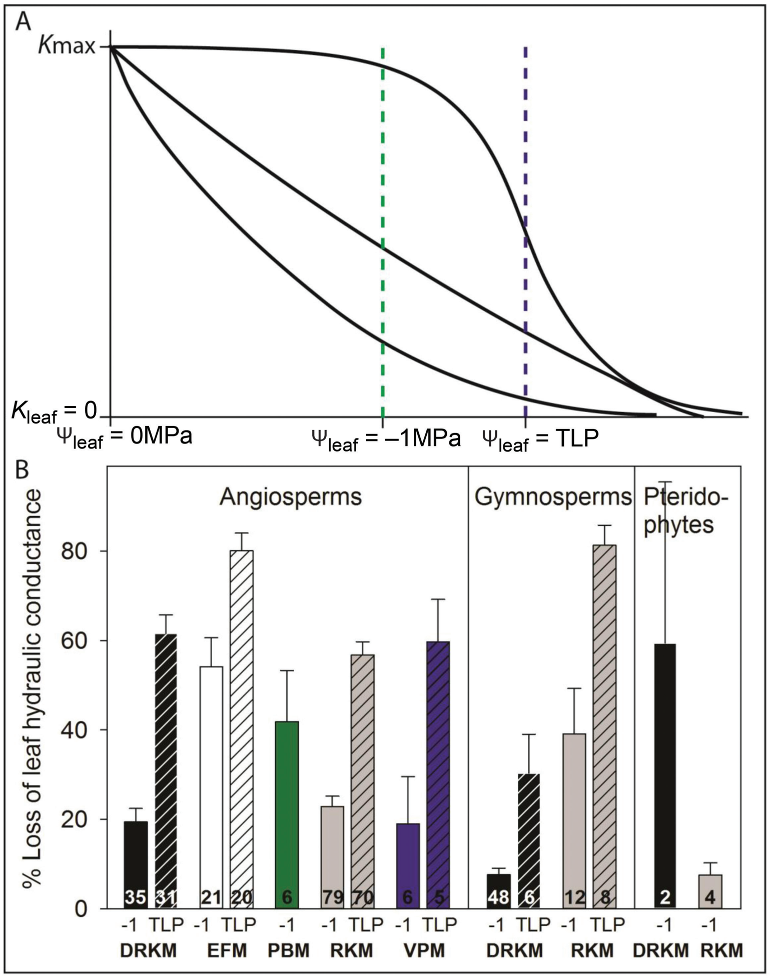

Fig. 1. The strong hydraulic vulnerability of leaves. (A) Examples of different responses of leaf hydraulic conductance to dehydration seen in the literature (sigmoidal versus linear versus exponential) showing strong but differential vulnerabilities at –1 MPa and turgor loss point. (B) Compiled data show the pronounced loss of hydraulic conductance (% decline of K_{leaf}) at –1 MPa (filled bars) and turgor loss point (striped bars) on average across species within lineages, with data separated according to the several methods used for determination of leaf hydraulic conductance (K_{leaf}). Numbers at the bottom of bars represent the number of species. Ψ_{leaf}, leaf water potential; DRKM, dynamic rehydration kinetics method; EFM, evaporative flux method; $K_{\max}$, leaf hydraulic conductance at full hydration; PBM, pressure bomb method; RKM, rehydration kinetics method; TLP, turgor loss point; VPM, vacuum pump method (data given in Supplementary Table S1). (This figure is available in colour at *JXB* online.)

the whole vein system decreases. Additionally, when the tensions in the conduits exceed the biomechanical resistance of cell walls, the xylem conduits can partially or fully collapse reducing hydraulic conductance.

Xylem embolism

The potential role of xylem embolism has been the principal focus of the bulk of studies on K_{leaf} decline with dehydration, and studies taking this singular perspective tended to conclude that xylem embolism occurs commonly and that it acts as the main driver of this decline. Studies of four types contributed to this perspective: acoustic emission studies in the 1960s and their current counterpart, optical visualization studies; dye uptake experiments, cryogenized scanning electron microscopy (cryo-SEM) studies, and xylem damage or sap modification studies (Table 1).

The acoustic emission method detects vibrations emitted in plant tissue as it dehydrates and was first developed on leaf petioles (Milburn and Johnson, 1966; West and Gaff, 1976; Crombie *et al.*, 1985), and has been widely used on stems and leaf midribs. These emitted ‘clicks’ were hypothesized to correspond to embolism formation in the xylem under tension, and, indeed, the number of ‘clicks’ or their amplitude with decreasing water potential correlated with K_{leaf} decline (Salleo *et al.*, 2000, 2001; Nardini and Salleo, 2003; Trifilò *et al.*, 2003*b*; Johnson *et al.*, 2009*a*; Charra-Vaskou *et al.*, 2012; Nolf *et al.*, 2016). However, this line of evidence was not sufficient to understand K_{leaf} decline for three reasons. First, several studies have indicated that acoustic emissions may correspond to events other than xylem conduit embolism, for example vibrations or embolisms originating in fibers, ray cells, collenchyma, or sclerenchyma cells in or around the xylem or in other tissues such as the pith and/or mesophyll (Sandford and Grace, 1985; Ritman and Milburn, 1988; Cochard and Tyree, 1990; Nolf et al., 2015, 2016), and/or nanobubble formation in xylem conduits (Schenk *et al.*, 2015). Indeed, there often are many more ‘clicks’ recorded than there are embolized conduits. Secondly, even if these acoustic emissions were caused only by the embolism of xylem conduits, the relationship between the number or amplitude of ‘clicks’

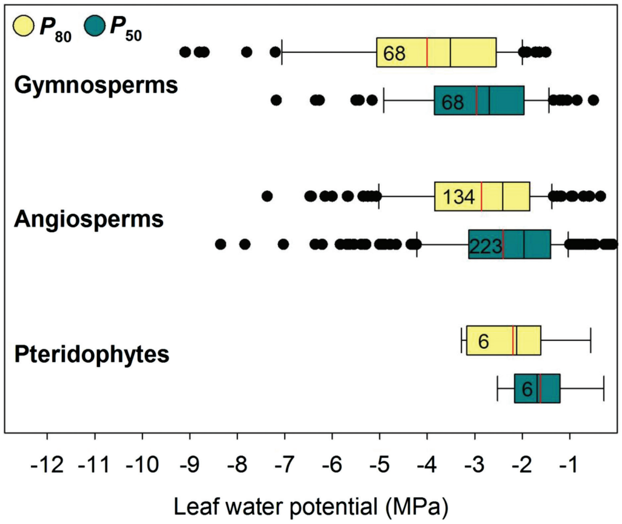

Fig. 2. Global analyses of the leaf water potential at which leaf hydraulic conductance declines by 50% and 80% (respectively, K_{leaf} P_{50} in blue and P_{80} in yellow) across major plant lineages. Species numbers are reported inside the box plots. Red and black bars inside the bar plots represent mean and median values, respectively. Dots outside the plots are outliers, showing the range of values observed.

and the actual loss of leaf xylem hydraulic conductance (K_x) is unknown. Because of the hierarchical and reticulate nature of leaf venation, the decline in K_x would depend not only on the number of embolized xylem conduits throughout the vein system, but also on their locations within the vein hierarchy, and on their sizes and their pit ultrastructure. Thirdly, comparing the signals for embolism with the decline of K_{leaf} is not sufficient to show a causal relationship, given that decline in outside-xylem hydraulic conductance (K_{ox}) is unknown. Recently, an optical method has been implemented, in which embolism spread within veins can be directly visualized in some leaves (Brodribb *et al.*, 2016*a*, *b*). A strong correspondence between P_{50} and the optical P_{50} was found across five angiosperm species, suggesting that leaf vein embolism might have driven K_{leaf} decline (Brodribb *et al.*, 2016*b*; Skelton *et al.*, 2017). However, just as described for the acoustic approach, observed embolism events cannot be directly linked to the decline of K_x or of K_{leaf}, as their effects would depend on the size and locations and pit ultrastructure of the embolized conduits, and, further, declines in K_{ox} may be occurring simultaneously. The acoustic and optical approaches are in their current state, qualitative, illustrative methods to explore the vulnerability of K_x or K_{leaf}, but do not yet enable quantitative estimation of decline in K_x or K_{leaf}.

Dye uptake studies provided a second line of apparent evidence for xylem embolism as the main driver of K_{leaf} decline with dehydration. Three different dyes have been supplied to leaf petioles for leaves forced to transpire or subjected to partial vacuum levels: fluorescein, phloxine B, and safranin. Early experiments showed that strongly dehydrated leaves no longer took up dye into their minor veins, and the 'functional' vein density, namely that of the dyed veins, which were presumed to be non-embolized, thus declined with K_{leaf}, suggesting that minor vein embolism would have caused K_{leaf} decline during dehydration (Salleo *et al.*, 2001; Nardini and Salleo, 2003; Trifilò *et al.*, 2003*a*, *b*; Laur and Hacke, 2014). However, the lack of dye uptake into minor veins could be due to some degree of embolism in the petiole or midrib and/or low water potential gradients through the transpiring leaf due to partial stomatal closure. Further, it is unclear how such embolism would relate to K_{leaf} decline for the same reasons explained above for acoustic and optical studies. Indeed, two studies found that K_{leaf} declined more steeply than functional vein density as determined by dye uptake into dehydrating leaves (Johnson *et al.*, 2012; Nolf *et al.*, 2016).

