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The dynamic multi-functionality of leaf water transport outside the xylem

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Summary

A surge of papers have reported low leaf vulnerability to xylem embolism during drought. Here, we focus on the less studied, and more sensitive, outside-xylem leaf hydraulic responses to multiple internal and external conditions. Studies of 34 species have resolved substantial vulnerability to dehydration of the outside-xylem pathways, and studies of leaf hydraulic responses to light also implicate dynamic outside-xylem responses. Detailed experiments suggest these dynamic responses arise at least in part from strong control of radial water movement across the vein bundle sheath. While leaf xylem vulnerability may influence leaf and plant survival during extreme drought, outside-xylem dynamic responses are important for the control and resilience of water transport and leaf water status for gas exchange and growth.

I. Introduction

Decades of studies have shown that the efficiency of water movement through leaves (leaf hydraulic conductance, K_{leaf} in mmol m^{-2} s^{-1} MPa^{-1}) is typically sensitive to early leaf dehydration (Scoffoni & Sack, 2017). In a comprehensive review of 56 studies of 310 species, K_{leaf} declined with dehydration by 0–96% (40% on average across species) before leaf wilting, across lineages and all measurement methods utilized (fig. 1 in Scoffoni & Sack, 2017). This sensitivity can be viewed as a dynamic, recoverable response. Given that embolism of the vein xylem requires long periods of low xylem tension to recover (Cochard & Delzon, 2013), or may presage or precipitate leaf death (Brodribb *et al.*, 2021), a reversibly dynamic response of K_{leaf} to environmental cues is likely achieved via changes in the outside-xylem part of K_{leaf}, or K_{ox}. The balance of data supports such a major role of K_{ox} in controlling responses of K_{leaf} to mild or moderate dehydration. In our compilation of the data of the 11 studies of 34 species that assessed K_{leaf} and, in addition, K_{ox}, and/or leaf xylem hydraulic conductance (K_{x}) or vein embolism, K_{ox} or K_{leaf} declined by 9–99% (66% on average) before the onset of vein embolism (Table 1). The pathways involved and the mechanisms for dynamic responses of K_{ox} are incompletely elucidated and remain a topic for exciting research. Indeed, the

Table 1 Compilation of studies that quantified the responses to leaf dehydration of leaf hydraulic conductance (K_{leaf}) and either or both of outside-xylem and xylem hydraulic conductances (K_{ox} and K_{x}, respectively) or leaf vein embolism.