Cryo-SEM of midrib (or needle) sections provided a third line of apparent evidence for xylem embolism driving K_{leaf} decline with dehydration (Woodruff *et al.*, 2007; Johnson *et al.*, 2009*a*; Charra-Vaskou *et al.*, 2012; Zhang *et al.*, 2014). The two earlier studies found embolized conduits for mildly dehydrated leaves of three gymnosperms and two angiosperms, apparently corresponding to the decline observed in K_{leaf}, but the two later studies reported embolized conduits occurring only at very dehydrated leaf water potentials in two gymnosperms, at a point when most of the decline in K_{leaf} had already occurred. This variation is most probably due to species differences in their vulnerability to xylem embolism. Further, while cryo-SEM can be applied very rigorously (Johnson *et al.*, 2012; Zhang *et al.*, 2016), it can be subject to artifacts associated with freezing under tension or etching during sample preparation that can generate the appearance of embolisms that would not occur *in vivo* (Cochard *et al.*, 2000). Beyond these concerns, as described previously, the numbers of embolized conduits would not necessarily correspond to a proportional decline in K_{leaf} as these would depend on conduit anatomy.

Studies using either mechanically damaged xylem or modified sap chemistry provided a fourth line of evidence apparently linking K_{leaf} decline with vein embolism. Two studies showed that damaging >90% of veins or cutting the midrib at the base of the leaf induced a steep decline in K_{leaf} (Huve *et al.*, 2002; Sack *et al.*, 2008). Similarly, leaves rehydrated in a solution with added surfactants were more susceptible to K_{leaf} decline with dehydration (Johnson *et al.*, 2012). These studies suggest that disruption of the leaf xylem can indeed cause strong declines in K_{leaf}, but do not prove that such dysfunction would occur during mild or moderate dehydration *in vivo*, when much of K_{leaf} has declined (Fig. 1).

In conclusion, although the notion of xylem embolism as the main driver of K_{leaf} decline with dehydration has dominated the field, the evidence has been generally correlative. More direct insights have been provided by very recent development of both *in vivo* imaging tools and hydraulic techniques to partition the vulnerabilities inside and outside the leaf xylem, which have not supported a major role for xylem embolism in driving K_{leaf} decline (see 'Leaf outside-xylem as the driver of K_{leaf} decline with dehydration').

Xylem collapse

The collapse of xylem conduit cell walls has also been hypothesized to drive decline in K_{leaf} during dehydration, but its commonness and importance remain controversial. Conduit collapse would theoretically reduce the lumen cross-sectional

Table 1. *Lines of evidence for xylem embolism and/or changes in outside-xylem pathways as the main driver of leaf hydraulic decline with dehydration given in chronological order*

In each approach, the correlation of the decline of leaf hydraulic conductance (K_{leaf}) with a signal or experimental treatment was used to infer a major role for xylem or outside-xylem pathways.

Line of evidence/approach used, in which a signal or experimental treatment correlated with K_{leaf} decline	Studies	Provides correlative or direct evidence supporting K_x decline as major determinant of K_{leaf} decline	Provides correlative or direct evidence supporting K_{ox} decline as major determinant of K_{leaf} decline	Does the method provide visual evidence of embolism?
(i) Acoustic emissions: the increase in emitted ‘clicks’ recorded in the midrib, hypothesized to correspond to embolism events, correlated with K_{leaf} decline with dehydration.	Milburn and Johnson, 1966; West and Gaff, 1976; Crombie *et al.*, 1985; Salleo *et al.*, 2000, 2001; Nardini and Salleo, 2003; Trifilò *et al.*, 2003*b*; Johnson *et al.*, 2009*a*; Charra-Vaskou *et al.*, 2012; Nolf *et al.*, 2016	Yes, correlative	NA	No
(ii) Dye uptake: the decrease in minor vein dye uptake assumed to be caused by embolism, correlated with K_{leaf} decline with dehydration.	Salleo *et al.*, 2001; Nardini *et al.*, 2003; Trifilò *et al.*, 2003*a*, *b*; Johnson *et al.*, 2012; Laur and Hacke, 2014; Nolf *et al.*, 2016	Yes, correlative	Yes, as the two most recent studies showed K_{leaf} decline before loss of dye uptake by veins	No (the lack of dye in minor veins could be due to stomatal closure and/or embolism in major veins or petiole).
(iii) Xylem disruption experiments: vein occlusion through either cutting or transpiration of solution containing surfactants caused a strong decline in K_{leaf}.	Huve *et al.*, 2002; Sack *et al.*, 2008; Johnson *et al.*, 2012	Only indirectly, as they do not provide evidence that xylem is vulnerable during dehydration.	NA	No
(iv) Cryo-SEM: % embolized conduits in the midrib match the decline in K_{leaf} with dehydration.	Woodruff *et al.*, 2007; Johnson *et al.*, 2009*a*	Yes, correlative	NA	Yes
(v) Aquaporin studies: the decline in K_{leaf} with dehydration corresponded to a decline in aquaporin expression. Aquaporin inhibition has been shown to decrease K_{leaf} by up to 75%.	Shatil-Cohen *et al.*, 2011; Pou *et al.*, 2013; Laur and Hacke, 2014; Sade *et al.*, 2014	NA	Yes, correlative	No
(vi) MicroCT: no or relatively few embolized conduits in the major veins are found at 50% loss of K_{leaf}. No embolized conduits were found in the minor veins. A spatially explicit model of the leaf veins demonstrated that the few embolized conduits were not sufficient to cause the decline observed in K_{leaf}.	Charra-Vaskou *et al.*, 2012; Bouche *et al.*, 2016; Scoffoni et al., 2017*a*, *b*	No, and modeling further supported a negligible causal role for xylem embolism in driving K_{leaf} decline	Yes, correlative	Yes
(vii) Optical visualization method: the percentage of ‘embolized vein pixels’ correlated with K_{leaf} decline in two studies, though two other studies showed that embolism occurred only after many hours of bench drying, i.e. long after stomatal closure	Brodribb *et al.*, 2016*a*, *b*; Hochberg *et al.*, 2017; Skelton *et al.*, 2017	Yes, correlative	NA	Yes, though the exact number and sizes of embolized conduits in given vein orders are not known.
(viii) Partitioning of hydraulic vulnerabilities: vulnerability in outside-xylem pathways explained >95% loss of K_{leaf} across 10 species, while in two species both outside-xylem and xylem pathways seemed to play a role (though these species were not light acclimated).	Trifilò *et al.*, 2016; Scoffoni *et al.*, 2017*a*	Yes, direct	Yes, direct	No

NA, not applicable.

area and conductivity, which could scale up to a reduction of K_x and K_{leaf}. Three studies have reported xylem conduit collapse using cryo-SEM imaging or X-ray micro-computed tomography (microCT): in needles of four adult and one juvenile pine species (Cochard *et al.*, 2004*a*; Bouche *et al.*, 2016), and in minor veins of *Quercus rubra* (Zhang *et al.*, 2016).

However, the commonness of xylem wall collapse is questionable (Table 2), as studies of seven species reported no vein tracheid collapse under increasing tensions using cryo-SEM observations or microCT (Brodribb and Holbrook, 2005; Johnson *et al.*, 2009*a*; Charra-Vaskou *et al.*, 2012; Zhang *et al.*, 2014; Bouche *et al.*, 2016). In conifers, however, transfusion tracheids outside the vein may exhibit strong collapse under increasing tensions (Brodribb and Holbrook, 2005; Zhang *et al.*, 2014), and the sensitivity of this tissue could protect the xylem from reaching negative water potentials that would induce cavitation. For dehydrating needles of *Taxus baccata*, cross-sectional area shrinkage of transfusion tracheids correlated with the observed K_{leaf} decline, while no vein xylem embolism was observed (Zhang *et al.*, 2014). Collapse of transfusion tracheids is not surprising given their parenchymatous origin (Aloni *et al.*, 2013; Bouche *et al.*, 2014), and their low degree of cell wall lignification compared with xylem tracheids (Zhang *et al.*, 2014).