Species	Family	Method for K_{leaf} and/or K_{ox} quantification	Method for K_{x} or embolism quantification	%K_{leaf} and %K_{ox} (denoted by * when available) decline before P_{12} or P_{e}	Water potential at P_{12} or P_{e} (MPa)	Reference(s)
Eucalyptus globulus	Myrtaceae	DRKM	OV	19%	−4.1	Brodribb *et al*. (2016)
Bursaria spinosa	Pittosporaceae	DRKM	OV	27%	−5.6	Brodribb *et al*. (2016)
Eucryphia moorei	Cunoniaceae	DRKM	OV	37%	−2.4	Brodribb *et al*. (2016)
Senecio minimus	Asteraceae	DRKM	OV	25%	−1.6	Brodribb *et al*. (2016)
Aleurites moluccana	Euphorbiaceae	RKM and VPM	VPM (cutting veins)	67% (RKM)–60% (VPM)	−3.0	Trifilò *et al*. (2016)
Magnolia grandiflora	Magnoliaceae	RKM and VPM	VPM (cutting veins)	80% (RKM)–86% (VPM)	−2.0	Trifilò *et al*. (2016)
Quercus rubra	Fagaceae	RKM and VPM	VPM (cutting veins)	86% (RKM)–75% (VPM)	−3.5	Trifilò *et al*. (2016)
Vitis labrusca	Vitaceae	RKM and VPM	VPM (cutting veins)	60%(RKM)– 63% (VPM)	−1.5	Trifilò *et al*. (2016)
Salvia canariensis	Lamiaceae	EFM	VPM (cutting veins)	35%/60%*	−0.21	Scoffoni *et al*. (2017)
Lantana camara	Verbenaceae	EFM	VPM (cutting veins)	9.1%/8.7%*	−0.31	Scoffoni *et al*. (2017)
Hedera canariensis	Araliaceae	EFM	VPM (cutting veins)	75%/80%*	−1.05	Scoffoni *et al*. (2017)
Cercocarpus betuloides	Rosaceae	EFM	VPM (cutting veins)	19%/12%*	−0.65	Scoffoni *et al*. (2017)
Quercus agrifolia	Fagaceae	EFM	VPM (cutting veins)	92%/93%*	−4.4	Scoffoni *et al*. (2017)
Comarostaphylis diversifolia	Ericaceae	EFM	VPM (cutting veins)	51%/53%*	−2.9	Scoffoni *et al*. (2017)
Magnolia grandiflora	Magnoliaceae	EFM	VPM (cutting veins)	76%/79%*	−2.0	Scoffoni *et al*. (2017)
Malosma laurina	Anacardiaceae	EFM	VPM (cutting veins)	52%/75%*	−0.70	Scoffoni *et al*. (2017)
Solanum lycopersicum 'Rhineland's Rhun'	Solanaceae	DRKM	OV	12%	−1.2	Skelton *et al*. (2017)
Arabidopsis thaliana Col-0	Brassicaceae	EFM	MicroCT	> 88%	> −0.87	Scoffoni *et al*. (2018)
Guzmania monostachia	Bromeliaceae	EFM	Dye uptake	30%	na	North *et al*. (2019)
Vitis vinifera L. 'Cabernet Sauvignon'	Vitaceae	EFM	MicroCT	95%	−1.3	Albuquerque *et al*. (2020)
Vitis vinifera L. 'Chardonnay'	Vitaceae	EFM	MicroCT	95%	−1.2	Albuquerque *et al*. (2020)
Triticum aestivum	Poaceae	RKM	OV	89%	−1.6	Corso *et al*. (2020)
Populus nigra L.	Salicaceae	EFM	VPM (cutting veins)	47%/52%*	−1.0	Trifilò *et al*. (2021)
Caragana korshinskii	Fabaceae	EFM	OV	77%	−4.0	Yao *et al*. (2021)
Caragana intermedia	Fabaceae	EFM	OV	78%	−4.0	Yao *et al*. (2021)
Caragana microphylla	Fabaceae	EFM	OV	93%	−4.0	Yao *et al*. (2021)
Caragana boisi	Fabaceae	EFM	OV	96%	−4.0	Yao *et al*. (2021)
Caragana stipitata	Fabaceae	EFM	OV	94%	−4.0	Yao *et al*. (2021)
Caragana sinica	Fabaceae	EFM	OV	99%	−4.0	Yao *et al*. (2021)
Festuca arundinacea	Poaceae	*In situ* EFM	OV	86%	−3.9	Jacob *et al*. (2022)
Phalaris aquatica	Poaceae	*In situ* EFM	OV	94%	−3.6	Jacob *et al*. (2022)
Chloris gayana	Poaceae	*In situ* EFM	OV	67%	−2.3	Jacob *et al*. (2022)
Digitaria eriantha	Poaceae	*In situ* EFM	OV	> 94%	−4.3	Jacob *et al*. (2022)
Themeda triandra	Poaceae	*In situ* EFM	OV	> 93%	−4.3	Jacob *et al*. (2022)

Species, methods for quantification of K_{leaf}, K_{ox}, K_{x}, and embolism are provided, and the %K_{ox} or K_{leaf} decline before the water potential corresponding to 12% K_{x} decline (P_{12}) or the onset of embolism (P_{e}). Percentage declines and water potentials were taken from published fitted response curves; when multiple curves were plotted for given species, values were averaged across curves. Notably, the contribution of K_{ox} decline to that of K_{leaf} during dehydration may be greater than indicated by its % decline, as its influence on K_{leaf} depends on the relative contribution of xylem and outside-xylem pathways to leaf hydraulic resistance. For example, though 9% of K_{ox} is lost at P_{12} in *Lantana camara*, K_{ox} decline explained 86% of that of K_{leaf} by the turgor loss point as most of the resistance lies outside the xylem in that species (%$R_{\mathrm{ox}} = 87$%; Scoffoni *et al*., 2017). Symbols: EFM, evaporative flux method; DRKM, dynamic rehydration kinetics method using a flowmeter; *In situ* EFM, measured from bag/unbagged leaf water potential and transpiration; RKM, rehydration kinetics method using pressure-volume curves; VPM, vacuum pump method; OV, optical vulnerability; MicroCT, micro-computed tomography.

benefits provided to the plant by dynamic responses of K_{ox} before stomatal closure can help explain evolutionary diversity across species in leaf water relations and gas exchange, and predict their responses to climate change. Here, we discuss the causes and potential benefits of a dynamic K_{ox} in response to multiple factors (Table 2), focusing on studies that show a sensitive K_{ox} or K_{leaf} in leaves hydrated above thresholds for turgor loss or vein embolism, which we assume to be mainly driven by outside-xylem changes. We highlight recent discoveries and future directions in this rapidly moving field.

Table 2 List of the hypothesized benefits of a multi-dynamic and rapidly responsive K_{ox} to dehydration.

Benefits of a multi-dynamic K_{ox}	Line of evidence	K_{leaf} method	Reference(s)
Regulation of gas exchange			
Protection from xylem embolism	Whole plant modeling showed that K_{ox} sensitivity to dehydration helps delay xylem embolism and mortality during drought	Evaporative flux method (balance method)	Scoffoni *et al*. (2018)
	MicroCT studies showed K_{leaf} decline is not explained by xylem embolism, suggesting that K_{ox} sensitivity acts to protect the xylem from embolism by preventing negative water potentials from building in the xylem, via stomatal control	Evaporative flux method (balance method)	Scoffoni *et al*. (2017, 2018); Albuquerque *et al*. (2020)
Increase in water use efficiency	Whole plant modeling showed that by amplifying stomatal closure, a dynamic K_{ox} improves plant water use efficiency and net CO_2 assimilation during drought periods	Evaporative flux method (balance method)	Scoffoni *et al*. (2018)
	More sensitive K_{leaf} to dehydration was reported in maize hybrids that exhibited greater growth and yield under both moist and dry soil conditions	Rehydration kinetics method	Gleason *et al*. (2021)
	ABA-insensitive bundle sheath and mesophyll cell mutants exhibited reduced WUE. Lack of ABA control of K_{leaf} led to higher K_{leaf} values, while photosynthetic rates remained similar	Evaporative flux method (LICOR method)	Yaaran *et al*. (2023)
Helping coordinate water transport and sugar export in veins	Application of glucose via leaf petioles led to significant decreases in K_{leaf}	Evaporative flux method (LICOR method)	Kelly *et al*. (2017)
Protecting the mesophyll from dehydration by subsaturated intercellular airspaces during transpiration	Leaves exposed to moderate-to-high air vapor pressure deficits exhibited below-saturation intercellular vapor pressure	N/A	Cernusak *et al*. (2018, 2019); Buckley & Sack (2019); Wong *et al*. (2022)
	Stomatal closure helped maintain saturation inside leaves at high VPD, whereas ABA-insensitive mutants could not close stomata and exhibited unsaturated airspaces		
	The unsaturation of leaf airspaces implies a very large water potential drop between the mesophyll symplast and adjacent airspaces		
	At high VPD, mesophyll cells adjacent to unsaturated airspaces remained hydrated and continued active photosynthesis		
Rapid response to abiotic factors			
Rapid adjustment to short time-scale changes in environmental conditions such as temperature, relative humidity and light, optimizing use of resources	Aquaporins activation enabled a rapid increase in leaf-specific hydraulic conductivity in Arabidopsis after exposure to low relative humidity while g_s remained constant	Transpiration *in vivo* and water potential	Levin *et al*. (2007)
	In Arabidopsis, K_{leaf} was reduced by 70% when measured under red light, relative to under red and blue light combined, and knockout mutants of the protein kinases phot1 and phot2 involved in blue light perception had significantly lower K_{leaf} than the wild-type under blue light, as did the wild-type when fed a kinase inhibitor	Evaporative flux method (LICOR method)	Grunwald *et al*. (2022)
	Several species rapidly increased their K_{leaf} upon exposure to high irradiance	Evaporative flux method and Rehydration kinetics method	Scoffoni *et al*. (2008) and references therein
	Species growing in habitats with greater potential evapotranspiration evolved a higher $K_{leaf} : g_s$ ratio	Evaporative flux method (flowmeter method)	Brodribb & Jordan (2008); Scoffoni *et al*. (2016)
	Lobeliad species growing under greater light environments exhibited greater $K_{leaf} : g_s$ ratios.	Evaporative flux method (flowmeter method)	Scoffoni *et al*. (2015)
	The high K_{leaf}/g_s observed in *Simarouba glauca* during the dry season would have buffered the effect of VPD on stomata	Rehydration kinetics method	Brodribb & Holbrook (2004)
	K_{ox} of *Acer saccharum* and *Quercus rubra* increased at higher temperature	High-pressure flowmeter	Sack *et al*. (2004)

Table 2 (Continued)

Benefits of a multi-dynamic K_{ox}	Line of evidence	K_{leaf} method	Reference(s)
Acceleration of tissue rehydration after periods of low soil moisture	Upon rehydration after exposure to moderate drought, K_{leaf} of potted species recovered overnight	Rehydration kinetics method	Blackman *et al*. (2009)
	In a study of Arabidopsis aquaporin mutants, control plants exposed to an 8-d dry down recovered faster in K_{leaf} than the mutants	Transpiration *in vivo* and water potential	Martre *et al*. (2002)
Prevention of flooding of the leaf airspaces of species exhibiting nocturnal positive pressure	In dark conditions, osmotic water permeability of bundle sheath cells associated with K_{leaf} is low	Evaporative flux method (LICOR method)	Grunwald *et al*. (2022)
Rapid response to biotic factors			
Prevention of fungal pathogens in the xylem sap from reaching the living cells	Application of chitin, a microbe-associated molecular pattern, led to a sharp decrease in osmotic water permeability in bundle sheath and mesophyll cells, decreasing K_{leaf} and closing stomata	Evaporative flux method (LICOR method)	Attia *et al*. (2020)