Spatially explicit modeling of the hydraulic impact of the collapse of xylem conduits and/or transfusion tissue is needed to support a causal impact on K_{leaf} decline. Notably, the collapse of xylem conduits occurred across species at very negative water potentials (from –1.8 MPa to –3.9 MPa across all six species; Cochard *et al.*, 2004*a*; Bouche *et al.*, 2016; Zhu *et al.*, 2016), typically below turgor loss point at which most of the loss in K_{leaf} has already occurred (Fig. 1). Further, a recent study with spatially explicit modeling of the leaf xylem showed that the hydraulic impact of xylem collapse in minor veins of angiosperms would be minimal and overshadowed by changes in outside-xylem pathways and embolism occurring in the midrib at those water potentials (Scoffoni *et al.*, 2017*b*). Indeed, minor veins appear to be generally overbuilt to resist tracheid collapse (Blackman *et al.*, 2010). More studies are needed, especially in angiosperms, to determine the commonness of xylem conduit collapse and its impact on K_{leaf}. Differences in anatomy and/or ontogenetic stages modifying conduit implosion pressures could explain the contrasting results observed so far.

Leaf outside-xylem as the driver of K_{leaf} *decline with dehydration*

In recent years, strong evidence based on novel imaging and hydraulic techniques has pointed to outside-xylem processes as the major driver of K_{leaf} decline (Table 1). The movement of water after leaving the xylem to the sites of evaporation is complex and dynamic, involving apoplastic, symplastic, and transmembrane liquid flow paths, and vapor diffusion in the intercellular airspaces.

Evidence for outside-xylem pathways driving K_{leaf} *decline with dehydration*

Even though the bulk of previous studies of hydraulic decline in leaves focused on the xylem as a source of mechanisms for leaf hydraulic decline, several of these studies acknowledged that outside-xylem processes would play a role, especially when xylem processes did not explain the observed decline in K_{leaf} (Huve *et al.*, 2002; Trifilò *et al.*, 2003*b*; Brodribb and Holbrook, 2006; Laur and Hacke, 2014). Indeed, K_{leaf} can decline early at high water potentials before embolism has been observed, following either a linear or non-linear trajectory (Brodribb and Holbrook, 2006; Scoffoni *et al.*, 2012;

Table 2. *Commonness of leaf xylem or transfusion tracheid collapse during leaf dehydration*

Species	Location of observed (or non-existent) collapse	Conduit collapse (Yes/No)	Collapse pressure/or strongest tension tested when no collapse found	References
Pieris japonica	Midrib	No (personal observation from figure in paper)	–2.5 MPa	Johnson *et al.*, 2009*a*
Pinus cembra	Midrib	Yes	–3.5 MPa	Cochard *et al.*, 2004*a*
Pinus mugo	Midrib	Yes	–3.4 MPa	Cochard *et al.*, 2004*a*
Pinus nigra	Midrib	Yes	–1.8 MPa	Cochard *et al.*, 2004*a*
Pinus pinaster	Midrib of seedling	Yes	–2.0 MPa	Bouche *et al.*, 2016
Pinus pinaster	Midrib of mature tree	No	–8.4 MPa	Bouche *et al.*, 2016
Pinus pinaster	Midrib	No (collapse observed post-embolism)	–4.0 MPa	Charra-Vaskou *et al.*, 2012
Pinus ponderosa	Midrib	No (personal observation from figure in paper)	–3.2 MPa	Johnson *et al.*, 2009*a*
Pinus sylvestris	Midrib	Yes	–2.6 MPa	Cochard *et al.*, 2004*a*
Podocarpus grayi	Midrib	No	–3.2 MPa	Brodribb and Holbrook, 2005
Podocarpus grayi	Transfusion tracheid	Yes	–2.3 MPa	Brodribb and Holbrook, 2005
Taxus baccata	Midrib	No	–4.7 MPa	Zhang *et al.*, 2014
Taxus baccata	Transfusion tracheid	Yes	–2.4 MPa	Zhang *et al.*, 2014
Quercus rubra	Minor veins	Yes	–2.5 MPa (some conduits show signs of buckling); –3.5 MPa (most conduits are highly deformed)	Zhang *et al.*, 2016

Sack *et al.*, 2016). This early decline in K_{leaf} with water potential (i.e. before turgor loss point, π_{tlp}) was hypothesized to arise from processes outside the xylem, given changes in cell volumes and turgor, and especially within the bundle sheath which represents the gateway for water movement to the mesophyll. This typical sequence of K_{leaf} declining before π_{tlp} explains the strong correlation of P_{50} and turgor loss point reported in studies of sets of diverse species (Blackman *et al.*, 2010; Scoffoni *et al.*, 2012; Villagra *et al.*, 2013; Martorell *et al.*, 2015; Bartlett *et al.*, 2016), and extended by our analysis of compiled data for 129 angiosperm species (Fig. 3). Computer modeling suggested that when outside-xylem pathways were more vulnerable to dehydration than xylary pathways, this vulnerability would protect vein xylem from developing strong tensions that would induce hydraulic failure (Scoffoni *et al.*, 2014; see Supplementary Appendix 1).

New evidence for outside-xylem processes driving the decline observed in K_{leaf} has recently emerged from novel imaging and hydraulic techniques. *In vivo* imaging of the leaf using microCT enables for the first time 2D and 3D snapshots within the leaf vein xylem during dehydration, and all showed that embolism formation generally began at water potentials substantially more negative than those at which K_{leaf} began to decline strongly, suggesting that outside-xylem processes were involved in the early decline of K_{leaf}. MicroCT studies on needles of *Pinus pinaster* showed that K_{leaf} had declined by >90% by the time 50% of tracheids embolized (Charra-Vaskou *et al.*, 2012; Bouche *et al.*, 2016). Similarly, a recent microCT study of four angiosperm species showed that only 6–14% of midrib conduits were embolized at water potentials by which K_{leaf} had declined by >60%, and no minor veins had embolized (Scoffoni *et al.*, 2017*a*). Thus far, microCT studies have focused on veins and tissue within a small sample of the leaf, and assumed the sample to be representative of the whole leaf. While optical visualization has shown that the timing of embolism is about the same throughout the midrib of a given species (Brodribb *et al.*, 2016*a*), a recent study on grapevines suggests that the base of the midrib might embolize earlier than the middle of the midrib, which is typically scanned for microCT (Hochberg *et al.*, 2017). Yet, the 3D snapshot of the leaf provides information of the numbers (and dimensions) of embolized conduits in different vein orders and can be used to parameterize a spatially explicit model of the leaf venation system such as K_LEAF (Cochard *et al.*, 2004*b*) to determine the hydraulic impact of embolism. A recent study found strong correspondence between simulated K_x values based on microCT, and K_x values measured hydraulically with the vacuum pump method (Scoffoni *et al.*, 2017*b*).

Hydraulic experiments enable the dissection of the decline of K_{leaf} with dehydration, as the increase in resistance (R_{leaf}=1/K_{leaf}) can be partitioned into additive terms, the hydraulic resistances of the xylem (R_x=1/K_x) and outside-xylem pathways (R_{ox}=1/K_{ox}). These experiments point to a primary role for K_{ox} decline in that of K_{leaf}. In a study of needles of *P. pinaster*, the water potential at 50% decline in K_x (measured with a centrifuge) was –1 MPa more negative than that of the entire needle (Charra-Vaskou *et al.*, 2012), suggesting that the outside-xylem compartment drove the decline of K_{leaf}. Further, in two studies of angiosperms, K_{ox} vulnerability curves were constructed by subtracting measured R_x (measured by cutting open minor veins to bypass the resistance of the outside-xylem and measuring the flow rate of water at different vacuum levels) from that of the entire leaf at decreasing water potentials (Trifilò *et al.*, 2016; Scoffoni *et al.*, 2017*a*). In one study, two of four species had their K_{ox} vulnerability curves matching that of K_{leaf}, while K_x was more tolerant to decline, indicating that outside-xylem pathways were the main driver of K_{leaf} decline (Trifilò *et al.*, 2016); the other two species showed similar declines in both K_x and K_{ox}. This could suggest that the dominant pathway causing the decline in K_{leaf} is species specific. However, all measurements in that study were performed under low light conditions, which would cause reductions in aquaporin activity (Cochard *et al.*, 2007), and in K_{ox} and its vulnerability to dehydration (Guyot *et al.*, 2012). In a second study, measurements were conducted under high irradiance for eight diverse angiosperms, and in all cases the decline in K_{ox} matched that of K_{leaf}, whereas xylem hydraulic conductance was more resistant to dehydration, with P_{50} for K_x on average –1.6 MPa more negative than that of K_{leaf} and K_{ox} (Scoffoni *et al.*, 2017*a*).