Strong changes in K_{leaf} in leaves hydrated above wilting point are assumed to reflect the responses of K_{ox}, given that embolism tends to occur past the stage of leaf wilting. g_s, stomatal conductance; K_{leaf}, leaf hydraulic conductance; K_{ox}, outside-xylem hydraulic conductance; VPD, vapor pressure deficit.

II. Localizing the K_{ox} response in bundle sheath cells

Outside the xylem, water moves through and/or around bundle sheath (BS) cells, then through the mesophyll, and finally through stomata, with the specific distribution of flows depending on species' anatomy (Fig. 1; Buckley *et al.*, 2015, 2017). In most hypostomatous species, palisade mesophyll is relatively hydraulically isolated, sustaining photosynthesis during mild dehydration (Yaaran *et al.*, 2023). Many recent studies of leaf water transport have focused on BS cells (Buckley *et al.*, 2015, 2017), yet the microroutes for water movement from the xylem into the BS remain unclear. Much like the endodermis in roots, leaf BS cells act as a barrier controlling water flow out of the xylem, particularly in response to environmental stressors (Lee *et al.*, 2009; Shatil-Cohen *et al.*, 2011). While the apoplastic route may be highly conductive for water (Buckley *et al.*, 2015), in many species, it is blocked at the BS by cell wall suberin/lignin deposits (Mertz & Brutnell, 2014; Taneda *et al.*, 2016). Across diverse species, those with lignified BS cell walls had > 50% lower K_{ox} and K_{leaf} (Ohtsuka *et al.*, 2018). However, the transmembrane pathway from xylem to BS might be equally important, regardless of cell wall lignification, as species without minor vein BS lignification exhibited greater K_{leaf} light enhancement (Ohtsuka *et al.*, 2018), which in turn is associated with activation of aquaporins in BS cell membranes (Grunwald *et al.*, 2022).

Indeed, recent studies have proposed a major mechanistic role for aquaporins in the control of water movement out of the xylem, and in leaf dynamic responses to the environment. Blue light activates H^+-ATPase pumps in BS membranes, causing acidification of xylem sap, which in turn activates aquaporins in the BS membrane, enhancing its permeability (Grunwald *et al.*, 2021, 2022). These results are consistent with a previous study of silver birch showing an enhancement of K_{leaf} in shade leaves under blue relative to red light (Sellin *et al.*, 2011), and many studies have reported a rapid enhancement of K_{leaf} across many species in response to increased irradiance, especially in well-hydrated leaves (e.g. Scoffoni *et al.*, 2008; Guyot *et al.*, 2012). During drought, ABA transported in xylem sap may be sensed at the BS membranes, triggering deactivation of aquaporins and reduction of membrane permeability for water transfer to the mesophyll (Shatil-Cohen *et al.*, 2011; Pantin *et al.*, 2013). The resulting reduced K_{leaf} would accelerate mesophyll dehydration, causing increased ABA production and potentially driving stomatal closure (Yaaran *et al.*, 2023). At the same time, ABA appears to *increase* symplastic flow from xylem to BS through plasmodesmata, possibly to help equilibrate water potential (Ψ) across cells (Yaaran *et al.*, 2023). More work is needed to disentangle the roles of the different outside-xylem pathways, and how these depend on membrane proteins and vary across species of diverse anatomies.

III. Benefits of a dynamic K_{ox}

Stomatal control and plant growth during mild and moderate drought

A high K_{ox} sensitivity would cause a high K_{leaf} sensitivity, which would protect midrib, petioles, and stems from embolizing (Zhang *et al.*, 2016, 2022; Scoffoni *et al.*, 2017; Albuquerque *et al.*, 2020), following the hydraulic vulnerability segmentation hypothesis (Tyree & Ewers, 1991). We note that at a given transpiration rate, K_{ox} or K_{leaf} decline would not directly ameliorate the water potential of leaf, stem or root xylem (Fig. 2) – except in the case that K_{ox} went to zero (i.e. becoming a 'circuit breaker' that completely stopped flow), which has not been observed. Rather, K_{ox} decline could play a major protective role by causing stomatal closure and thus reducing transpiration rate, which would allow Ψ throughout the plant to recover. A sensitive K_{ox} could help amplify stomatal closure (Buckley, 2005), provided the location where Ψ is 'sensed', leading to active regulation of stomatal aperture, is downstream from the xylem (Buckley *et al.*, 2017). It is unclear where sensing occurs; some evidence supports the mesophyll (McAdam & Brodribb, 2018), while other data suggest the vasculature (Kuromori *et al.*, 2014). Modeling shows that stomatal closure in droughted wheat and Arabidopsis could be 9–65% attributable to K_{leaf} dynamics (Scoffoni *et al.*, 2018; Corso *et al.*, 2020); this is because, if K_{leaf} is

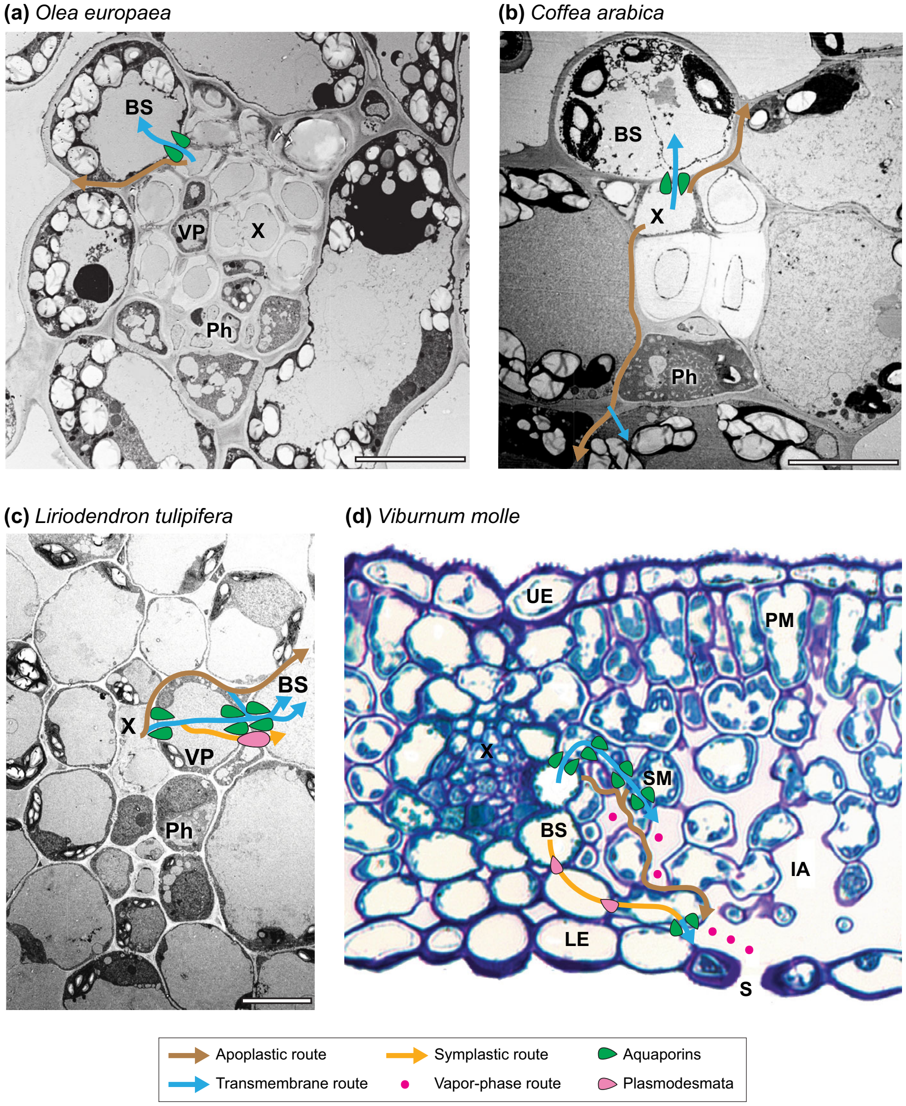
(a) *Olea europaea*
(b) *Coffea arabica*
(c) *Liriodendron tulipifera*
(d) *Viburnum molle*