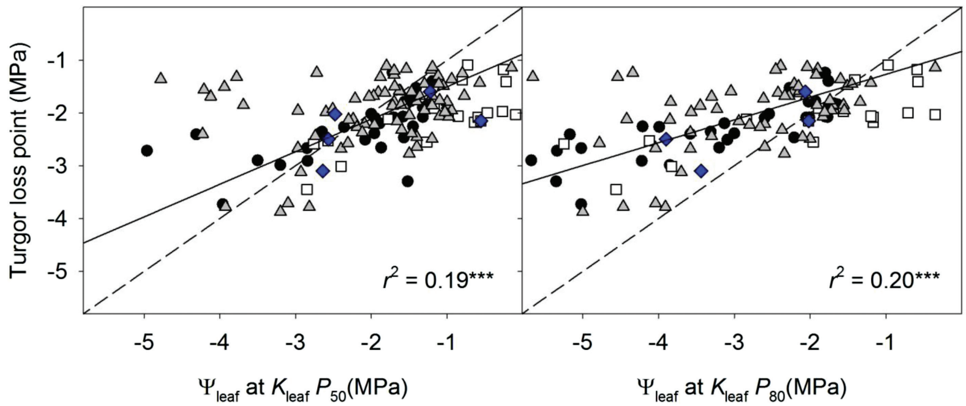

Fig. 3. Co-ordination of the water potential at which leaf hydraulic conductance (K_{leaf}) declined by 50% (left panel) and 80% (right panel) with bulk leaf turgor loss point. The 1:1 line is represented by black dashes. Colors represent the K_{leaf} method used for given species: dynamic rehydration kinetics method (DRKM; black dots), evaporative flux method (EFM; white squares), rehydration kinetics method (RKM; gray triangles), and vacuum pump method (VPM; blue diamonds). ***P<0.001.

Across species on average, 98% of the decline in K_{leaf} by P_{50} was caused by outside-xylem pathways (Scoffoni *et al.*, 2017*a*). Future work performed on more species and under high light conditions is needed to determine whether outside-xylem pathways are generally the main driver of K_{leaf} decline with dehydration across species, or whether the principal cause of the decline is species specific.

Mechanisms for the decline in K_{ox}

Three main mechanisms have been proposed to explain K_{ox} decline with dehydration: changes in cell turgor, changes in membrane permeability, and changes in the evaporation sites within the leaf. A recent study found a strong correlation between leaf (and cell) shrinkage before turgor loss point and the decline in K_{leaf} with dehydration (Scoffoni *et al.*, 2014). The authors suggested that the effect of leaf shrinkage on K_{leaf} decline could be physical in nature, if the reduction of cell size and cell connectivity during leaf shrinkage increased the resistance to water flow outside the xylem. However, recent simulations using a spatially explicit model of the leaf outside-xylem hydraulic pathways showed that reduction of cell size or connectivity would have little impact on changes in K_{ox}. Indeed, all else being equal, cell shrinkage would increase K_{ox} (by 4–55% for the eight species tested) rather than decrease it, as it would reduce the pathlength of water movement both horizontally (reducing the distance between veins) and vertically (reducing the distance from vein to stomata) (Scoffoni *et al.*, 2017*a*). Thus, the effect of cell and leaf shrinkage on K_{ox} decline is most probably indirect, potentially via an influence on membrane permeability.

The decline of membrane permeability with dehydration would arise from known changes in the activity of aquaporins, the protein channels primarily responsible for flow across the hydrophobic lipid bilayer of cell membranes. Aquaporins play a fundamental role in the hydraulic regulation of both leaves and roots in response to environmental factors such as water, temperature, light, and nutrient availability (Maurel *et al.*, 2015). Living cells in the leaf veins, such as vascular parenchyma and bundle sheath, thus play a major role in controlling K_{leaf} during dehydration (Ache *et al.*, 2010; Prado *et al.*, 2013; Sade *et al.*, 2014). Aquaporins can deactivate in response to changes in turgor (Kim and Steudle, 2007; Lee *et al.*, 2009), and/or in response to abscisic acid (ABA) signals from the vein xylem and phloem (Shatil-Cohen *et al.*, 2011; Pantin *et al.*, 2013; Prado *et al.*, 2013; Sade *et al.*, 2015), which would induce a decline in K_{leaf}. The decline in K_{leaf} in droughted poplar plants was associated with a significant decrease in aquaporin expression (Laur and Hacke, 2014). A role for aquaporins in the decline of K_{leaf} at mild dehydration was also suggested by a study testing the effect of irradiance on hydraulic vulnerability, which showed that across four species, K_{leaf} in hydrated leaves increased under higher irradiance, but declined more abruptly with water potential than for low-light-acclimated leaves (Guyot *et al.*, 2012). These results are in accordance with recent simulations using a spatially explicit model of leaf outside-xylem pathways, showing that the most likely mechanism for the observed decline in K_{ox} at turgor loss point was the reduction of membrane permeability in the presence of an apoplastic barrier at the bundle sheath (Scoffoni *et al.*, 2017*a*). Indeed, some species have been reported to show suberized or lignified cell walls at the bundle sheath cells, which would probably impede apoplastic flow (Lersten, 1997; Taneda *et al.*, 2016). Consequently, bundle sheath cells could act as a selective barrier against toxic ions from entering the mesophyll and inducing leaf necrosis, analogous to the root endodermis (Shatil-Cohen and Moshelion, 2012). However, not all species seem to exhibit such apoplastic barriers at the bundle sheath cells (Lersten, 1997; Taneda *et al.*, 2016), and another possible explanation in K_{ox} decline could relate to changes occurring in water transport through the vascular parenchyma. Further, bundle sheath walls may be fully lignified, unlike the Casparian strip in which only radial and transverse walls are lignified (Taneda *et al.*, 2016). In that case, given the large resistance imposed by the lignified bundle sheath cell walls, the effect of membrane aquaporins would be lower, and would depend on their localization within the cell membrane (e.g. their proximity to pits where secondary cell walls are greatly reduced). Future work is needed to resolve the chemistry of cell walls and aquaporin ultralocalization.

A final hypothesis is that changes in evaporation sites throughout the leaf as it dehydrates could explain changes in K_{leaf} (Brodribb and Holbrook, 2006). However, recent modeling suggested that the sites of evaporation were not likely to change from full turgidity to turgor loss point given a similar temperature gradient across the leaf (Buckley *et al.*, 2017). Spatially explicit simulations of the leaf mesophyll and bundle sheath showed that sites of evaporation within the leaf were not changed when decreasing membrane permeability and imposing an apoplastic barrier to water flow at the bundle sheath cells. Additionally, that study showed that the main factor influencing the sites of evaporation in the leaf is tissue porosity, with greater evaporation from deeper within the mesophyll in leaves with a greater fraction of airspace. However, porosity generally did not change much for 12 species from full leaf turgidity to turgor loss point (i.e. increasing or decreasing by <15% for most species (Scoffoni *et al.*, 2014; Buckley *et al.*, 2017).

Recovery of K_{leaf} decline with rehydration

Many studies have shown that a range of species can recover rapidly and on a daily basis in K_{leaf} and thus gas exchange, especially when exposed to mild dehydration (Lo Gullo *et al.*, 2003, 2005; Brodribb and Holbrook, 2004; Blackman *et al.*, 2009; Brodribb and Cochard, 2009; Johnson *et al.*, 2009*b*). Recovery in K_{leaf} has been shown to be quite rapid (1 h at most) in 4/10 diverse angiosperm species dehydrated to around turgor loss point, yet 6/10 species did not recover at all during that short time scale (Scoffoni *et al.*, 2012). When plants are exposed to strong drought, such that K_{leaf} declines by >80% (at which point embolism might occur), lasting depression of gas exchange results, and longer times are required to recover hydraulic function (Blackman *et al.*, 2009; Brodribb and Cochard, 2009). ABA may control stomatal closure after strong drought even after the recovery of leaf water potential, potentially allowing plants the time to repair xylem embolism under no tension (Lovisolo *et al.*, 2008).

A major advantage of having vulnerable outside-xylem pathways is that this would protect xylem from reaching tensions that would cause embolism formation (Supplementary Appendix 1), and outside-xylem pathways might recover in conductance faster than xylem pathways, enabling faster recovery in biochemical functioning. Such a mechanism is consistent with the vulnerability segmentation hypothesis, according to which more distal and more easily replaceable parts in the plant show stronger vulnerability, and therefore protect more proximal parts from strong tensions and embolisms (Tyree and Ewers, 1991; Pivovaroff *et al.*, 2014; Scoffoni *et al.*, 2017*b*). In stems, the refilling of embolized conduits is a slow and expensive process, especially in the absence of root pressure (Stiller *et al.*, 2003; Brodersen and McElrone, 2013; Knipfer *et al.*, 2015). In one early study on leaf veins of sunflowers, the refilling of embolized xylem conduits was reported to occur in under 1 h and even under tension (Nardini *et al.*, 2008). However, in that study, embolized conduits were not directly visualized. Future studies should test these findings with *in vivo* micro-CT to check whether indeed embolized conduits did refill, or if another phenomenon or artifacts from the hydraulic method could account for the rapid recovery of K_{leaf} in that study. For example, a study found that the reversal of hydraulic decline in the rehydrated needle of *Taxus baccata* related to recovery from collapse of transfusion tracheids, rather than to refilling xylem embolism (Zhang *et al.*, 2014). In angiosperms, collapsed xylem conduits in minor veins of *Quercus rubra* showed complete recovery after rehydrating for 2 min (Zhang *et al.*, 2016). In angiosperm leaves, K_{ox} decline and fast recoverability would act dynamically to protect the xylem from strong tensions inducing embolisms (Zhang *et al.*, 2016; Scoffoni *et al.*, 2017*a*). Future studies should investigate the causes of K_{leaf} recovery across species.