Fig. 1 Pathways for water movement out of the minor vein xylem to stomata. Transmission electron micrograph of minor veins of three diverse species: *Olea europaea* (Oleaceae, a), *Coffea arabica* (Rubiaceae, b), and *Liriodendron tulipifera* (Magnoliaceae, c). Cross-sectional light microscopy of *Viburnum molle* (Viburnaceae; d). Because the secondary walls of xylem conduits are lignified, water will move through primary wall in xylem pits and/or in between the helical thickenings of the secondary wall to the bundle sheath (BS) or vascular parenchyma. From there, water could move apoplastically (brown lines) and/or across the BS membrane through aquaporins (blue lines). Alternatively, if the water moves through vascular parenchyma before reaching the BS, it could enter BS cells symplastically through plasmodesmata (orange lines). Once at the BS, water can either follow a symplastic (via plasmodesmata; orange), transmembrane (via aquaporins, blue), apoplastic (brown), and/or vapor-phase (pink dots) routes. Notably, water evaporates into intercellular airspaces from exposed cell walls, and water moving symplastically must cross one or more membranes via aquaporins to reach the cell walls. Bars, 10 μm. BS, bundle sheath; LE, lower epidermis; Ph, Phloem; PM, palisade mesophyll; S, stomata; SP, spongy mesophyll; UE, upper epidermis; VP, vascular parenchyma; X, xylem. Aquaporins are represented as green shapes, plasmodesmata as pink shapes.

more sensitive to Ψ than stomatal conductance (g_s), the ratio of water demand (controlled by g_s) to supply (K_{leaf}) increases during dehydration, amplifying the decline in Ψ_{leaf} and, with it, stomatal closure (Scoffoni *et al.*, 2018; Albuquerque *et al.*, 2020). By amplifying stomatal closure, a dynamic K_{ox} can improve intrinsic water use efficiency, that is, reducing g_s more than carbon assimilation (A), and thus increasing A/g_s, and enhance cumulative carbon gain integrated over growth throughout a drought under field conditions, while preventing higher xylem tensions that cause embolism (Scoffoni *et al.*, 2018). Crop productivity would particularly benefit from a dynamic K_{ox}, as water stress can severely reduce yields, especially during reproductive stages (Senapati *et al.*, 2019; Cohen *et al.*, 2021). More sensitive K_{leaf} to dehydration was reported in maize hybrids that exhibited greater growth and yield under both wet and dry soil conditions (Gleason *et al.*, 2021). A sensitive K_{ox} would also help mitigate effects of increased and prolonged atmospheric drought, which can reduce photosynthesis (Grossiord *et al.*, 2020) and drive the formation of xylem embolism (Schönbeck *et al.*, 2022).

The importance of the vulnerability of K_{ox} to dehydration in determining stomatal closure would depend on how these responses are governed by mesophyll or epidermal hydration status. ABA is produced in dehydrating mesophyll (McAdam & Brodribb, 2018), and affects BS and guard cells, reducing K_{ox} and g_s semi-independently (Shatil-Cohen *et al.*, 2011; Pantin *et al.*, 2013). Indeed, a literature review concluded that species varied in their coordination of K_{leaf} and g_s decline during dehydration, with some species exhibiting roughly similar sensitivity in K_{leaf} and g_s decline (Scoffoni & Sack, 2017). In the genus *Caragana*, species native to arid habitats exhibited higher K_{leaf}, less sensitivity of K_{leaf} to dehydration, enhanced ABA accumulation and more sensitive stomatal responses to dehydration than species from moist habitats, thus increasing water use efficiency even without K_{leaf} sensitivity to dehydration (Yao *et al.*, 2021). Future work is needed to disentangle the diversity in g_s and K_{leaf} responses to dehydration, how these responses are driven by changes in K_{ox}, and how they scale up to impacting plant growth under varying environmental conditions (Torres-Ruiz *et al.*, 2015).

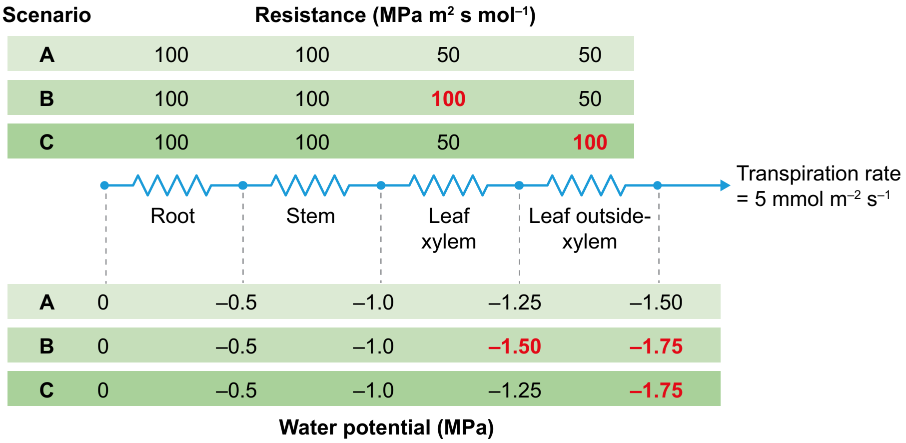

Fig. 2 At a given transpiration rate, changes in the hydraulic resistance of leaf pathways do not *directly* affect xylem water potentials elsewhere in the plant. For example, if leaf xylem resistance is doubled from 50 MPa m^2 s mol^{-1} (scenario A) to 100 MPa m^2 s mol^{-1} (scenario B), water potential declines only for locations downstream of the leaf xylem; it does not change in locations upstream of the leaf xylem, such as the stem or root. Similarly, if the resistance of leaf outside-xylem pathways is doubled from 50 MPa m^2 s mol^{-1} (scenario A) to 100 MPa m^2 s mol^{-1} (scenario C), water potential does not change in the leaf xylem, nor in any locations farther upstream. Water potential for each location was calculated as the water potential of the location immediately proximal to (upstream from) it, minus the product of transpiration rate and the resistance between the two locations. For example, the water potential at the base of the petiole (i.e. between the stem and leaf xylem) was -0.5 MPa – (0.005 mol m^{-2} s^{-1}) × (100 MPa m^2 s mol^{-1}) = -1.0 MPa. Calculations assumed constant soil water potential of 0 MPa, constant leaf transpiration rate of 5 mmol m^{-2} s^{-1}, and steady-state flows among compartments. Bold and red water potential values are shown when modified due to the imposed change in resistance in each scenario (also in bold/red).

Rapid adjustments to short-term abiotic/biotic changes

A dynamic K_{ox} would help species adjust to rapid changes in internal and external conditions. In Arabidopsis, aquaporin activation enabled rapid increases in hydraulic conductance after exposure to low humidity while stomatal conductance remained stable, such that Ψ_{leaf} only decreased by 0.25 MPa when VPD was increased by 1.85 kPa, ensuring adequate water supply to meet increased transpirational demand and avoid tissue dehydration (Levin *et al.*, 2007). Indeed, a high K_{ox}/g_s can enhance both short-term acclimation and long-term adaptation. Higher $K_{leaf} : g_s$ ratios were found in species from habitats with greater potential evapotranspiration (Brodribb & Jordan, 2008; Scoffoni *et al.*, 2015, 2016), and in tropical species facing a dry season (Brodribb & Holbrook, 2004). Leaf warming can also increase K_{ox}, consistent with aquaporin-mediated membrane permeability (Sack *et al.*, 2004). Additionally, the rapid enhancement of K_{leaf} in response to irradiance in many species (Scoffoni *et al.*, 2008) has been associated with a blue light response, helping keep stomata open when energy is most available (Grunwald *et al.*, 2022). A low K_{leaf} under low light via aquaporin deactivation (and thus decreased membrane permeability) may also prevent flooding of leaf airspaces when Ψ is high at night (Grunwald *et al.*, 2022). Other potential benefits of dynamic K_{leaf} include coordinating water transport and sugar export in veins (Kelly *et al.*, 2017), preventing fungal pathogens in the xylem sap from reaching living cells (Attia *et al.*, 2020), and accelerating tissue rehydration after periods of high VPD or low soil moisture (Martre *et al.*, 2002; Blackman *et al.*, 2009).

The association of K_{ox} dynamics with protection of leaf mesophyll from unsaturated leaf intercellular airspaces

Recent work has suggested that leaf intercellular airspaces are not saturated with water vapor when leaves are exposed to dry air (Cernusak *et al.*, 2018, 2019; Wong *et al.*, 2022). Unsaturation implies very low Ψ in the mesophyll airspaces, at the same time as a fairly high Ψ must be maintained in the mesophyll symplast if cells are continuing to photosynthesize. Indeed, the co-occurrence of unsaturation and active photosynthesis implies a very large drop in Ψ – up to 30 MPa – between the mesophyll symplast and the adjacent airspaces (Buckley & Sack, 2019). This drop in Ψ raises the question of how a low K_{ox} may be related to airspace unsaturation, that is, as a potential cause, by limiting water supply to the airspaces, and/or as a mechanism for protecting the mesophyll from low airspace Ψ.

The possibility of airspace unsaturation poses a quandary for modeling leaf water transport. The most sophisticated models to date have assumed that the spatial pattern of Ψ across the xylem and epidermis can be reasonably approximated with a single scalar field, and that flow outside the xylem occurs through four parallel and continuously anastomosing pathways (airspaces, apoplast, symplast, and transmembrane), such that mesophyll and adjacent airspaces have similar Ψ (Rockwell *et al.*, 2014; Buckley *et al.*, 2015, 2017). In other words, each point or 'grid cell' in these models represents a small volume in which it is assumed reasonable to average Ψ among three phases (airspaces, apoplast, and symplast). Yet, airspace unsaturation implies instead that symplastic water is largely sequestered from water in the airspaces and adjacent apoplast, generating two very different Ψ fields. Such isolation could result from very low membrane permeability, due to low aquaporin activity (Wong *et al.*, 2022), and/or from large decreases in mesophyll cell wall water permeability at high VPD. Distinguishing adjacent symplast and airspace phases in these models would require vastly increasing their spatial resolution and hence computational requirements.