K_{leaf} decline in relation to stem and whole-plant vulnerability

Understanding plant drought responses requires precise knowledge of the vulnerability of each organ. Formulated >20 years ago, the hydraulic vulnerability segmentation hypothesis stated that most distal parts of the plant should be more vulnerable to hydraulic failure than basal parts (Tyree and Ewers, 1991). Being most vulnerable and expendable, leaves would thus protect the stems from reaching tensions that would induce catastrophic xylem hydraulic failure. Several studies have directly tested this hypothesis (Supplementary Table S1). A recent meta-analysis of 69 woody species suggested that species of dry environments showed vulnerability segmentation, but that species of wet environments had branch xylem equal to or more vulnerable than leaves, and concluded that other mechanisms were needed to protect the entire system from failure (Zhu *et al.*, 2016). To update and improve that analysis, we compiled additional data, but removed data for which K_{leaf} P_{50} values were obtained from stem hydraulic conductivities, unpublished data, and data for which K_{leaf} and K_{stem} vulnerability had been combined from different papers. We found that 51/63 angiosperms and 9/10 gymnosperms for which data were available, native to biomes from semi-desert to tropical wet forests, exhibited more vulnerable leaves than stems (Supplementary Table S1). Across 63 angiosperms, K_{leaf} and K_{stem} P_{50} were co-ordinated (Fig. 4), indicating an overall convergence across organs in hydraulic vulnerability to drought. The high degree of scatter explains why this co-ordination would not be resolved in studies of smaller species sets (Hao *et al.*, 2008) and suggests variation in the adaptation of whole-plant hydraulic design to tolerate drought. For example, in a cold desert ecosystem, species with shallow roots experienced greater water stress and had wider leaf to stem safety margins (i.e. K_{leaf} P_{50}–K_{stem} P_{50}), with leaves conferring greater protection of the stem during extreme drought (Bucci *et al.*, 2013).

Notably, data in the literature for leaf versus stem P_{50} (Fig. 4) need to be interpreted with caution for two reasons. First, most studies comparing leaf and stem hydraulic vulnerability used the early 'two-point' rehydration kinetics method under conditions that would have reduced accuracy for estimation of $K_{\max}$ and leaf hydraulic vulnerability; that is, (i) not correcting for the dynamic changes of capacitance with dehydration (Blackman and Brodribb, 2011) and (ii) measuring leaves under low irradiance, which can lead to underestimation of $K_{\max}$ and leaf hydraulic vulnerability (Scoffoni *et al.*, 2008; Guyot *et al.*, 2012). Secondly, stem hydraulics measurements can be highly prone to artifacts, especially in species with long vessels; simply cutting the stems under water to relieve tension 2–3 nodes from the segment to be measured can induce embolism formation (Wheeler *et al.*, 2013) and overestimation of stem vulnerability. The 12 species exhibiting more vulnerable stems than leaves exhibited suspiciously high stem

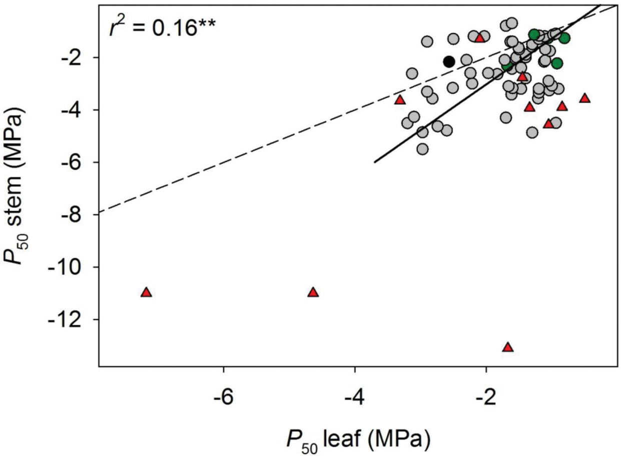

Fig. 4. Co-ordination of leaf and stem water potential at 50% of initial hydraulic conductance (P_{50}-leaf and P_{50}-stem, respectively) for 63 angiosperm species. Gymnosperms were excluded from this analysis as data were available for only 10 species for both leaf and stem vulnerabilities. However, when plotted with the angiosperms, gymnosperms appear to behave similarly (red triangles). Colors represent the K_{leaf} method used for given species: pressure bomb method (PBM, green circles); rehydration kinetics method (RKM, gray circles). Black dashes represent the 1:1 line. **P=0.001

P_{50} values (on average across species –1.37 ± 0.15 MPa and a minimum of –0.7 MPa). Future studies need to test for vulnerability segmentation with a more robust K_{leaf} method, and confirm such highly vulnerable stem P_{50} values by other means such as microCT.

Even given these findings, such a simple comparison of the P_{50} of stems and leaves is not in itself sufficient to test the vulnerability segmentation hypothesis. For example, a recent study on tomato argued that herbs may not show vulnerability segmentation (Skelton *et al.*, 2017), based on the similar P_{50} values of stem, leaf, and root. However, vulnerability segmentation may operate even if P_{50} values are similar across organs. Given that hydraulic decline in the leaf will reduce tensions in the stem and root during transpiration (Supplementary Appendix 1), K_{leaf} decline will reduce tension (and embolism formation) in the stem for a given soil water potential. Indeed, the water potentials in a given organ at a given time depend on the transpiration rate and the distribution of resistances in the plant, and these can only be estimated using a whole-plant model including vulnerability in all compartments, or measured using psychrometers throughout the plant. Several studies reported that at mid-day water potentials leaves would experience hydraulic decline, but stems would not, giving support to the hypothesis that leaves act as a 'safety-valve' (Bucci *et al.*, 2008; Villagra *et al.*, 2013; Pivovaroff *et al.*, 2014).

The very few studies that have examined the partitioning of vulnerabilities including roots reported them to have similar vulnerability to leaves (Bucci *et al.*, 2013; Johnson *et al.*, 2013, 2016; Skelton *et al.*, 2017). Such strong vulnerability in roots could help prevent reverse water flow from the soil during drought. Notably, roots and leaves both exhibit xylem and outside-xylem hydraulic pathways, and vulnerable outside-xylem pathways in each of these organs would potentially drive vulnerability at the entire organ level that would protect the xylem pathways (in roots and leaves, as well as stems) from embolisms. Recent work using X-ray microCT on roots showed that the formation of cortical lacunae in fine roots induced strong declines in hydraulic conductance that protected the root xylem from tensions that would cause embolism (Cuneo *et al.*, 2016). Future studies are needed to compare leaf and root vulnerabilities across species and habitats.

Impact of K_{leaf} decline on gas exchange and drought tolerance

An adequate water supply is fundamental to the maintenance of gas exchange, especially during soil or atmospheric drought. Consequently, maximum K_{leaf} (K_{max}) evolves to match stomatal conductance (g_s) and the light-saturated photosynthetic CO_2 assimilation rate (A_{max}) both across major lineages of plants (Brodribb *et al.*, 2007) and within lineages of closely related species (Scoffoni *et al.*, 2016).

Because K_{leaf} decline with dehydration could strongly influence gas exchange rates, three important questions have been investigated in the past 15 years. (i) Is there a trade-off between leaf hydraulic safety and efficiency? (ii) Is K_{leaf} decline a main cause of the depression observed in gas exchange at mid-day or during soil drought, and how do species vary in their response? (iii) Does K_{leaf} vulnerability act physically to force stomatal closure or is it involved in signaling to drive stomatal closure to prevent further leaf dehydration, plant hydraulic failure, and irreversible damage to the photosynthetic apparatus?

Trade-off between leaf hydraulic efficiency and leaf hydraulic safety

A trade-off between leaf hydraulic safety and efficiency has been reported previously for C_4 grasses (Ocheltree *et al.*, 2016), but was not found in several studies of phylogenetically diverse species sets (Sack and Holbrook, 2006; Chen *et al.*, 2009; Blackman *et al.*, 2010; Scoffoni *et al.*, 2012; Nardini and Luglio, 2014). In our new compilation of published data for 137 angiosperms, we found a weak yet significant trade-off between K_{max} and P_{50} (Fig. 5). This weak trade-off was found when including data collected for leaf hydraulic vulnerability using either the evaporative flux method (EFM; r^2=0.30, P=0.011) or the rehydration kinetics method (RKM; r^2=0.18, P<0.001), but not the dynamic rehydration kinetics method (DRKM), which may be due to the lack of vulnerable species

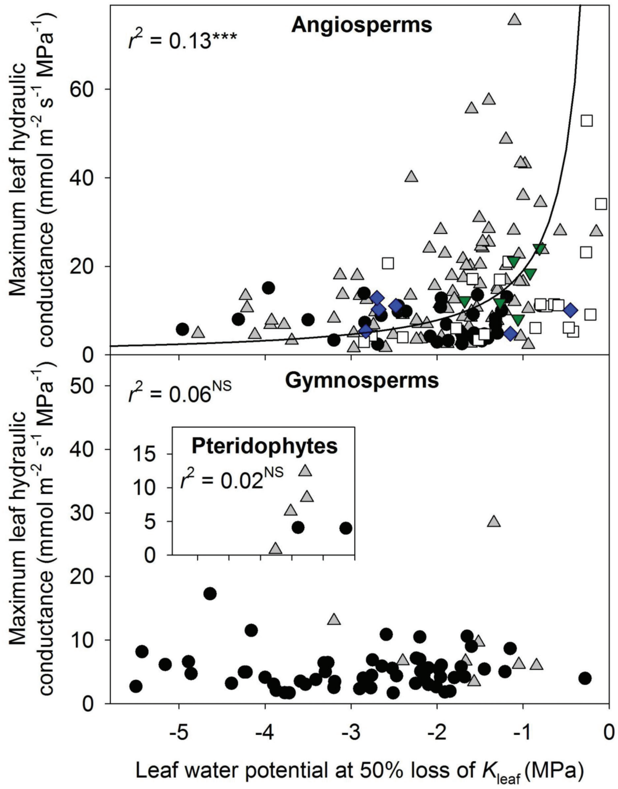

Fig. 5. The leaf hydraulic efficiency–safety trade-off across angiosperms (top panel) but not for gymnosperms (bottom panel) or pteridophytes (inset). Colors represent the K_{leaf} method used for given species: dynamic rehydration kinetics method (DRKM, black dots), evaporative flux method (EFM, white squares), pressure bomb method (PBM; inverted green triangles); rehydration kinetics method (RKM, gray triangles), and vacuum pump method (VPM, blue diamonds). ***P=0.001; ^{NS}P>0.05.

in that data set (r^2=0.007, P=0.64; Supplementary Table S1). In contrast, in our analyses of compiled data, K_{max} was not correlated with P_{50} across 66 gymnosperms or across 6 pteridophytes (Fig. 5; Supplementary Table S1). The significant trade-off between maximum K_{leaf} versus P_{50} suggests that underlying anatomical and/or biochemical traits can have opposite influences on maximum conductance and hydraulic safety, and/or that ecological adaptation tends to select these traits in opposite directions. However, a high degree of scatter exists, especially at high water potentials (Fig. 5), similarly to the relationship for maximum stem hydraulic conductance versus stem P_{50} (Gleason *et al.*, 2016), making P_{50} a weak predictor of K_{max}. Such a large variation reflects the enormous diversity in the anatomy of the leaf xylem and outside-xylem pathways, and in the biochemistry of the bundle sheath and mesophyll cells, such that evolving one trait to increase drought tolerance at the expense of maximum hydraulic conductance could be compensated by evolution of another trait.

Leaf hydraulic vulnerability as a determinant of gas exchange responses during drought

Photosynthetic rates decline strongly around mid-day, when temperature and vapor pressure deficit (VPD) reach peak values and stomata close (e.g. Stocker, 1956; Schulze *et al.*, 1974; Roessler and Monson, 1985; Koyama and Takemoto, 2014). Leaf hydraulics studies in the last two decades suggested that this mid-day depression in leaf gas exchange could be related to K_{leaf} decline. The underlying theory is that during increasing transpiration, any hydraulic decline would amplify the drop in leaf water potential, actuating stomatal closure by a passive mechanism (i.e. simple loss of turgor in the guard cells) and/or active mechanisms, i.e. ion pumping out of guard cells, whether or not in response to signals such as ABA (Brodribb and McAdam, 2011; McAdam and Brodribb, 2014). Though several studies have shown a strong correspondence in the daily patterns of K_{leaf} with those of g_s and A_{max} (Brodribb and Holbrook, 2004; Lo Gullo *et al.*, 2005; Brodribb and Holbrook, 2007), other studies found the responses did not match in some species (Lo Gullo *et al.*, 2005; Johnson *et al.*, 2013).

Our compilation of the published data shows a diversity of ways in which K_{leaf}, A_{max}, and g_s responses are coordinated during leaf dehydration. The 50 species that have been studied either in diurnal gas exchange in the field or in bench dehydration experiments exhibited three types of response of K_{leaf} and gas exchange to dehydration, with implications for drought tolerance (Fig. 6; Supplementary Table S1). We propose a framework to explain these patterns, based on whether K_{leaf} decline is less than, similar to, or greater than that of g_s as leaf water potential declines. Notably, these three types of behavior described above were found regardless of the specific K_{leaf} measurement methods (Supplementary Table S1).

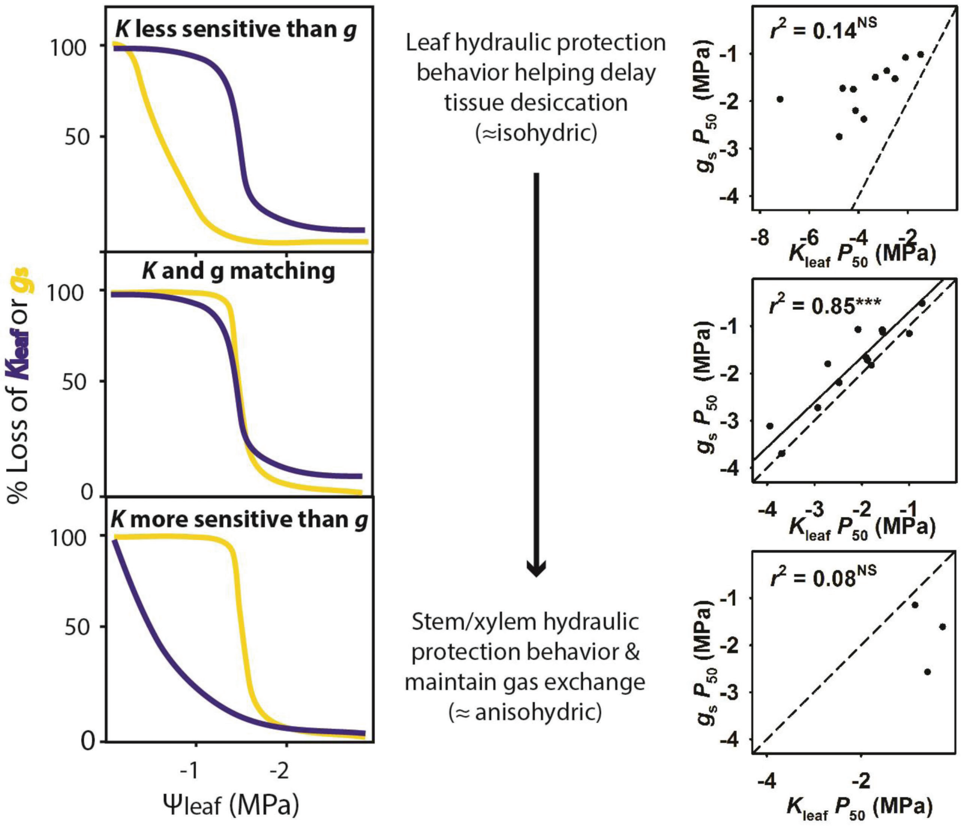

Fig. 6. Summary of the spectrum of responses of leaf hydraulic conductance (K_{leaf}) and stomatal conductance (g_s) to decreasing leaf water potential (Ψ_{leaf}). Panels on the right show the co=ordination between leaf water potential at 50% loss of K_{leaf} (P_{50}) and the water potential at 50% and 95% loss of stomatal conductance (g_s) for the three types of behavior. Black dashes represent the 1:1 line. ***P=0.001; $^{\text{NS}}P$>0.05.

These types of co-ordination can be related to the frequently used dichotomy between 'isohydric' and 'anisohydric species', bearing in mind that recent work shows that species show a continuum between iso- and anisohydry (Meinzer *et al.*, 2016) and that these categories, which have been typically related only to stomatal responses, need more explicit definitions, including consideration of a wider range of processes. Indeed, the stomatal behaviors that these terms refer to would depend mechanistically on K_{leaf} responses to leaf water potential.