If leaf airspaces are unsaturated, then how does having two different Ψ gradients outside the xylem (one through the symplast and one through the airspaces) relate to K_{ox}? Here, we must distinguish between operational and aspirational definitions of K_{ox}. Operationally, K_{ox}, like K_{leaf}, is always measured using a bulk Ψ_{leaf}, a practical necessity for studying temporal and species variation. However, bulk Ψ_{leaf} measurements would be unlikely to detect airspace unsaturation; the quantity of water in the apoplast and airspaces is very small compared with that in the symplasm, so when a leaf is excised and equilibrated for pressure chamber measurement, even very low airspace Ψ would relax to high values without causing much water loss from the mesophyll symplast. Thus, the operational measurement of K_{ox} is likely dominated by mesophyll Ψ ($\psi_{\mathrm{mesophyll}}$):

$$K_{\mathrm{ox}}(\mathrm{operational}) \approx \frac{\mathrm{Transpiration}}{\left(\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{mesophyll}}\right)}.$$

Were we to define K_{ox} *aspirationally* as referring to pathways that end in the airspaces, then

$$\begin{aligned} K_{\mathrm{ox}}(\mathrm{ending\ at\ airspaces}) &= \frac{\mathrm{Transpiration}}{\left(\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{airspaces}}\right)} \\ &= \frac{\mathrm{Transpiration}}{\left(\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{mesophyll}}\right) + \left(\Psi_{\mathrm{mesophyll}} - \Psi_{\mathrm{airspaces}}\right)} \\ &= K_{\mathrm{ox}}(\mathrm{operational}) \\ &\quad \times \frac{\left(\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{mesophyll}}\right)}{\left(\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{mesophyll}}\right) + \left(\Psi_{\mathrm{mesophyll}} - \Psi_{\mathrm{airspaces}}\right)}. \end{aligned}$$

Unsaturation implies that $\Psi_{\mathrm{mesophyll}} - \Psi_{\mathrm{airspaces}}$ is very large compared with $\Psi_{\mathrm{xylem}} - \Psi_{\mathrm{mesophyll}}$, so the aspirational value of K_{ox} would be much smaller than the operational value. Such a large decline in K_{ox} would only be possible via either tight transmembrane control of water movement, which could occur via aquaporin deactivation, or via a dramatic increase in the resistance for water movement across mesophyll cell walls, by yet-unknown mechanisms (Wong *et al.*, 2022). A role for aquaporins would be consistent with that previously observed in BS cell membranes, as discussed above. Thus, a low value of (aspirational) K_{ox} arising from low membrane permeability would be both a cause of airspace unsaturation, and a mechanism to protect the mesophyll from dehydration due to surrounding dry airspaces.

We note that which definition of K_{ox} is preferred – operational vs aspirational – depends on our research question. For example, the operational definition is arguably preferable when mesophyll water status is of particular interest, as in studies of bulk leaf water relations, relative water content, turgor, and photosynthetic function. Similarly, if BS cells are the central player in the multidynamic functions of K_{leaf}, then bulk Ψ_{leaf} would likely mirror these changes well. Conversely, if airspace unsaturation or guard cell water relations is of particular interest, then we would like to know the conductance all the way to airspace and the guard cells. Technological advances enabling quantification of Ψ *in vivo* at leaf level, using intercellular gel sensors (Jain *et al.*, 2021; Rockwell *et al.*, 2022), or at the cellular level, using intracellular protein biosensors (Cuevas-Velazquez *et al.*, 2021) may soon enable estimation of K_{ox} dynamics according to both definitions.

IV. Conclusions

K_{leaf} can be dynamic, exhibiting high values at Ψ near zero, and declining sensitively as Ψ_{leaf}, temperature, or irradiance decline. A large body of evidence suggests this dynamic responsiveness occurs in the outside-xylem pathways and is associated with the activation/deactivation of aquaporins. Biochemical control of K_{leaf} would allow for fast 'recovery' of K_{leaf} when conditions improve, and help leaves rapidly adjust to biotic and abiotic changes and improve water use efficiency and growth. Future work is needed to understand the variation in outside-xylem pathways across species and how it scales up to explaining the diversity of K_{leaf} responses to dehydration, and, ultimately, the impact of K_{ox} declines on stomatal control, sugar export, and productivity as climate changes.

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Competing interests

None declared.

Author contributions

CA, TNB, and LS contributed ideas and helped write the manuscript, with CS leading the effort.

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