Species in which K_{leaf} is less sensitive than g_{s} as leaf water potential declines would have the advantages associated with 'isohydry'; that is, in which g_{s} declines at relatively mild leaf dehydration, enabling Ψ_{leaf} to remain high. We propose that such behavior would require not only early g_{s} decline but also K_{leaf} to be maintained. Thus, because, assuming boundary layer conductance >>g_{s},

$$\Psi_{\mathrm{leaf}} = \Psi_{\mathrm{soil}} - \frac{\mathrm{VPD} \times g_{\mathrm{s}}}{K_{\mathrm{plant}}} \qquad (1)$$

where Ψ_{soil} represents the soil water potential and K_{plant} the plant hydraulic conductance, reducing g_{s} with constant K will enable the plant to maintain Ψ_{leaf} under increasing VPD at a given Ψ_{soil}, or with declining Ψ_{soil} at a given VPD. Such a '*K* less sensitive than *g*' behavior would protect leaf tissues from desiccating below damaging thresholds of Ψ_{leaf} under changing VPD, which would be especially beneficial in high light and/or dry environments. Indeed, in our data compilation, angiosperms exhibiting this strategy either thrive in high light environments or were C_4 grasses ('*K* less sensitive than *g*'; Fig. 6; Supplementary Table S1). The '*K* less sensitive than *g*' behavior would thus help prevent tissue desiccation by maintaining leaf water potential, and potentially enable leaves to recover gas exchange more rapidly when conditions ameliorate.

The opposite type of behavior, namely *K* more sensitive than *g*, would contribute to anisohydric responses; that is, maintaining stomata open with declining soil water potential such that diurnal CO_2 uptake continues, though at the risk of sustaining hydraulic failure. In species that maintain g_{s} high despite declining Ψ_{leaf}, a highly sensitive K_{leaf} could act as a hydraulic bottleneck, reducing the tension in the stem and root xylem, potentially protecting from embolism (Supplementary Appendix 1). Such protection from embolism would arise for the leaf xylem as well, if most of the decline in K_{leaf} occurs outside the xylem. We found that the species exhibiting the '*K* more sensitive than *g*' behavior were very sensitive to dehydration in their K_{leaf} (Fig. 6). Species with such a high leaf hydraulic vulnerability would need to maintain an adequate g_{s} even after their hydraulic supply has declined (<50% of maximum K_{leaf}), and this may come at the cost of achieving very high g_{s} in hydrated leaves. Indeed, the mean operating g_{s} value across all species showing this behavior was 152 ± 25 mmol m^{-2} s^{-1}, significantly lower than species exhibiting matching behavior (see below), which averaged 324 ± 41 mmol m^{-2} s^{-1} (one-way ANOVA; P=0.016). This behavior could be especially beneficial for species in which K_{leaf} recovers daily and at low metabolic cost.

Notably, species with such behavior often have very high K_{leaf} values measured for well-hydrated excised leaves at water potentials greater than –0.5 MPa. Such high K_{leaf} values merit additional discussion, as a number of studies begin their vulnerability curves below this point, or even below –1 MPa. Some might even ask whether such high K_{leaf} values in well-hydrated leaves arise as an artifact of the calculation of K_{leaf} as the ratio of transpiration to leaf water potential difference (i.e. if leaf water potential were hard to measure precisely close to full hydration). However, carefully equilibrating leaves prior to leaf water potential measurement, and the use of a dissecting scope and digital gauge with a pressure chamber can considerably reduce measurement errors. Indeed, high K_{leaf} values have been found across all measurement methods, and studies comparing methods have found estimated parameters to match (Sack *et al.*, 2002; Hernandez-Santana *et al.*, 2016; Trifilò *et al.*, 2016). Another question is: what is the significance of such high K_{leaf} values measured for excised leaves at water potentials greater than –0.5 MPa? Such high values would not necessarily occur *in vivo* because hydraulic resistance throughout the plant and soil would reduce leaf water potential below this level during transpiration. However, the design of leaves with high K_{leaf} at full hydration would enable substantial hydraulic capacity to be maintained even given strong hydraulic decline. Further, a high K_{leaf} for hydrated leaves might be beneficial once stomata close, contributing to rapid leaf rehydration. Overall, the fact that leaves do not operate at such high K_{leaf} values *in vivo* does not preclude such high values in fully hydrated leaves, or obviate their functional significance, in the same way that a Ferrari can operate at 340 km h^{-1} and, indeed, this is a major feature of its design, even if never observed operating at that speed in traffic. Finally, consideration of K_{leaf} values for hydrated leaves greatly improves the accuracy of vulnerability curve fitting and the estimation of vulnerability curve parameters (Guyot *et al.*, 2012), whereas excluding points for hydrated leaves on the basis that they do not occur *in vivo*, and beginning vulnerability curves with leaves dehydrated to below –1 MPa runs a risk of underestimating K_{max} and hydraulic vulnerability.

The third type of behavior, '*K* and *g* matching', would be intermediate in conferring the benefits of the other two behaviors (i.e. protecting the leaf from water potential decline and protecting the xylem from embolism). The continuous tracking of K_{leaf} and gas exchange to leaf water potential could act to balance water supply and demand, thus stabilizing leaf water potential, and enabling species to avoid hydraulic failure in the xylem, though this could come at the cost of a decrease in the integrated diurnal CO_2 uptake of plants. As expected from these matching patterns, we found across all species exhibiting this pattern a significant correlation of P_{50} and the water potential at which g_{s} declined by 50% and 95% (g_{50} and g_{95}, respectively; Fig. 6)

It is important to consider that the coupled declines of g_{s} and K_{leaf} would not necessarily scale directly to the dynamics of photosynthesis, in cases when g_{s} and A_{max} become decoupled during drought. Two types of responses of A_{max} to declining leaf water status have been observed: a linear decline at high relative water content or water potential, or maintenance at a high value with dehydration until an abrupt decline at low relative water content or water potential (Boyer, 1970, 1971; Lawlor and Cornic, 2002; Stiller *et al.*, 2003; Muller *et al.*, 2011). More studies are needed to investigate the response of A_{max} to declining water potential across more numerous and diverse species. Further work will be needed to disentangle these different behaviors (i.e. based on the sequence of decline of K_{leaf}, g_s, and A_{max}) across species and habitats, and their mechanistic and evolutionary bases.

Leaf hydraulic vulnerability as a potential trigger for stomatal closure

The investigation of hydraulic decline with dehydration and its implications for gas exchange has produced insights into the mechanisms of stomatal closure. Several have hypothesized that stomata may close early to reduce tensions and thus prevent embolism formation in stems (e.g. Cochard, 2002; Cochard *et al.*, 2002; Brodribb *et al.*, 2003; Vilagrosa *et al.*, 2003), and this behavior has been supported by studies on grapevine (Hochberg *et al.*, 2017) and meta-analyses across diverse species (Bartlett *et al.*, 2016). As described above, K_{leaf} decline might further contribute to such a response, by escalating the decline in leaf water potential during transpiration in addition to reducing tension in the stem for a given transpiration rate (Supplementary Appendix 1). Others have hypothesized that embolism formation in leaf veins may be a direct physical signal causing stomatal closure (Nardini and Salleo, 2000; Salleo *et al.*, 2001; Lo Gullo *et al.*, 2003; Nardini and Salleo, 2003; Brodribb and Holbrook, 2004). However, if K_{leaf} decline with dehydration is caused by changes in the outside-xylem pathways, a direct hydraulic effect triggering stomatal closure might instead be achieved through the reduction of cell water status (Brodribb and Holbrook, 2003; Blackman *et al.*, 2009; Guyot *et al.*, 2012). Such a signal for stomatal closure based on the decline in K_{ox} would be advantageous for protecting plants from xylem failure and irreversible damage to their photosynthetic apparatus at very negative water potentials (Tyree and Sperry, 1988; Brodribb and Holbrook, 2003, 2006; Blackman *et al.*, 2009; Ganthaler and Mayr, 2015; Gleason *et al.*, 2017).

The fact that species show a strong variation in the coordination of hydraulic decline and stomatal closure with dehydration suggests that at least some species have other mechanisms beyond K_{leaf} decline as causes of stomatal closure (Fig. 6; Supplementary Table S1). Stomatal closure could thus relate to ABA signaling that would be differently sensitive to K_{leaf} decline across species, and/or to changes in the turgor or osmotic concentrations of the guard cells and epidermal pavement cells, rather than bulk leaf cell turgor (Guyot *et al.*, 2012). Two studies hypothesized that ABA could contribute to K_{leaf} decline by reducing bundle sheath cell permeability, in addition to reducing stomatal conductance (via the guard cells) (Pantin *et al.*, 2013*a*, *b*). Future studies are required to clarify the potential role of hydraulic decline in driving stomatal closure.

Ecological consequences of K_{leaf} decline with dehydration

Some studies have proposed that K_{leaf} vulnerability may be related to leaf habit and, even more broadly, that K_{leaf} vulnerability would exert a control on plant distributions in wet versus dry forests or biomes, but evidence has been mixed (Blackman *et al.*, 2012, 2014; Scoffoni *et al.*, 2012; Nardini and Luglio, 2014; Gleason *et al.*, 2016). To test these hypotheses, we compiled all available data across studies and performed a global analysis of leaf P_{50} variation with leaf habit and biome (Figs 7, 8).

Our analyses supported a linkage of K_{leaf} vulnerability with leaf habit. One previous study reported no differences in P_{50} between three evergreen and three deciduous species (Chen *et al.*, 2009), and another reported that three deciduous tree species had less negative P_{50} values than two evergreen species (Villagra *et al.*, 2013). Our analysis of 215 species showed that among angiosperms, evergreen woody species had significantly more negative P_{50} values than deciduous woody species and herbs (–2.62 versus –1.68 versus –0.79 MPa, respectively; one-way ANOVA $P<0.001$; Fig. 7). This finding is consistent with the expectation that species with long-lived leaves that would undergo more droughts during their lifetime would benefit from greater resistance to hydraulic decline than species with leaves that only remain on the plant for several months. The benefit might be especially strong if K_{leaf} decline is not fully reversible (Scoffoni *et al.*, 2012) especially with repeated dehydration; that is, if leaves show 'K_{leaf} decline fatigue', analogous to the cavitation fatigue showed by stems (Hacke *et al.*, 2001), a hypothesis remaining to be tested. Notably, achieving higher resistance to hydraulic decline might come at an economic cost if it requires the construction of cells that can withstand higher tensions.

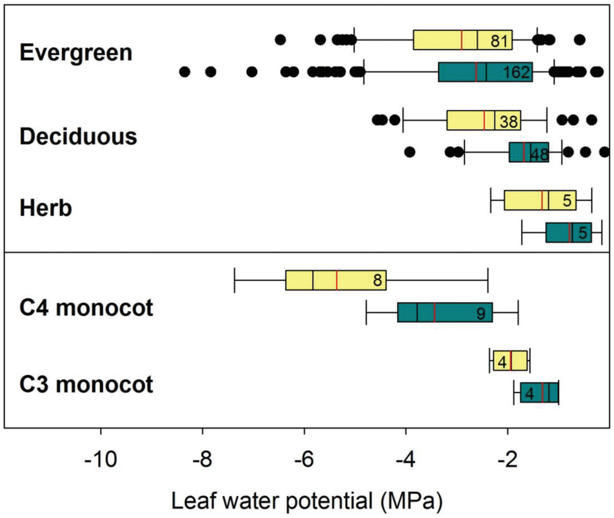

Fig. 7. Global analyses of the leaf water potential at which leaf hydraulic conductance declined by 50% (blue) and 80% (yellow) (K_{leaf} P_{50} and K_{leaf} P_{80}, respectively) for angiosperm species differing in leaf habit. Species numbers are reported inside the box plots. Red and black bars inside the bar plots represent mean and median values, respectively. Dots outside the plots are outliers, showing the range of values observed.

We tested for a shift of K_{leaf} vulnerability with photosynthetic type in grasses. Few studies have been performed for grasses; our analyses of 12 species show that C_4 species had significantly more negative P_{50} than C_3 species (–3.44 MPa versus –1.32 MPa, respectively; one-way ANOVA P=0.003; Fig. 7). C_4 species tend to occur in drier and more exposed environments, and have adapted their photosynthesis for higher water use efficiency. Our data suggest that they may have also evolved the ability to maintain hydraulic integrity at more negative water potentials. Future work should test these in an explicit phylogenetic framework, and considering both photosynthetic types across a range of habitat specializations.

Our global analyses revealed variation in K_{leaf} P_{50} values with biome water availability, as previously reported for 84 dicot species (Nardini *et al.*, 2012) and here extended to 216 dicot species (Fig. 8). We found significant differences in K_{leaf} P_{50} and P_{80} between Mediterranean/dry temperate, temperate, and tropical biomes (one-way ANOVA; P<0.001), with the most negative K_{leaf} P_{50} values in biomes with low water availability. However, each biome contained species that spanned a wide range of K_{leaf} P_{50} values, indicating strong functional differentiation within biomes. Indeed, microclimate niches for these species might be a stronger driver of variation in K_{leaf} P_{50}. Consistent with that hypothesis, two studies with >90 species showed a strong correlation between K_{leaf} P_{50} and the minimum water availability experienced by a species in its distributional range, with higher variation in K_{leaf} P_{50} in the driest extremes (Blackman *et al.*, 2012, 2014).

K_{leaf} vulnerability may also contribute to ecological differentiation or convergence among species co-existing within given communities. One study showed that exotic *Khaya senegalensis* had lower K_{leaf} vulnerability, and less reduction in g_s during drought than two native species possessing deeper roots (Arndt *et al.*, 2015). One study showed that two tropical rainforest lianas possessed similar hydraulic vulnerability to their host tree *Anacardium excelsum* (Johnson *et al.*, 2013).

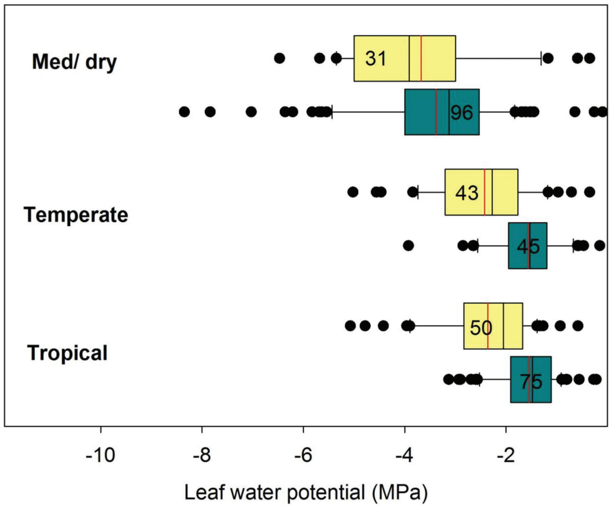

Fig. 8. Global analyses of the leaf water potential at which leaf hydraulic conductance declined by 50% (blue) and 80% (yellow) (K_{leaf} P_{50} and K_{leaf} P_{80}, respectively) across biomes for dicotyledonous angiosperm species. Species numbers are reported inside the box plots. Red and black bars inside the bar plots represent mean and median values, respectively. Dots outside the plots are outliers, showing the range of values observed.

As discussed in the section 'Impact of K_{leaf} decline on gas exchange and drought tolerance' above, drought tolerance depends importantly on the co-ordination of the declines of K_{leaf} and g_s with water potential. Thus, the plasticity of both K_{leaf} and g_s responses to leaf hydration requires further research. Two studies have suggested that species can modify their K_{leaf} P_{50} through osmotic adjustment during drought, to allow plants to operate at higher K_{leaf} values and maintain gas exchange rates under these stressful conditions (Holloway-Phillips and Brodribb, 2011; Martorell *et al.*, 2015), and K_{leaf} P_{50} shifted by up to 0.5 MPa and 0.6 MPa more negative in two grapevine cultivars grown throughout the summer under water deficit (Martorell *et al.*, 2015). Similarly, one study suggested groundwater conditions could have an impact on leaf hydraulics (Pan *et al.*, 2016): *Populus euphratica* exhibited higher K_{max} values and slightly more vulnerable leaves when grown further from rivers. Such a higher K_{max} would have an advantage under drier conditions for species that conduct rapid gas exchange when water is available but 'gear-down' during shortage (Maximov, 1931; Grubb, 1998; Pan *et al.*, 2016). Differences within and across biomes need to be investigated in an integrated way with models to decipher the mechanisms and implications for variation in the drought tolerance of the hydraulic system.

Future directions

Our analyses and syntheses highlighted avenues for research that will be critical to resolve completely the mechanisms and implications of K_{leaf} decline across crop cultivars and diverse species. Additional studies are needed of the partitioning of the decline in leaf hydraulic conductance to test the generality of recent findings pointing towards vulnerability in the outside-xylem pathways as the primary driver of whole leaf hydraulic decline with dehydration. In-depth anatomy of both the xylem and outside-xylem is still not well understood. Very few studies have looked at the nature of conduits (vessels versus tracheids), their diameters and length distributions across vein orders, or their pit dimensions, and how these vary across species or relate to leaf xylem vulnerability. Further, additional quantitative anatomy is needed for the tissues outside the xylem, along with the chemistry of cell walls, namely lignin in parenchyma and bundle sheath cells, and aquaporin activity across diverse species sets. Finally, further resolution is needed of the linkages among leaf hydraulic vulnerability, stomatal control, gas exchange and productivity, and survival of drought.

Supplementary data

Supplementary data are available at *JXB* online.

Appendix 1. The influence of leaf hydraulic decline in alleviating stem xylem tension.

Table S1. Data for all traits discussed in the study.

Table S2. Literature survey of the percentage hydraulic resistance outside the xylem (%R_{ox}).

Acknowledgements

We thank Tim Brodribb, Tom Buckley, Hervé Cochard, Steven Jansen, Dan Johnson, and Kate McCulloh for helpful insights during the preparation of this manuscript, as well as three anonymous reviewers that have helped greatly improved the quality and clarity of this manuscript. This work was funded by the US National Science Foundation (Award #1457279).

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